G. CONCLUSIONS

The detailed investigation of the transcriptional regulation of the three *H. pylori* virulence factors flagella, chaperones and pathogenicity island has shed new light on the functions of the putative regulatory proteins of the *H. pylori* genome annotated by Tomb *et al*. (1997). Although the actual number of these regulatory proteins appears to be very low (see Table 1), the analyses carried out in this thesis have revealed that many of them play central roles in the coordinate regulation of virulence gene expression. Table 6 summarises the functions that could be assigned for some of the *H. pylori* regulators on the basis of the experimental data obtained.

Interestingly, most of the regulatory proteins investigated appear to participate directly or indirectly in the regulation of motility. Coordinate expression of flagellar strucural genes involves all three sigma factors (σ^{28}, σ^{54} and σ^{80}, Fig. 27) of *H. pylori* as well as a specialised two component signal transduction system (HP244/FlgR; see E.2/F.2) and possibly other regulators. This relative abundance of regulatory functions suggests a crucial role of the flagellar apparatus in the initial colonisation of the gastric epithelium. Most likely, the differential expression of the flagellar structural components in response to certain environmental parameters produces flagella which are optimally suited for motility in a given microenvironment. The bacteria might in this way be able to modify the structure of their flagella according to specific environmental cues in order to optimise their chances for the achievement of their ecological niche on the surface of the gastroepithelial cells. Intriguingly, motility of *H. pylori* depends also on the HspR repressor, which controls the expression of the major chaperones including the putative adhesion factors DnaK and GroEL (see E.3, F.3). This surprising finding may indicate that HspR represents a regulatory link connecting chemotaxis and adhesion functions in this bacterium. Transcriptional regulators that mediate a switch between the phase of swimming and the phase of adhesion to epithelial cells have been described for other pathogenic bacteria. In the well studied system *Vibrio cholerae* the membrane-anchored ToxR regulator activates transcription of the genes coding for cholera toxin, the TCP pilus, an outer membrane adhesin (OmpU), and a protein of unknown function called TcpI. This protein shows strong similarity to the Tsr methyl accepting chemotaxis protein of *E. coli* (reviewed in DiRita, 1995) and its deletion results in enhanced motility, whereas its overexpression by means of ToxR mediated transcriptional activation leads to repression of motility functions. Harkey *et al*. (1994) proposed a model in which ToxR, under appropriate *in vivo* conditions, mediates a switch from motility to colonisation by activating the expression of adhesion and colonisation factors and downregulating at the same time motility by activation of TcpI, which probably titrates the methylation signal away from the chemotaxis system. It is tempting to speculate that in *H. pylori* the HspR protein might exert a similar role, possibly in cooperation with

the HrcA protein (see Fig. 28), which is likely to participate in regulation of HspR dependent genes and which seems to share topological features with ToxR (see F.3). Support for this hypothesis comes from preliminary *in vitro* binding studies that indicate a role for HspR in the transcriptional regulation of a putative operon coding for a methylaccepting chemotaxis protein and a glycosyltransferase (I. Delany, pers. communication). The HspR-mediated differential expression of these genes in response to specific environmental signals in the course of the infectious process might mediate a switch between motility and adhesion functions.

number	putative function	experimentally assigned function
sigma factors		
HP88	**RNA polymerase sigma-70 factor (*rpoD*)**	**RNA polymerase sigma-80 factor**
HP714	**RNA polymerase sigma-54 factor (*rpoN*)**	**transcription of flagellar basal body and hook genes**
HP1032	**alternative transcription initiation factor, sigma-F (*fliA*)**	**transcription of *flaA***
signal-transducing histidine kinases		
HP164	signal-transducing protein, histidine kinase	
HP244	signal-transducing protein, histidine kinase (*atoS*)	(cognate sensor kinase for FlgR)
HP1364	signal-transducing protein, histidine kinase	
response regulators		
HP166	response regulator (*ompR*)	
HP703	**response regulator**	**transcriptional activator of flagellar basal body and hook genes (FlgR)**
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*)**	**transcriptional repressor of chaperone genes (HspR), regulation of motility**
HP1027	ferric uptake regulation protein (*fur*)	
HP1287	transcriptional regulator (*tenA*)	
HP1572	regulatory protein DniR	
HP111*	transcriptional repressor (*hrcA*)	
HP835*	nucleoprotein (HU/IHF)	

Table 6: Putative and experimenally proven regulatory functions of *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). Transcriptional regulators whose functions have been assigned experimentally in the present thesis are in bold letters. The asterisk indicates additional putative proteins that could participate in transcriptional regulation of virulence gene expression.

Another interesting feature arising from the transcriptional analyses of the different virulence factors is the relative importance of DNA topology and DNA context in the regulation of some virulence-associated genes. Drug-induced changes in DNA topology were shown to alter transcription from the FlgR-regulated *flaB* promoter (Fig. 18), and osmolarity changes, that are known to influence the overall supercoil-state of the chromosomal DNA (Higgins *et al.*, 1988; Hulton *et al.*, 1990), derepress expression of the HspR-regulated *cbpA* and *groESL* operons (Fig. 23). Furthermore, DNA context and the alteration of local DNA structures by structural DNA-binding proteins like the HU-IHF homolog (HP835, Table 6), are likely to be involved in transcriptional regulation of the *cagAB* genes of the pathogenicity island (see F.1.). These observations might suggest, that a global network is present in *H. pylori* that controls the expression of certain genes by specifically changing the local DNA topology and chromatin structure in response to changes in the environment. In the case of the flagellar and chaperone genes this network might cooperate with specific DNA-binding proteins such as FlgR and HspR, whereas in the case of the genes encoded by the *cag* pathogenicity island it might act directly on the local DNA context. Such a putative direct interaction might be a consequence of the relatively recent acquisition of the *cag* pathogenicity island by horizontal gene transfer and the lack of a cognate regulator on the island, that could productively interact with the transcriptional machinery of *H. pylori* to coordinate expression of the *cag* genes. In the absence of a specific regulator the global regulatory network of *H. pylori* might be used to regulate transcription of the *cag* genes in response to certain environmental stimuli.