B. INTRODUCTION

In 1983 the Australian pathologist Robin Warren and the microbiologist Barry Marshall isolated a spiral, microaerophilic, Gram-negative bacterium from a gastric biopsy of a patient suffering from duodenal ulcer and proposed that the bacterium was the cause of the disease (Warren and Marshall, 1983). In the years following this discovery, the results of a huge amount of research carried out in laboratories all over the world have confirmed this hypothesis and have identified *Helicobacter pylori* as the major cause of many gastroduodenal disorders including chronic superficial gastritis, chronic active gastritis, peptic ulcer, and gastric adenocarcinoma (Blaser, 1987; Dick, 1990; Nomura *et al*., 1991; Parsonnet *et al*., 1991). Epidemiological studies revealed that *H. pylori* is one of the most important human gastrointestinal pathogens, infecting 25-50% of the population in industrialised countries, and 80% or more of the population in the developing world (Taylor and Blaser, 1991). Infection of the stomach with *H. pylori* appears to occur early in childhood, probably via faecal-oral or oral-oral transmission from person to person (Thomas *et al*., 1992; Megraud, 1995; Blaser, 1993). Following infection, the bacterium colonises the gastric mucosa and causes an acute gastritis that progresses into chronic gastritis (also called bacterial or type B gastritis), a condition that usually persists for life (Fig. 1). In the years following the initial infection the generally asymptomatic chronic gastritis may progress into more severe forms of the disease, such as peptic ulcer, MALT (mucosa-associated-lymphoid tissue)-lymphoma, or the near complete loss of gastric epithelial cell function observed in atrophic gastritis. The latter condition is considered a precancerous lesion that over the course of decades might result in the development of adenocarcinoma (Nomura *et al*., 1991; Parsonnet *et al*., 1991). Because of the long period of time that usually passes between *H. pylori* infection and the onset of disease, the term "slow bacterium" has been proposed to reflect the pathogen's ability to avoid the defence mechanisms of the host and to survive in the gastric mucosa for years or decades (Blaser, 1993).

Many of the bacterial factors that are responsible for the establishment of such a persistent infection have been characterised in some detail in the last few years. One of the most important factors involved in the initial phase of infection are the flagella, that allow the bacteria to move in the stomach lumen and in the viscous mucus layer overlying the gastric epithelium. *H. pylori* cells normally possess from two to six unipolar flagella that consist of a central flagellar filament and a surrounding membranous sheath that protects the acid-labile filament from the low pH of the stomach (Suerbaum, 1995). The filament is a co-polymer of the flagellin subunits FlaA (Leying *et al*., 1992; Haas *et al*., 1993) and FlaB (Suerbaum *et al*., 1993), with FlaA being the predominant subtype and FlaB the minor subtype, localised close to the basis of the flagellum (Kostrzynska *et al*., 1991). Both flagellins have similar molecular mass (53 kDa) and share considerable amino acid

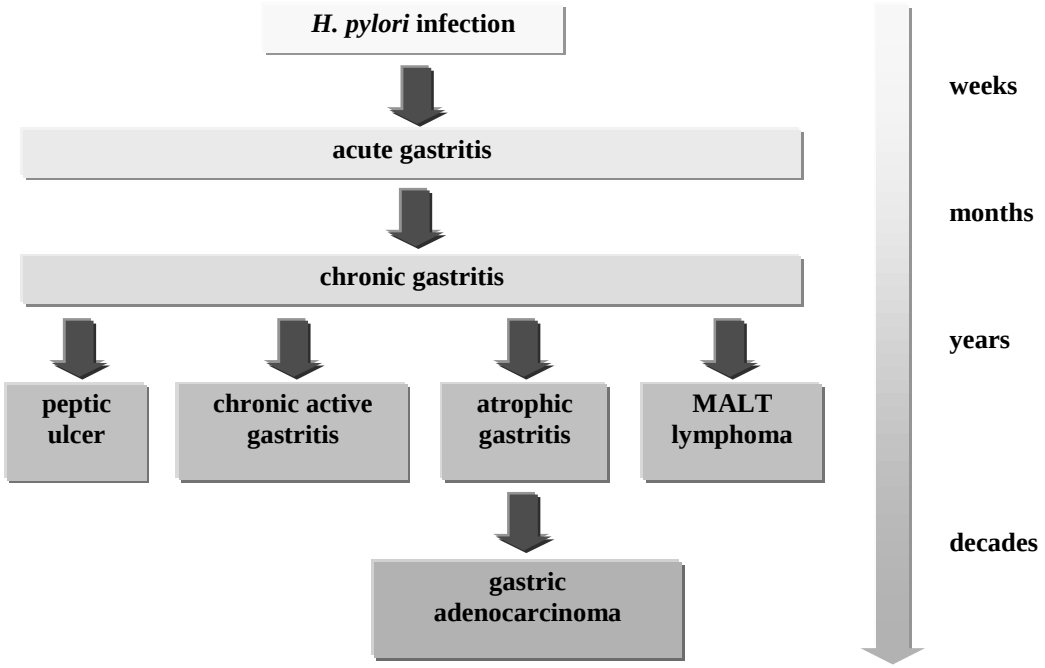

Fig. 1: Schematic representation of the progression of *Helicobacter pylori* infection and associated diseases.

homology (58% identity), but the respective genes are unlinked on the chromosome (Suerbaum *et al*., 1993). Studies with isogenic mutants of either *flaA* or *flaB* have revealed that both genes are necessary for full motility (Josenhans *et al*., 1995) and for establishment of a persistent *H. pylori* infection in the gnotobiotic piglet model (Eaton *et al*., 1996). Besides the flagellin genes, a few other loci involved in formation of the flagellar apparatus have been characterised. These include the *flgE* gene which codes for the structural component of the flagellar hook, that connects the filament to the flagellar basal disk (O'Toole *et al*., 1994). Mutants in this gene are nonmotile and aflagellate, but continue to produce both flagellin monomers in apparently normal amounts. Another gene essential for flagellar biosynthesis is *flbA*, which codes for a membrane protein with sequence homology to the FlbF/LcrD family of motility and virulence associated proteins (Schmitz *et al*., 1997). Isogenic *flbA* mutants are nonmotile and fail to express both FlaA and FlaB, while the production of FlgE is severely reduced. Recently, an operon coding for two putative flagellar export proteins (FliI and FliQ) was also characterised (Jenks *et al*., 1997; Porwollik *et al*., 1999). Both proteins were found to be necessary for flagellum synthesis and motility and isogenic mutants showed reduced production of flagellins and hook protein.

The second bacterial factor essential for colonisation of the gastric epithelium is represented by the urease enzyme, which hydrolyses urea into ammonia and carbon dioxide (Labigne *et al*., 1991; Cussac *et al*., 1992). Experiments with isogenic mutants obtained by gene disruption have shown that urease is essential for colonisation in the gnotobiotic piglet model (Eaton and Krakowka, 1994) and the nude mouse model (Tsuda *et al*., 1994). Furthermore, there is evidence that the ammonia ions produced by urease contribute to the tissue damage observed in *H. pylori* infections. The ammonia ions are *per se* not toxic but the hydroxide ions that are generated from

ammonia by equilibration with water seem to exert a strong cytotoxic effect on gastric epithelial cells (Barer *et al.*, 1990; Smoot *et al*., 1990). The urease enzyme is a large (~550 kDa) multimeric complex containing six copies of each of the two different structural subunits UreA (26.5 kDa) and UreB (61 kDa) and several Ni^{2+} ions (Dunn *et al*., 1990). Although synthesised and localised in the cytoplasm of *H. pylori*, part of the urease enzyme ultimately accumulates on the bacterial surface (Dunn *et al*., 1990, Bode *et al*., 1993a), where it is thought to contribute to the short term survival of *H. pylori* during its passage through the acidic milieu of the stomach lumen by producing a neutral microenvironment. In close association with the urease enzyme, the HspB (Hsp60) heat shock protein, the *H. pylori* homologue of GroEL can also be found on the bacterial surface (Dunn *et al*., 1992; Evans *et al*., 1992). This protein forms a macromolecular complex with structural similarities to the urease holoenzyme (Austin *et al.*, 1992), and it was postulated that it exerts a specialised role in stabilisation and/or export of the enzyme. The mechanisms that account for the unusual extracytoplasmic localisation of both urease and HspB are not well understood; an altruistic lysis model, in which cytoplasmic proteins released from dead cells become reabsorbed to the surface of intact cells, as well as a specific and selective export mechanism have been proposed by different authors (Phadnis *et al.*, 1996; Dunn *et al.*, 1997; Vanet and Labigne, 1998). Urease activity is strictly dependent on insertion of two Ni^{2+} ions into the active site of each of the six UreB subunits contained in the complex. In the cytoplasm of *H. pylori* cells, this insertion process seems to be accomplished by five accessory proteins (UreI, UreE, UreF, UreG, and UreH) that are encoded by genes localised within the *ure* gene cluster. A number of other proteins are however involved in transport of Ni^{2+} ions and subsequent control of their availability to the urease apoenzyme. Selective import of nickel ions from the medium into the cytoplasm seems to be mediated by an ABC transport system with specificity for nickel ions (Hendricks and Mobley, 1997) and by NixA, a high-affinity nickel transport protein located in the cytoplasmic membrane (Mobley *et al*., 1995; Bauerfeind *et al*., 1996). Intracellular binding and storage of nickel ions appears to be accomplished by Hpn, a 60 amino acids protein with 28 histidine residues (Gilbert *et al*., 1995), and HspA, the *H. pylori* GroES homologue, which contains an unusual histidine-rich motif at its C-terminus (Suerbaum *et al*., 1994; Kansau *et al*., 1996).

Once the *H. pylori* cells have reached the mucus layer on the gastric epithelium, they have to adhere tightly to the epithelial cells in order to establish a persistent infection. Several adhesion molecules have been described although none of them has been proven to be essential for colonisation in the animal model. These include HpaA, a N-acetyl-neuraminyllactose binding lipoprotein (Evans *et al*., 1993; O'Toole *et al*., 1995), BabA, a fucosylated Lewis b histo-blood group antigen binding adhesin (Borén *et al*., 1993; Ilver *et al*., 1998), AlpA and AlpB, two porin-like outer membrane proteins (Odenbreit *et al*., 1999), and KatA, the *H. pylori* catalase, which mediates attachment to a phosphatidylethanolamine receptor on epithelial cells (Lingwood *et al*., 1992; Odenbreit *et al*., 1996). In addition, specific adhesin molecules have been postulated for extracellular matrix components like laminin, fibronectin, collagen, and heparan sulfate, that might become exposed to the bacteria after injury of the gastric epithelium (Trust *et al*., 1991; Ascencio *et*

al., 1993). Furthermore, it was suggested that the putative urease-stabilising HspB heat shock protein on the bacterial surface might also contribute to adhesion of *H. pylori* by binding to sulfated glycolipids on epithelial cells (Huesca *et al*., 1996). A similar role might be exerted by Hsp70, the *H. pylori* homologue of DnaK, which can also be detected on the cell surface (Huesca *et al*., 1996; Cao *et al*., 1998).

After successful colonisation of the gastric mucosa, *H. pylori* must avoid the immune response of the host that would otherwise result in clearance of the bacterium from the stomach. One strategy adopted by *H. pylori* to deceive the immune system consists of the molecular mimicry of host proteins by antigens on the bacterial surface. The most striking example of such molecular mimicry is the lipopolysaccharide of *H. pylori*, which contains Lewis x (Le^x) and/or Lewis y (Le^y) epitopes (mono- and di-fucosylated glycoconjugates) that closely resemble the Lewis antigens on the epithelial cells of the gastric mucosa (Aspinall *et al*., 1996; Wirth *et al*., 1996; Appelmelk *et al*., 1997). This similarity together with the remarkably low toxicity of *H. pylori* LPS (1000-fold less toxic than the LPS of other Gram-negative bacteria; Muotiala *et al*., 1992) may be the reason why the immune system is not stimulated to the degree necessary for clearance. Other surface proteins that may facilitate the persistence of *H. pylori* infection by a similar camouflage mechanism include the HspB protein, which shows extensive homology to the human Hsp60 protein (Macchia *et al*., 1993), a mucin degrading metalloprotease with homology to human carbonic anhydrase (Smith *et al*., 1994), and a heavy metal transporting ATPase with similarity to the H^+,K^+-ATPase of the gastric mucosa (Ge *et al*., 1995; Melchers *et al*., 1996). The molecular mimicry between the bacterial and human antigens may also explain the occurrence of gastric autoantibodies in patients suffering from *H. pylori* associated pathologies. Especially anti-Hsp60 antibodies and anti-H^+,K^+-ATPase antibodies are often detected in the sera of *H. pylori* infected persons (Barton *et al*., 1998; Claeys *et al*., 1998).

Other mechanisms that could contribute to avoidance of the immune response may involve superoxide dismutase (Spiegelhalder *et al*., 1993) and/or catalase (Odenbreit *et al*., 1996), two *H. pylori* enzymes that might detoxify reactive oxygen radicals produced by polymorphonuclear leukocytes. Immune evasion may also be aided by the modification of cell morphology by *H. pylori*. Various reports have shown that prolonged culture of *H. pylori in vitro* leads to conversion from the normal bacillary form to a coccoid form with different ultrastructural, physiological and biochemical properties (Bode *et al*., 1993b; Benaissa *et al*., 1996; Costa *et al*., 1999). Coccoid forms could be observed also in biopsies from the human gastric mucosa (Chan *et al*., 1994), and it was proposed that they might represent a dormant and/or environmentally resistant phase of the bacteria, that can revert to the pathogenic bacillary phase in response to certain stimuli. However, such a reversion could neither be observed *in vitro* nor could it be induced *in vivo* in the gnotobiotic piglet model (Eaton *et al*., 1995); its significance for *H. pylori* virulence is therefore still unclear.

After years or decades of chronic infection many *H. pylori* carriers tend to develop severe gastrointestinal diseases including atrophic gastritis, peptic ulcer or MALT lymphoma (Fig. 1). The epithelial tissue damage associated with these pathologies is the consequence of a multifactorial process, that involves a variety of bacterial factors. One of the most important among these factors is the vacuolating cytotoxin VacA, which is secreted into the supernatant of some *H. pylori* isolates and which induces the formation of vacuoles in epithelial cells (Leunk *et al*., 1988). VacA is a high molecular weight ring-shaped oligomeric protein of about 900 kDa composed of six or seven subunits of a 87-95 kDa polypeptide (Cover and Blaser, 1992; Lupetti *et al*., 1996). The *vacA* gene (Cover *et al*., 1994; Schmitt and Haas, 1994; Telford *et al*., 1994) encodes a 140 kDa precursor protein, that undergoes cleavage of both an N-terminal 33 amino acids signal sequence and a 45 kDa C-terminal domain to yield the mature ≈90 kDa monomer, which is secreted into the supernatant. This monomer is then proteolytically processed to generate an N-terminal 37 kDa fragment and a C-terminal 58 kDa fragment (Telford *et al*., 1994). Both fragments remain associated after cleavage (Telford *et al*., 1994), suggesting that they may represent two distinct cytotoxin subunits. After binding to the epithelial cells, the cytotoxin becomes internalised (Garner *et al*., 1995; de Bernard *et al*., 1995) and induces acidic vacuoles in the cytoplasm by blocking the intracellular membrane trafficking during the late endosome pathway (Papini *et al*., 1994). Convincing experimental data are available suggesting that this vacuolisation contributes to the mucosal damage observed in *H. pylori* infected individuals. VacA producing strains are more frequently isolated from patients with peptic ulcer disease than from *H. pylori* infected individuals without peptic ulceration (Figura *et al*., 1989; Tee *et al*., 1995). Accordingly, in a mouse model of infection intragastric administration of sonicates from $VacA^+$ *H. pylori* strains induces epithelial damage, whereas administration of sonicates from either wild type $VacA^-$ strains or isogenic *vacA*$^-$ mutants fails to cause this damage (Ghiara *et al*., 1995). Furthermore, intragastric administration of purified VacA to mice yields gastric epithelial vacuolation and focal necrosis (Telford *et al*., 1994).

Although the *vacA* gene is present in virtually all *H. pylori* strains (Cover *et al*., 1994; Schmitt and Haas, 1994; Telford *et al*., 1994), only 50% of them express an active VacA cytotoxin (Leunk *et al*., 1988; Atherton *et al*., 1995). These strains also express the so called Cytotoxin associated antigen CagA, a surface exposed protein of about 120-130 kDa, that is strongly recognised by sera from infected individuals (Crabtree *et al*., 1992; Covacci *et al*., 1993; Tummuru *et al*., 1993). Epidemiological studies have revealed a high correlation between the occurance of antibodies against CagA and the presence and/or degree of the gastroduodenal lesions in infected patients. CagA and VacA producing bacteria are frequently associated with severe pathologies including peptic ulcer and adenocarcinoma while CagA and VacA negative bacteria are usually found in asymptomatic carriers (Covacci *et al*., 1993; Weel *et al*., 1996). Non-toxigenic strains that do not produce CagA usually lack the *cagA* gene (Xiang *et al*., 1995), but production of the active cytotoxin is not dependent on *cagA*, as isogenic mutants are not affected in synthesis or toxigenicity of the VacA protein (Tummuru *et al*., 1994). Detailed molecular analyses have shown that the *cagA* gene is part of a large chromosomal region of about 40 kb (the *cag* region) that has the typical

features of a pathogenicity island (Censini *et al*., 1996; Covacci *et al*., 1997; Akopyants *et al*., 1998). This island has a G+C content of 35%, which is significantly different from the 38-45% G+C values of the rest of the *H. pylori* genome, indicating an acquisition by horizontal gene transfer, possibly mediated by a phage or plasmid. Two 31 bp direct repeats are present at both ends of the *cag* island and duplicate sequences are absent within, suggesting that the island had originally inserted as a single unit into the *H. pylori* chromosome. Fig. 2 shows that *cag* codes for more than 30 putative proteins with features similar to components of type IV secretion systems involved in delivery of macromolecules targeted to host cells.

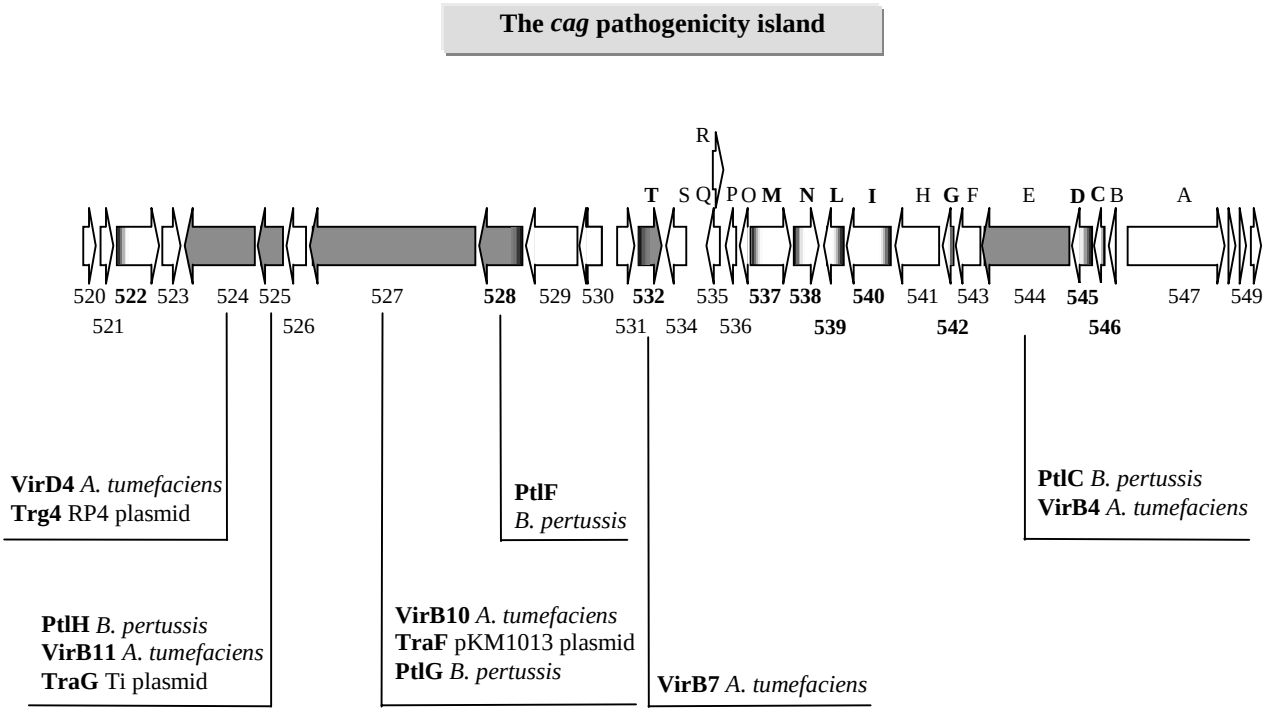

Fig. 2: The *cag* pathogenicity island. Arrows point to the direction of transcription of the indicated genes. Numbers refer to the annotated genome sequence published by Tomb *et al*. (1997), letters to the nomenclature suggested by Censini *et al.* (1996). Proteins with predicted leader peptides are indicated by bold letters and/or numbers and shaded arrows. Grey arrows indicate homologues of proteins of the type IV secretion machineries from *Bordetella pertussis* ("Ptl"), *Escherichia coli* ("Tra"), and *Agrobacterium tumefaciens* ("Vir").

In particular, homologues of the Vir proteins from *Agrobacterium tumefaciens* (Christie, 1997), which mediate the mobilisation and transfer of tumor inducing (Ti) DNA from the bacterial to the plant cell, and of the Ptl proteins from *Bordetella pertussis* (Weiss *et al*., 1993), which are responsible for translocation of the Pertussis toxin to the eukaryotic cell, could be identified (Censini *et al*., 1996). On the basis of these homologies it was hypothesised that the *cag* pathogenicity island may function to secrete molecules involved in *H. pylori*-host cell interactions. Several *cag* proteins have been shown to be essential for the induction of IL-8 secretion from gastric epithelial cells (Censini *et al*., 1996; Tummuru *et al*., 1995) and for activation of the

transcription factor NF-κB (Munzenmaier *et al*., 1997; Glocker *et al*., 1998), suggesting that *cag* may play a role in chronic inflammation. In addition, *cag* proteins seem to induce the tyrosine phosphorylation of a 145 kDa protein and the remodeling of the cytoskeleton in the epithelial cells which results in pedestal formation (Segal *et al*., 1997). Similar effects are mediated by the factors encoded by the LEE pathogenicity island from enteropathogenic *Escherichia coli* (EPEC) (Rosenshine *et al*., 1996), suggesting a similar mechanism of action.

Molecular genetic analyses of different *H. pylori* strains have revealed that the structure of the *cag* pathogenicity island varies from strain to strain. It can either be present in its entire length, or it can be split into two halves (*cag*I and *cag*II) by the insertion of an intervening sequence flanked on both sides by IS605, a transposable element (Censini *et al*., 1996; Akopyants *et al*., 1998). IS605 encodes two putative transposases, TnpA and TnpB, that might participate in rearrangement and/or horizontal transfer of the *cag* PAI. In fact, intermediate strains that carry multiple insertions of IS605 and inversions or partial deletions of *cag*I and/or *cag*II can be isolated, as well as strains that have completely lost the pathogenicity island (Censini *et al*., 1996). Strains containing *cag* and strains missing *cag* can be isolated from the same patient (van der Ende *et al*., 1996), suggesting that loss and acquisition of the pathogenicity island is part of the infectious process. Instability of *cag* might act as a regulatory mechanism to reduce the virulence of the bacteria in response to certain defence mechanisms of the host, and might therefore contribute to the long term persistance in the gastric mucosa (Hacker *et al*., 1997).

Similar regulatory mechanisms might be expected also for the expression of other *H. pylori* virulence factors, that are involved in earlier stages of the infectious process. By analogy with most other pathogenic bacteria it is reasonable to hypothesise that expression of these factors is tightly regulated in order to maximise the chances for a successful infection of the host. Environmental parameters that signal the entry of the bacterium into the host organism, such as elevated temperature, low iron concentrations or high osmolarity, might be received by specialised sensor proteins and transduced to regulator proteins that activate transcription of certain subsets of virulence genes. Surprisingly, the sequencing of the entire genome of a *H. pylori* strain (Tomb *et al*., 1997) has revealed the presence of a very low number of putative proteins, that could participate in such a regulatory network (Table 1). In particular, only four inferred proteins with putative helix-turn-helix DNA-binding motifs have been detected, compared to the 34 found in *Haemophilus influenzae* and the 148 found in *Escherichia coli*. Furthermore, only three putative proteins with convincing similarity to signal-transducing sensor proteins and only three sigma factors could be identified (Table 1). This paucity of regulatory functions has been interpreted as a consequence of the restricted ecological niche of the bacterium and the lack of competition with other microorganisms in the gastric mucosa. *H. pylori* may not possess a habitat other than the human stomach and the regulatory network that in other microbial pathogens signals the transfer from the environment into a host organism may therefore not be necessary in this bacterium. However, the human stomach represents a rather variable environment, including strong pH gradients between the

number	putative function
sigma factors	
HP88	**RNA polymerase sigma-70 factor (*rpoD*)**
HP714	**RNA polymerase sigma-54 factor (*rpoN*)**
HP1032	**alternative transcription initiation factor, sigma-F (*fliA*)**
signal-transducing histidine kinases	
HP164	signal-transducing protein, histidine kinase
HP244	signal-transducing protein, histidine kinase (*atoS*)
HP1364	signal-transducing protein, histidine kinase
response regulators	
HP166	response regulator (*ompR*)
HP703	**response regulator**
HP1021	response regulator
HP1043	response regulator
HP1365	response regulator
transcriptional regulators	
HP48	transcriptional regulator (*hypF*)
HP727	transcriptional regulator, putative
HP1025	**putative heat shock protein (*hspR*)**
HP1027	ferric uptake regulation protein (*fur*)
HP1287	transcriptional regulator (*tenA*)
HP1572	regulatory protein DniR

Table 1: Transcriptional regulation in *Helicobacter pylori.* Numbers and putative functions of the open reading frames have been assigned according to the annotated genome sequence of *H. pylori* strain 26695 published by Tomb *et al.* (1997). Regulatory proteins analysed in the present thesis are in bold letters.

gastric mucosa and the lumen as well as considerable differences in viscosity and nutrient availability between these two compartments. Expression of *H. pylori* virulence genes must therefore be regulated in response to these differences in order to optimise the possibilities for establishment of a successful infection. For example, virulence factors that are needed for survival at low pH and initial colonisation of the gastric mucosa must be turned on during the passage through the gastric lumen, while other factors needed for establishment of a chronic infection including tissue-damaging and immune-evasive factors have to be upregulated upon contact with the gastric epithelium. In order to gain insight into the molecular mechanisms that mediate these putative regulatory events, several virulence factors have been analysed in detail in the present thesis regarding their transcriptional regulation. In an attempt to obtain a general overview of the basic mechanisms of virulence gene transcription in *H. pylori* three different virulence determinants, that are believed to be representative for different stages of the infectious process and different pathogenic mechanisms, have been selected for this analysis. As an essential factor for the early phase of infection, including the initial passage through the gastric lumen and the colonisation

of the gastric mucus layer the flagella have been chosen; the chaperones including the putative adherence factors GroEL and DnaK, as well as the Nickel binding protein GroES, have been selected as representatives of the phase following the initial infection, which is characterised by adhesion to the epithelial tissue and persistence in the mucus layer; and finally the cytotoxin associated antigen CagA has been selected as a representative of the proteins encoded by the *cag* pathogenicity island, which may be responsible for the inflammation and tissue damage that can be observed in the chronic phase of infection years or decades after the initial colonisation.

Available evidence suggests that pathogenicity islands usually encode their own regulators, which turn on expression of their genes in response to changes in the environmental parameters that reflect a certain ecological habitat in the host or a certain stage in the infectious process. For example, the genes encoded by the pathogenicity island 1 (SPI1) of *Salmonella enterica* sv. *typhimurium,* necessary for invasion of epithelial cells, are induced by the cognate transcription factor HilA when the external pH, oxygen, and osmolarity coditions resemble those encountered in the small intestine (Bajaj *et al*., 1996). Surprisingly, sequence analysis of the *H. pylori cag* pathogenicity island revealed no such putative transcriptional regulator, and no convincing evidence is available that points towards environmentally regulated expression of *cagA* or other genes of the pathogenicity island. In a very systematic approach the region between the *cagA* gene and the upstream divergently transcribed *cagB* gene was therefore analysed in detail to identify the DNA elements involved in transcriptional regulation (Chapters E.1/F.1). Primer extension experiments and deletion analyses as well as transcriptional fusions to a reporter gene were used to identify the transcriptional start sites and the sequences responsible for full activation of the respective promoters. These analyses revealed that three overlapping promoters regulate expression of the divergent *cagA* and *cagB* genes. Full activation of the *cagA* promoter requires sequences up to -70 and binding of the C-terminal portion of the α subunit of RNA polymerase to an UP-like element located between -40 and -60, while full activation of the major *cagB* promoter requires sequences upstream of -96 which overlap with the *cagA* promoter. The data presented suggest that the promoters of the pathogenicity island represent a class of minimum promoters, that ensure a basic level of transcription, while full activation requires regulatory elements or a defined promoter context.

Regarding the flagella, available experimental data suggest that expression of the two flagellin genes is regulated by different sigma factors (σ^{54} in the case of *flaB* and σ^{28} in the case of *flaA*), pointing towards a differential regulation of both genes by environmental conditions. In support of such a hypothesis is the finding that *flaB* expression in the closely related organism *Campylobacter coli* can be modulated by certain growth conditions (Alm *et al*., 1993). Another surprising peculiarity of the *H. pylori* system is inferred by the fact that mutations in the flagellar hook protein FlgE do not prevent the synthesis of either FlaA nor FlaB (O'Toole *et al*., 1994). This finding is in sharp contrast with the situation in enteric bacteria, where the flagellin can only be synthesised when the assembly of the basal body-hook complex is completed. Interestingly, like *flaB*, the *flgE* gene is preceded by a consensus sequence similar to a σ^{54}-recognised promoter,

suggesting that both genes are regulated coordinately by the same factors and might constitute members of a larger regulon of flagellar structural and biosynthetic genes. In chapters E.2/F.2 of the present thesis a series of basal body and hook genes is identified as part of such a regulon and evidence is provided, showing that these genes are all regulated by the same master transcriptional factor, the NtrC-homologue FlgR. In addition, this regulator is shown to act as a repressor of transcription of the σ^{28}-regulated *flaA* promoter, while changes in DNA topology are shown to repress transcription of the σ^{54}-regulated *flaB* promoter. These data indicate that regulation of flagellar gene expression in *H. pylori* shows similarities to that in *Salmonella* spp. and *Caulobacter crescentus.* A model is presented that tentatively explains how *H. pylori* has united features of both systems to evolve a regulatory network for the control of flagellum synthesis.

Apart from a few reports that indicate pH and temperature dependence of DnaK and GroESL induction (Huesca *et al*., 1996, 1998; Yokota *et al*., 1994), little is known about the molecular mechanisms that regulate expression of chaperones in *H. pylori*. In the best studied system *Escherichia coli*, transcription of the *groESL* and *dnaK* operons is under the control of the alternative sigma factor σ^{32} (RpoH). Translation of *rpoH* mRNA and stability of σ^{32} increase as a consequence of environmental stress and the resulting elevated level of σ^{32} leads to induction of the chaperone genes (Gross, 1996). In *Bacillus subtilis* chaperone gene expression is σ^{A}-dependent and negatively regulated by the repressor protein HrcA which binds to an inverted repeat in the promoter region (Schulz and Schumann, 1996). This inverted repeat, designated CIRCE (for Controlling Inverted Repeat of Chaperone Expression; Zuber and Schumann, 1994), has been reported to be present in a variety of Gram-positive and a few Gram-negative bacteria including *Chlamydia trachomatis* (Tan *et al*., 1996) and *Agrobacterium tumefaciens* (Segal and Ron, 1993), suggesting that this regulatory mechanism is widespread among eubacteria. In *Streptomyces* spp. the two *groESL* operons are controlled by a tandemly arranged CIRCE element while the *dnaK* operon encodes its own autoregulatory repressor (Bucca *et al*., 1995; Grandvalet *et al*., 1997). This repressor, HspR, shows sequence similarities to the GlnR family of transcription factors and binds to three partially related inverted repeats in the promoter region (Bucca *et al.*, 1995). The consensus motif of the three binding sites has been designated HAIR for HspR Associated Inverted Repeat. Recently, it has been demonstrated that the *S. albus* G *clpB* gene is also regulated by a HAIR element, and an analysis of available sequence data has revealed the presence of this motif in a variety of bacterial species including *Mycobacterium* spec. and *H. pylori* (Grandvalet *et al*., 1999). Furthermore the publication of the complete genome sequence of *H. pylori* (Tomb *et al.*, 1997) has revealed the absence of a σ^{32} homologue and the presence of a homologue of the *Streptomyces* HspR protein (Table 1). In chapters E.3/F.3 of the present thesis it is reported that expression of at least three *H. pylori* chaperone encoding operons, including the *groESL* and *dnaK* operons, is under the control of the vegetative sigma factor σ^{80} and is negatively regulated by the *H. pylori* HspR homologue. *In vitro* studies with purified recombinant HspR protein established that the protein represses transcription by binding to large DNA regions centered around the transcription initiation site on one promoter, and around -85 and -120, respectively, on the other two promoters. In contrast

to the situation in *Streptomyces* spp., where transcription of HspR-regulated genes is induced in response to heat shock, transcription of the HspR-dependent genes in *H. pylori* is not inducible with thermal stimuli. Instead, two of the three operons regulated by the *H. pylori* HspR repressor are inducible by osmotic shock.

Taken together, the transcriptional analyses carried out suggest that *H. pylori* has reduced the total number of specific regulatory proteins to a basic level that may ensure coordinate regulation of those factors that are necessary during the initial phase of infection including motility and adhesion functions. The importance of DNA topology and/or context for transcription of many virulence gene promoters may on the other hand indicate the presence of a global regulatory network, that influences transcription of certain subsets of virulence genes in response to specific changes in the microenvironment.