Mutational and transcriptional analysis of the *Campylobacter jejuni* flagellar biosynthesis gene *flhB*

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A *Campylobacter jejuni* gene encoding a homologue of the flagellar biosynthesis gene *flhB* was identified downstream of the peroxide stress defence gene *ahpC*. Insertional mutagenesis of the *flhB* gene rendered *C. jejuni* non-motile, with most cells aflagellate, although a small number expressed truncated flagella. The absence of FlhB also appeared to affect cell shape, as the majority of cells were straight rather than curved rods. Transcription of the flagellin gene *flaA* was significantly reduced in the *C. jejuni flhB* mutants, which also did not express significant amounts of flagellin proteins, indicating that FlhB is an essential protein for subsequent expression of flagellar genes. The transcription start site of the *flhB* gene, as determined by primer extension, was located 91 bp upstream of the *flhB* start codon, but no recognizable promoter sequence could be identified immediately upstream of this transcription start site. Transcriptional *flhB::lacZ* reporter gene fusions confirmed that the *flhB* gene has its own promoter region, is expressed at very low levels and is transcribed independently of *ahpC*, and that its transcription is not regulated by iron or growth phase.

Keywords: *Campylobacter*, motility, transcription

INTRODUCTION

The human pathogen *Campylobacter jejuni* is recognized as one of the major causes of bacterial gastroenteritis, and is considered to be an important public health and economic burden (van Vliet & Ketley, 2001). The high level of motility conferred by flagella, together with the spiral, curved shape, allow *C. jejuni* to penetrate the mucus layer overlying the intestinal epithelium (Szymanski *et al.*, 1995). Flagella and motility have also been implicated in adhesion and invasion of *C. jejuni* (Wassenaar *et al.*, 1991), and are required for intestinal colonization in chicken colonization models (Nachamkin *et al.*, 1993; Wassenaar *et al.*, 1993).

The determination of the complete genome sequence of *C. jejuni* has allowed the identification of putative flagellar genes in *C. jejuni* (Parkhill *et al.*, 2000). Comparison with flagellar genes of the *Enterobac-*

teriaceae indicated that regulation and biosynthesis of flagella in *C. jejuni* is likely to be different from that of the *Enterobacteriaceae* (Chilcott & Hughes, 2000; Macnab, 1996; Parkhill *et al.*, 2000), more closely resembling the organization predicted for the related pathogen *Helicobacter pylori* (O'Toole *et al.*, 2000; Tomb *et al.*, 1997). *C. jejuni* contains the flagellar sigma factors σ^{28} and σ^{54} (Jagannathan *et al.*, 2001; Wösten *et al.*, 1998a) and a homologue of the *H. pylori flgR* gene, which encodes a σ^{54} -associated activator of transcription of the flagellar hook, basal body and FlaB flagellar filament genes (Jagannathan *et al.*, 2001; Spohn & Scarlato, 1999).

So far, relatively few *C. jejuni* genes involved in flagellar biosynthesis have been investigated. In recent studies using random transposon mutagenesis or site-directed mutagenesis, it was demonstrated that mutations in at least 19 genes led to altered or absent motility of *C. jejuni* (Colegio *et al.*, 2001; Hendrixson *et al.*, 2001; Jagannathan *et al.*, 2001). One of these *C. jejuni* genes is *flhB* (Hendrixson *et al.*, 2001), which encodes a protein that in *Salmonella enterica* serovar Typhimurium is

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Fig. 1. Schematic diagram representing the genomic region containing the *flhB* gene. The position and orientation of the *cat* cassette inserted into the *BclI* site of *flhB* and the resulting mutants are shown. Promoters of *flhB* and *ahpC* are indicated by arrows, and a putative terminator structure is indicated by a stem-loop symbol.

involved in the flagellar protein export pathway, and in the hook-length-dependent switch in export specificity from basal-body-hook proteins to filament and associated proteins (Kutsukake *et al.*, 1994; Kutsukake, 1997; Minamino *et al.*, 1994; Minamino & Macnab, 1999, 2000; Williams *et al.*, 1996). As in *C. jejuni*, mutation of *flhB* in *H. pylori* resulted in a non-motile phenotype (Foyne *et al.*, 1999). Synthesis of the flagellar filament proteins FlaA and FlaB, and the hook protein FlgE, was almost completely abolished in the *H. pylori flhB* mutant, and transcription of the *flaA*, *flaB* and *flgE* genes was significantly reduced (Allan *et al.*, 2000).

Examination of the nucleotide sequence downstream of the peroxide stress defence gene *ahpC* (Baillon *et al.*, 1999) revealed the presence of the motility-associated genes *flhB* and *motB* (Fig. 1). In this study, we have characterized the transcriptional organization and regulation of *flhB*, and show that mutation of *flhB* affects *C. jejuni* motility and flagellin expression.

METHODS

Bacterial strains and growth conditions. The *C. jejuni* strains used in this study are 81116 (NCTC 11828), 480 (NCTC 12744) (National Collection of Type Cultures) and the *C. jejuni* 81116 flagellar mutants R2 (FlaA⁻ FlaB⁻), R3 (FlaA⁺ FlaB⁻) and R4 (FlaA⁻ FlaB⁺) (Wassenaar *et al.*, 1991). *C. jejuni* strains were cultured in Mueller-Hinton (MH) media (Oxoid) at 37 °C under microaerobic conditions (5% O₂, 5% CO₂, 90% N₂). Antibiotics were used at the following final concentrations: 10 µg vancomycin ml⁻¹, 50 µg kanamycin ml⁻¹, and 20 µg chloramphenicol ml⁻¹, when appropriate. Iron-restricted growth conditions were achieved by supplementing MH medium with Desferal (Sigma) to a final concentration of 20 µM (van Vliet *et al.*, 1998b). For motility tests, *C. jejuni* grown overnight in MH broth at 37 °C under microaerobic conditions was diluted in MH broth to approximately 100 cells ml⁻¹, and, subsequently, 100 µl of this cell suspension was added to 5 ml soft agar [MH broth containing 0.3% (w/v) agar, cooled to 45 °C], poured onto soft agar plates [MH broth containing 0.4% (w/v) agar], and incubated for 2 days microaerobically at 37 °C.

Recombinant DNA techniques. Plasmid preparations, restriction endonuclease digestions, ligations and transformations of

Escherichia coli were performed according to standard procedures (Sambrook *et al.*, 1989). All restriction and modifying enzymes were purchased from Gibco-BRL and used according to the manufacturer's instructions. Genomic DNA was isolated by the IsoQuick nucleic acid extraction kit (ORCA Research). A PCR was performed using Vent polymerase (New England Biolabs) or Taq polymerase (Life Technology) according to the manufacturer's instructions. Allelic exchange mutagenesis of *flhB* was performed by insertion of the chloramphenicol-resistance (*cat*) cassette of plasmid pAV35 (van Vliet *et al.*, 1998b) in both transcriptional orientations into the unique *BclI* restriction site of the *flhB* gene (Fig. 1). The resulting plasmids, pAV107 and pAV108, were introduced into *C. jejuni* 81116 using electroporation (van Vliet *et al.*, 1998a), and yielded *C. jejuni* strains AV33 and AV34 (*flhB*::Cm^R) (Fig. 1). Correct replacement of the wild-type *flhB* gene by the *flhB*::*cat* gene was verified by Southern blotting (not shown).

RNA isolation and analysis. Total RNA was isolated from *C. jejuni* overnight cultures as described previously (Oelmüller *et al.*, 1990). Primer extension was performed using primers PE1 and PE2 (5'-CTATCTTTTGGACGTGGGTTTC-3' and 5'-CTGCCGCATCTTGAGACTTTGG-3') essentially as described previously (Baillon *et al.*, 1999). For Northern hybridization, RNA was transferred, using standard protocols (Sambrook *et al.*, 1989), to Amersham Hybond-N⁺ nylon membranes and hybridized with PCR-amplified *flaA*- and *flhB*-specific probes. The *flaA* and *flhB* probes consisted of parts of the *flaA* gene (positions 1269232–1270532 on the *C. jejuni* genome; Parkhill *et al.*, 2000) and the *flhB* gene (positions 303149–304169 on the *C. jejuni* genome; Parkhill *et al.*, 2000), respectively. All probes were labelled with [α -³²P]dATP by using the Radprime DNA labelling kit and random hexanucleotide primers (Life Technology).

Reporter gene assays. The *C. jejuni* promoter probe vector pMW10 contains a promoterless *lacZ* gene, and has previously been used to quantify promoter activity in *C. jejuni* (Baillon *et al.*, 1999; van Vliet *et al.*, 2000; Wösten *et al.*, 1998b). Overlapping fragments containing the *flhB* start codon and various parts of the upstream region were amplified by PCR, checked for the absence of PCR incorporation errors by DNA sequencing, and subsequently cloned into the *Bam*HI site of pMW10 (Table 1). As positive controls, the *C. jejuni* 81116 *ahpC* (Baillon *et al.*, 1999) and *flaA* promoters were used (Table 1). The constructs were transformed into *C. jejuni* strain 480, and β -galactosidase activity was measured as described previously (Baillon *et al.*, 1999; van Vliet *et al.*, 2000; Wösten *et al.*, 1998b), after approximately 7.5 h growth, at an OD₆₀₀ of approximately 0.4. The β -galactosidase activities were expressed in Miller units (Sambrook *et al.*, 1989) and were derived from three independent experiments.

Protein analysis. *C. jejuni* wild-type and *flhB* mutants were grown on MH agar for 24 h, fractionated into periplasmic, cytoplasmic and membrane-bound proteins (van Vliet *et al.*, 1998b) and subjected to SDS-PAGE (Sambrook *et al.*, 1989). Gels were either stained with PAGE blue 83 (Fluka) or immunoblotted and subsequently incubated with mouse monoclonal antibodies P3B2 and O2D4, which recognize both *Campylobacter* flagellin A and flagellin B (Constantinidou *et al.*, 1996; Jagannathan *et al.*, 2001).

Electron microscopy. *C. jejuni* wild-type and *flhB* mutants were grown on MH agar for 24 h, negatively stained in 1.25% (w/v) sodium phosphotungstate (pH 7.0), and examined with a Jeol JEM1200EX transmission electron microscope.

Table 1. Promoter activity of *flhB* promoter–*lacZ* fusions

Clone	<i>lacZ</i> fusion to:	Position*	β -Galactosidase activity† after growth in:	
			Iron-replete medium‡	Iron-restricted medium‡
pCM1	Promoter <i>flhB</i> with complete <i>ahpC</i> gene and promoter	302 160–303 147	6.1 ± 0.5	8.6 ± 0.2
pCM2	Promoter <i>flhB</i> , with large part of <i>ahpC</i> gene	302 421–303 147	11.9 ± 1.1	11.5 ± 0.6
pCM3	Promoter <i>flhB</i> (–75 to +140)§	302 933–303 147	12.0 ± 1.2	10.6 ± 1.1
pCM4	Promoter <i>flhB</i> (+5 to +140)§	303 012–303 147	1.7 ± 0.5	2.0 ± 0.6
pMW10	None	–	1.8 ± 0.5	1.9 ± 0.2
pAV197	Promoter region <i>ahpC</i>	302 157–302 402	367.6 ± 34.0	973.4 ± 49.7
pAV150	Promoter <i>flaA</i>	124–508	269.2 ± 19.1	249.7 ± 22.3

* Position of the DNA fragment in the *C. jejuni* genome sequence (Parkhill *et al.*, 2000).

† β -Galactosidase activity (in Miller Units, $\pm$ SD) after 7.5 h growth. Results shown are derived from three independent experiments.

‡ Iron-replete medium consisted of MH broth; iron-restriction was achieved by supplementation of MH broth with 20 μ M Desferal.

§ Relative to experimentally determined transcription start site.

|| Position in the *C. jejuni* 81116 promoter sequence (GenBank accession no. AF202168) (Nuijten *et al.*, 2000).

RESULTS

Identification and mutation of a *C. jejuni flhB* homologue

Examination of the sequence downstream of the *C. jejuni* 81116 *ahpC* oxidative stress defence gene had previously revealed the presence of a gene encoding an FlhB homologue (Baillon *et al.*, 1999). The predicted *C. jejuni* FlhB protein is 37% identical, and 56% similar, to the FlhB protein of *H. pylori* and shows strong conservation of hydrophobic and hydrophilic domains when compared with the FlhB protein of *S. enterica* serovar Typhimurium (not shown).

The *flhB* gene of *C. jejuni* strain 81116 was mutated by insertion of a *cat* cassette in both transcriptional orientations into the unique *Bcl*I restriction site of *flhB* (Fig. 1), followed by allelic replacement of the wild-type *flhB* gene with the interrupted gene. This resulted in the isolation of *C. jejuni* strains AV33 and AV34 (81116 *flhB*::*Cm*^R) (Fig. 1). Correct replacement of the wild-type *flhB* gene with its interrupted counterpart was confirmed by Southern blot analysis (data not shown). Mutants AV33 and AV34, which had the *cat* cassette in opposite orientations, gave identical flagellar phenotypes in all tests (not shown). This indicates there was no polar effect on flagellar gene expression from the insertion of the *cat* cassette.

Mutation of *flhB* affects *C. jejuni* motility and cell shape

The relative motility of *flhB* mutants was compared with that of *C. jejuni* 81116 wild-type and the aflagellate, non-motile mutant R2 (FlaA[–] FlaB[–]) (Wassenaar *et al.*, 1991). *C. jejuni* 81116 had spread to the edge of soft-agar plates after 3 days incubation, whilst the R2 mutant had produced small, distinct colonies. Like the R2 mutant,

the *flhB* mutants were non-motile, although the colonies were slightly larger than those of R2 (data not shown). Electron microscopy of the *C. jejuni flhB* mutants (Fig. 2) showed that the *flhB* mutants lacked flagella and often lost the spirally curved morphology typical of wild-type *C. jejuni* 81116. Occasionally, cells with truncated flagella and a spirally curved morphology were observed among *flhB* mutants (Fig. 2b), but we were unable to identify these as colonies showing motility on motility plates and hence could not characterize them further (data not shown).

Mutation of *flhB* affects flagellin expression and transcription

Expression of the flagellar proteins FlaA and FlaB was absent in the *flhB* mutants, as assessed using monoclonal antibodies (Fig. 3a). This indicates that the non-motile phenotype of the *flhB* mutants is caused, at least in part, by blocked expression of flagellin protein. Repression of flagellin expression appeared to be mediated at the transcriptional level, since flagellin mRNA levels were significantly reduced in the *flhB* mutants (Fig. 3b), though not absent as in the flagellar mutants R2 and R4 (Fig. 3b). Since the *flaA* probe used shows a certain degree of cross-reactivity with the highly similar *flaB* gene (Nuijten *et al.*, 1990), it is likely that the mRNA hybridizing to the *flaA* probe represents low levels of *flaB* expression. In contrast, transcription of the flagellar motor gene *motB* was not significantly affected by any of the mutations (Fig. 3b).

Identification of the *flhB* promoter

The transcription start point for *flhB* was determined by primer extension. This gave a weak but reproducible primer extension signal located 91 bp upstream of the

Fig. 2. Electron microscopy of *C. jejuni* 81116 and *flhB* mutant AV34. Cells were grown for 24 h and then negatively stained with potassium phosphotungstate (pH 7.0). (a) *C. jejuni* wild-type 81116. Most cells have a spirally curved morphology and full-length flagella. (b) *C. jejuni flhB* mutant AV34. Most cells are elongated, straight, and lack flagella. One cell exhibits a spirally curved morphology and truncated flagella (indicated by arrowheads). Bars, 0.5 µm.

flhB ATG codon (Fig. 4). Typical σ^{70} or σ^{28} promoter sequences were not obvious in the sequence directly upstream of the *flhB* transcription start point, but 39 bp upstream of the transcription start point a typical σ^{54} promoter sequence, GG-N₁₀-GC, was identified, as reported for the *Campylobacter coli flhB* and *flgE* genes (Guerry *et al.*, 1992; Kinsella *et al.*, 1997). This promoter-like sequence, however, is too far upstream from the transcription start for it to be involved in this activity (Buck *et al.*, 2000). The weak primer extension signal indicated that *flhB* is transcribed at a very low

Fig. 3. Analysis of (a) Fla protein levels and (b) *flaA* and *motB* transcript levels in *C. jejuni* wild-type, *flhB* mutants AV33 and AV34, and flagellar mutants R2, R3 and R4. (a) Western immunoblot of *C. jejuni* whole-cell protein probed with the flagellum-specific monoclonal antibody P2D3. Marker sizes are given in kDa on the right, while on the left the FlaA protein is indicated. The monoclonal antibody or conjugate used displays a low level of cross-reactivity with the major outer-membrane protein (MOMP), as indicated on the left. (b) Northern hybridization of RNA isolated from *C. jejuni* cells in exponential growth phase, probed with *flaA*-specific (top panel) and *motB*-specific (lower panel) probes. Lanes: 1, *C. jejuni* 81116; 2, *C. jejuni* AV33; 3, *C. jejuni* AV34; 4, *C. jejuni* R2; 5, *C. jejuni* R3; 6, *C. jejuni* R4.

level, or that the transcript is unstable, and this was confirmed by the failure to detect an *flhB* transcript on Northern hybridization (not shown).

The pMW10-based *lacZ* reporter gene system (Wösten *et al.*, 1998b) was used to identify the *flhB* promoter and

Fig. 4. Characterization of the *flhB* promoter region. (a) Primer extension analysis of *C. jejuni* mRNA with the *flhB*-specific oligonucleotide PE1. The lane marked PE contains the primer extension product, whereas the lanes marked A, C, G and T contain a DNA sequencing reaction initiated with primer PE1 used for the primer extension reaction. (b) Sequence and annotation of the *ahpC*-*flhB* intergenic region. The transcription start site is indicated as +1 in bold typeface and is underlined. The stop codon of *ahpC* and the start codon of *flhB* are shown in bold typeface; DNA sequences resembling the *C. jejuni* σ^{54} consensus sequence are underlined, highlighted, and labelled as -12 and -24. (c) Strategy for determining the promoter of *flhB*. Overlapping fragments of the *C. jejuni* *ahpC*-*flhB* region were transcriptionally fused to the promoterless *lacZ* gene of pMW10 (Table 1).

to quantify transcription of *flhB*, including any co-transcription with the upstream, iron-repressed *ahpC* gene. Overlapping fragments were generated, containing either the direct upstream region of *flhB*, or, in addition to this, the upstream *ahpC* gene with and without its iron-regulated promoter (Fig. 4c, Table 1). The *flaA* promoter and the *ahpC* promoter were used as positive controls (Table 1). Expression of the *flhB*::*lacZ* fusions was only slightly, though reproducibly, higher than

background levels, and was up to 100-fold lower than activity from the *ahpC*::*lacZ* fusion and 25-fold lower than the *flaA*::*lacZ* fusion (Table 1). The inclusion of the *ahpC* gene alone (pCM2) or the *ahpC* gene and promoter (pCM1) upstream of the putative *flhB* promoter region did not significantly affect expression of the *flhB*::*lacZ* fusion when compared with pCM3, indicating that transcriptional readthrough from the *ahpC* promoter does not seem to play a role in transcription of *flhB*. Conversely, removal of the sequences upstream of the transcription start point (pCM4) lowered β -galactosidase activity to background levels. Growth in iron-restricted media only affected β -galactosidase activity of the *ahpC*::*lacZ* fusion (Baillon *et al.*, 1999), but not that of any of the *flhB*::*lacZ* or the *flaA*::*lacZ* fusions (Table 1), again confirming that there was no influence of the iron-regulated *ahpC* promoter on *flhB* transcription.

DISCUSSION

Flagellar biogenesis has been extensively studied in *S. enterica* serovar Typhimurium and *E. coli* (Chilcott & Hughes, 2000; Macnab, 1996), and it has been shown that the FlhB protein plays a central part therein. FlhB is involved in hook-length control and switch of export substrate as part of the flagellar export apparatus (Kutsukake *et al.*, 1994; Kutsukake, 1997; Minamino *et al.*, 1994; Minamino & Macnab, 1999, 2000; Williams *et al.*, 1996). However, experimental evidence as well as the availability of complete genome sequences from several bacteria have indicated that models developed for *S. enterica* serovar Typhimurium do not always correspond to those of other bacteria (O'Toole *et al.*, 2000; Parkhill *et al.*, 2000; Tomb *et al.*, 1997). Here, we have initiated characterization of flagellar biogenesis in *C. jejuni* by studying the role and transcription of its *flhB* gene.

The FlhB protein seems to play a central role in flagellar biogenesis of *C. jejuni*, since the *flhB* mutants used in this study did not assemble flagella. Similar observations were made with *H. pylori* *flhB* mutants, which were also non-motile and lacked flagella (Foyne *et al.*, 1999). The *flhB* mutants of *C. jejuni* strain 81116 also generally exhibited a change in cell shape, from spiral cells with a relatively short aspect ratio to a straighter and apparently longer form. No explanation is apparent for this, but in polar flagellate bacteria where a new pole requires a new flagellum, there clearly must be co-ordination between flagellar insertion and cell division. Furthermore, it has been shown in the *Enterobacteriaceae* that cell-division events are affected by mutations in the *flhD* flagellar master-regulator, and it is possible that a cell-division gene(s), with implications for determination of aspects of cell shape, might be affected in aflagellate mutants (Chilcott & Hughes, 2000; Prüß & Matsumura, 1997).

Flagellin expression was significantly decreased in the *C. jejuni* *flhB* mutant, and this was mediated at the transcriptional level. This repression of flagellin tran-

scription and expression has also been described in mutants lacking other predicted components of the flagellar export apparatus in either *H. pylori*, *C. coli* or *C. jejuni* (Allan *et al.*, 2000; Miller *et al.*, 1993; O'Toole *et al.*, 2000; Park *et al.*, 2000). In contrast, mutants in the flagellar hook-encoding gene *flgE* in *Helicobacter mustelae*, *H. pylori* and *C. coli* are also non-motile and aflagellate, but express flagellin at normal levels so that it accumulates intracellularly (Kinsella *et al.*, 1997; O'Toole *et al.*, 1994, 2000). This suggests that full activation of flagellin genes in *Campylobacter* requires the proper assembly of the flagellar-specific export apparatus.

The levels of *flhB* in *C. jejuni* were too low to allow detection in Northern hybridization experiments, and therefore we utilized reporter gene analysis to identify promoter sequences driving *flhB* transcription. The reporter gene experiments confirmed that levels of *flhB* transcripts are very low, since the β -galactosidase activities of *flhB::lacZ* fusions were only slightly higher than background values. Transcription of *flhB* is not affected by the presence or absence of the upstream *ahpC* gene and promoter, thus demonstrating that the *flhB* gene is transcribed at a low level from its own promoter, and indicating there is an (as yet unidentified) transcriptional stop between the *flhB* and *ahpC* genes. Taken together, these results demonstrate that *flhB* is transcribed at a low level, that the *flhB* promoter is located in the *ahpC*–*flhB* intergenic region, that transcription starts at the experimentally determined transcription start point, and that it is independent of the iron-repressed promoter of the upstream-located *aphC* gene. In conclusion, the *flhB* gene is essential for flagellar biogenesis in *C. jejuni*, and is likely to function as a component of the flagellar export apparatus.

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REFERENCES

Allan, E., Dorrell, N., Foyes, S., Anyim, M. & Wren, B. W. (2000). Mutational analysis of genes encoding the early flagellar components of *Helicobacter pylori*: evidence for transcriptional regulation of flagellin A biosynthesis. *J Bacteriol* **182**, 5274–5277.

Baillon, M.-L. A., van Vliet, A. H. M., Ketley, J. M., Constantinidou, C. & Penn, C. W. (1999). An iron-regulated alkyl hydroperoxide reductase (AhpC) confers aerotolerance and oxidative stress resistance to the microaerophilic pathogen *Campylobacter jejuni*. *J Bacteriol* **181**, 4798–4804.

Buck, M., Gallegos, M. T., Studholme, D. J., Guo, Y. & Gralla, J. D. (2000). The bacterial enhancer-dependent σ^{54} (σ^N) transcription factor. *J Bacteriol* **182**, 4129–4136.

Chilcott, G. S. & Hughes, K. T. (2000). Coupling of flagellar gene expression to flagellar assembly in *Salmonella enterica* serovar

typhimurium and *Escherichia coli*. *Microbiol Mol Biol Rev* **64**, 694–708.

Colegio, O. R., Griffin, T. J., Grindley, N. D. & Galan, J. E. (2001). In vitro transposition system for efficient generation of random mutants of *Campylobacter jejuni*. *J Bacteriol* **183**, 2384–2388.

Constantinidou, C., Hellyer, T. J., Richardson, P. T. & Penn, C. W. (1996). Cross-reactivities and strain-specificity of monoclonal antibodies to *Campylobacter jejuni* and *Helicobacter pylori* flagellins. In *Campylobacters, Helicobacters and Related Organisms*, pp. 663–666. Edited by D. G. Newell, J. M. Ketley & R. A. Feldman. New York: Plenum Press.

Foyes, S., Dorrell, N., Ward, S. J., Zhang, Z. W., McColm, A. A., Farthing, M. J. & Wren, B. W. (1999). Functional analysis of the roles of FlhQ and FlhB in flagellar expression in *Helicobacter pylori*. *FEMS Microbiol Lett* **174**, 33–39.

Guerry, P., Alm, R. A., Power, M. E. & Trust, T. J. (1992). Molecular and structural analysis of *Campylobacter* flagellin. In *Campylobacter jejuni: Current Status and Future Trends*, pp. 267–281. Edited by I. Nachamkin, M. J. Blaser & L. S. Tompkins. Washington, DC: American Society for Microbiology.

Hendrixson, D. R., Akerley, B. J. & Dirita, V. J. (2001). Transposon mutagenesis of *Campylobacter jejuni* identifies a bipartite energy taxis system required for motility. *Mol Microbiol* **40**, 214–224.

Jagannathan, A., Constantinidou, C. & Penn, C. W. (2001). Roles of *rpoN*, *fliA*, and *flgR* in expression of flagella in *Campylobacter jejuni*. *J Bacteriol* **183**, 2937–2942.

Kinsella, N., Guerry, P., Cooney, J. & Trust, T. J. (1997). The *flgE* gene of *Campylobacter coli* is under the control of the alternative sigma factor σ^{54} . *J Bacteriol* **179**, 4647–4653.

Kutsukake, K. (1997). Hook-length control of the export-switching machinery involves a double-locked gate in *Salmonella typhimurium* flagellar morphogenesis. *J Bacteriol* **179**, 1268–1273.

Kutsukake, K., Minamino, T. & Yokoseki, T. (1994). Isolation and characterization of FlhK-independent flagellation mutants from *Salmonella typhimurium*. *J Bacteriol* **176**, 7625–7629.

Macnab, R. M. (1996). Flagella and motility. In *Escherichia coli and Salmonella: Cellular and Molecular Biology*, pp. 123–145. Edited by F. C. Neidhardt. Washington, DC: ASM Press.

Miller, S., Pesci, E. C. & Pickett, C. L. (1993). A *Campylobacter jejuni* homolog of the LcrD/FlhF family of proteins is necessary for flagellar biogenesis. *Infect Immun* **61**, 2930–2936.

Minamino, T. & Macnab, R. M. (1999). Components of the *Salmonella* flagellar export apparatus and classification of export substrates. *J Bacteriol* **181**, 1388–1394.

Minamino, T. & Macnab, R. M. (2000). Domain structure of *Salmonella* FlhB, a flagellar export component responsible for substrate specificity switching. *J Bacteriol* **182**, 4906–4914.

Minamino, T., Iino, T. & Kutsukake, K. (1994). Molecular characterization of the *Salmonella typhimurium flhB* operon and its protein products. *J Bacteriol* **176**, 7630–7637.

Nachamkin, I., Yang, X. H. & Stern, N. J. (1993). Role of *Campylobacter jejuni* flagella as colonization factors for three-day-old chicks: analysis with flagellar mutants. *Appl Environ Microbiol* **59**, 1269–1273.

Nuijten, P. J. M., van Asten, A. J. A. M., Gastra, W. & van der Zeijst, B. A. M. (1990). Structural and functional analysis of two *Campylobacter jejuni* flagellin genes. *J Biol Chem* **265**, 17798–17804.

Nuijten, P. J., van den Berg, A. J. G., Formentini, I., van der Zeijst, B. A. M. & Jacobs, A. A. C. (2000). DNA rearrangements in the flagellin locus of an *flaA* mutant of *Campylobacter jejuni* during colonization of chicken ceca. *Infect Immun* **68**, 7137–7140.

- Oelmüller, U., Krüger, N., Steinbüchel, A. & Friedrich, C. (1990). Isolation of prokaryotic RNA and detection of specific mRNA with biotinylated probes. *J Microbiol Methods* **11**, 73–81.
- O'Toole, P. W., Kostrzynska, M. & Trust, T. J. (1994). Non-motile mutants of *Helicobacter pylori* and *Helicobacter mustelae* defective in flagellar hook production. *Mol Microbiol* **14**, 691–703.
- O'Toole, P. W., Lane, M. C. & Porwollik, S. (2000). *Helicobacter pylori* motility. *Microbes Infect* **2**, 1207–1214.
- Park, S. F., Purdy, D. & Leach, S. (2000). Localized reversible frameshift mutation in the *flhA* gene confers phase variability to flagellin gene expression in *Campylobacter coli*. *J Bacteriol* **182**, 207–210.
- Parkhill, J., Wren, B. W., Mungall, K. & 18 other authors (2000). The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* **403**, 665–668.
- Prüß, B. M. & Matsumura, P. (1997). Cell cycle regulation of flagellar genes. *J Bacteriol* **179**, 5602–5604.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- Spohn, G. & Scarlato, V. (1999). Motility of *Helicobacter pylori* is coordinately regulated by the transcriptional activator FlgR, an NtrC homolog. *J Bacteriol* **181**, 593–599.
- Szymanski, C. M., King, M., Haardt, M. & Armstrong, G. D. (1995). *Campylobacter jejuni* motility and invasion of Caco-2 cells. *Infect Immun* **63**, 4295–4300.
- Tomb, J. F., White, O., Kerlavage, A. R. & 39 other authors (1997). The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* **388**, 539–547.
- van Vliet, A. H. M. & Ketley, J. M. (2001). Pathogenesis of enteric *Campylobacter* infection. *J Appl Microbiol* **90**, 45S–56S.
- van Vliet, A. H. M., Wood, A. C., Henderson, J., Wooldridge, K. G. & Ketley, J. M. (1998a). Genetic manipulation of enteric *Campylobacter* species. *Methods Microbiol* **27**, 407–419.
- van Vliet, A. H. M., Wooldridge, K. G. & Ketley, J. M. (1998b). Iron-responsive gene regulation in a *Campylobacter jejuni* *fur* mutant. *J Bacteriol* **180**, 5291–5298.
- van Vliet, A. H. M., Rock, J. D., Madeleine, L. N. & Ketley, J. M. (2000). The iron-responsive regulator Fur of *Campylobacter jejuni* is expressed from two separate promoters. *FEMS Microbiol Lett* **188**, 115–118.
- Wassenaar, T. M., Bleumink-Pluym, N. M. C. & van der Zeijst, B. A. M. (1991). Inactivation of *Campylobacter jejuni* flagellin genes by homologous recombination demonstrates that *flaA* but not *flaB* is required for invasion. *EMBO J* **10**, 2055–2061.
- Wassenaar, T. M., van der Zeijst, B. A. M., Ayling, R. & Newell, D. G. (1993). Colonization of chicks by motility mutants of *Campylobacter jejuni* demonstrates the importance of flagellin A expression. *J Gen Microbiol* **139**, 1171–1175.
- Williams, A. W., Yamaguchi, S., Togashi, F., Aizawa, S. I., Kawagishi, I. & Macnab, R. M. (1996). Mutations in *fliK* and *flhB* affecting flagellar hook and filament assembly in *Salmonella typhimurium*. *J Bacteriol* **178**, 2960–2970.
- Wösten, M. M. S. M., Boeve, M., Gaastra, W. & van der Zeijst, B. A. M. (1998a). Cloning and characterization of the gene encoding the primary σ -factor of *Campylobacter jejuni*. *FEMS Microbiol Lett* **162**, 97–103.
- Wösten, M. M. S. M., Boeve, M., Koot, M. G., van Nuenen, A. C. & van der Zeijst, B. A. M. (1998b). Identification of *Campylobacter jejuni* promoter sequences. *J Bacteriol* **180**, 594–599.

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