Mini-Review

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Pumping iron: mechanisms for iron uptake by *Campylobacter*

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Campylobacter requires iron for successful colonization of the host. In the last 7 years, a wealth of data has been generated allowing detailed molecular characterization of *Campylobacter* iron-uptake systems. Several exogenous siderophores have been identified as sources of ferric iron for *Campylobacter*. Ferri-enterochelin uptake requires both the outer-membrane receptor protein CfrA and the inner-membrane ABC transporter system CeuBCDE. Ferrichrome has been shown to support growth of some *Campylobacter jejuni* strains and the presence of homologues of *Escherichia coli* *fhuABD* genes was proposed; the Cj1658–Cj1663 system appears to be involved in the uptake of ferri-rhodotorulic acid. In addition to siderophores, the importance of host iron sources was highlighted by recent studies demonstrating that *C. jejuni* can exploit haem compounds and the transferrins using ChuABCDZ and Cj0173c–Cj0178, respectively. An additional putative receptor, Cj0444, present in some, but not all, strains has not yet been characterized. Following diffusion through the outer membrane, inner-membrane transport of ferrous iron can occur via the FeoB protein. While it may be assumed that all systems are not essential, there is growing evidence supporting the need for multiple iron-uptake systems for successful host colonization by *Campylobacter*. In light of this, comparative molecular characterization of iron systems in all *Campylobacter* strains is necessary to gain further insight into the pathogenesis of members of this genus.

Introduction

For many micro-organisms, iron acquisition is essential for colonization and infection of the host. Iron is a cofactor in many proteins involved in metabolism and basic cellular pathways in both pathogens and their hosts. Under aqueous aerobic conditions at physiological pH, iron is predominantly present as Fe^{3+} , forming insoluble ferric oxide hydrates that are poorly available for use by cells (solubility 1.4×10^{-9} M). Many host iron-binding and transport processes deliver Fe^{3+} to cells; in turn these processes can be hijacked by pathogens.

Unlike Fe^{2+} , which can passively diffuse through the outer-membrane porins (limit ~600 Da), Fe^{3+} uptake using siderophores (low-molecular-mass, high-affinity iron-binding compounds) by Gram-negative pathogens begins by binding of the iron source to a specific iron-regulated ligand-gated outer-membrane receptor protein (Miethke & Marahiel, 2007). Following binding, a conformational change facilitates transport through the receptor pore using energy transduced from the cytoplasmic membrane to the outer membrane by the ExbBD-TonB protein complex (Chakraborty *et al.*, 2007). Once within the periplasm, ferri-siderophore or free iron is sequestered by a periplasmic binding protein and delivered to an inner-membrane ATP-binding cassette (ABC)

transporter system consisting of permease protein subunits, which form an inner-membrane pore, coupled with an ATP-binding protein (Davidson & Maloney, 2007). ATP is hydrolysed to provide the energy to drive transport across the inner membrane into the cytoplasm, where iron bound to siderophores may be liberated by reduction or by siderophore degradation (Faraldo-Gómez & Sansom, 2003). Host iron-binding or transport proteins such as haem-containing proteins or ferri-transferrins can also be used as Fe^{3+} sources. Haem compounds are bound directly by specific outer-membrane receptors or delivered to the receptors by haemophores (Tong & Guo, 2009). Transferrin-bound iron may be acquired by receptor-mediated release, proteolytic cleavage or reduction to Fe^{2+} , which dissociates from the protein (Cornelissen, 2003). Following transport across the outer membrane, uptake of haem or iron from the transferrins proceeds as described above.

Since the 1980s *Campylobacter* spp. have emerged as the most prevalent food-borne enteric pathogens in the Western world, constituting a major economic burden. *Campylobacter* is commensal in many animals, notably birds, but colonization of the human intestinal tract results in disease (Skirrow, 1994; Humphrey, 2006). Enteritis is the most frequent outcome of human infection.

Campylobacter pathogenesis, recently reviewed by Poly & Guerry (2008), is not as well understood as the pathogenesis of other bacteria, but motility, chemotaxis, adhesion, invasion, toxin production and the acquisition of iron have all been demonstrated to be important. In addition, aspects of *Campylobacter* virulence, including growth, invasion of Caco-2 cells, disruption of cultured Caco-2 cell monolayers and cellular tight junction breakdown, are enhanced in the presence of the catecholamine host stress hormone nor-adrenaline (Cogan *et al.*, 2006). The observed modulation of virulence in a stressed host would be expected to lead to increased colonization and disease (Cogan *et al.*, 2006).

Genomics, transcriptomics and the regulation of iron uptake by *C. jejuni*

Campylobacter jejuni uses highly adapted specific mechanisms for scavenging iron from a range of sources in the human host to enable its survival and proliferation (Palyada *et al.*, 2004; Naikare *et al.*, 2006). The importance of iron is suggested by the large number of genes dedicated to iron uptake, regulation and homeostasis in the relatively small genomes of *C. jejuni* strains NCTC 11168 (Parkhill *et al.*, 2000), 81-176 (Hofreuter *et al.*, 2006) and 81116 (Pearson *et al.*, 2007). Moreover, comparative genomics indicates variation in iron-associated genes among the sequenced strains. A representation of the structure of all known *Campylobacter* iron-uptake systems, their compo-

nents and sources is shown in Fig. 1; the chromosomal organization of iron-uptake genes is represented in Fig. 2, and Table 1 provides a directory of genes associated with iron uptake in *C. jejuni*.

C. jejuni is exposed to aerobic conditions during transmission and to reactive oxygen species (ROS) during host cell invasion. Uptake of iron by all cells must be tightly regulated to avoid the combination of excess iron and oxygen that generates toxic ROS (Andrews *et al.*, 2003; Massé & Arguin, 2005). In times of iron limitation, genes encoding proteins responsible for iron uptake must be expressed to allow cells to acquire enough iron for survival; conversely, when cells become iron-replete, iron-uptake genes must be repressed to avoid the accumulation of excess iron, which may lead to the production of damaging ROS. Two separate but possibly overlapping iron-responsive regulatory systems are encoded within the *C. jejuni* genome, regulating both iron acquisition and oxidative stress defence. The regulatory protein Fur is primarily responsible for regulation of genes involved in iron uptake; the peroxide stress regulator protein is PerR (van Vliet *et al.*, 1998, 1999). Two studies have demonstrated differences in the transcriptome and proteome of *C. jejuni* NCTC 11168 in iron-restricted and high-iron conditions (Palyada *et al.*, 2004; Holmes *et al.*, 2005). Expression levels of many iron-transport genes as well as genes proposed to encode the iron-storage proteins ferritin (*cft*) and bacterioferritin (*bfr*), the PerR regulon (*kata*, *ahpC* and *perR*), thioredoxins

Fig. 1. Iron-uptake systems of *C. jejuni*. Ferric iron sources are typically bound by a surface ligand-gated porin, which transports the iron source into the periplasm using energy derived from proton-motive force transferred from the cytoplasmic membrane (CM) to the outer membrane (OM) by the energy transduction system ExbBD-TonB. Once within the periplasm, iron sources are delivered to the CM ABC transporter systems by the periplasmic binding proteins. Ferrous iron diffuses through the OM, only requiring transport across the CM. See Fig. 2 for colour key.

Fig. 2. Genes involved in iron uptake in *C. jejuni*. The diagrams indicate the genomic organization and functions of the components of systems involved in the uptake of iron from (a) ferri-enterochelin, (b) ferrichrome (hypothetical), (c) ferri-rhodotorulic acid, (d) haem and (e) ferri-transferrins, and (f) in the uptake of ferrous iron; (g) represents the outer-membrane receptor gene *cj0444*. Gene names, sizes and context are based on NCTC 11168; when information from other strains is included, this is indicated in the figure. Diagrams are to scale and are based on the genomic structure taken from <http://xbase.bham.ac.uk/campydb/>. Dashed lines indicate a lack of close linkage; solid lines indicate the presence of an intergenic spacer region of greater than 10 bp. Genes with increased relative transcript levels under iron limitation are indicated by the fold change shown beneath the gene; none of the genes demonstrated decreased relative transcript levels when iron was limited (Holmes *et al.*, 2005). Where the change is not known or has not been reported, the spaces beneath the genes are blank.

(*cj1664*, *cj1665*, *trxB*) and oxidoreductases (*cj0559*, *cj1287c* and *cj1516*; oxidative stress defence) and genes of the energy-transduction systems (*exbB1*–*exbD1*, *exbB2*–*exbD2*, *tonB1*, *tonB2* and *tonB3*) were shown to be higher under iron limitation, but interestingly not the expression level of *fur* itself (Holmes *et al.*, 2005). Upon addition of iron, iron-acquisition genes were downregulated. Components of iron-uptake systems that showed enhanced expression under iron limitation also had increased transcript levels in a *fur* mutant under iron-replete conditions, indicating derepression of

these genes in the absence of Fur. The *fur* mutant strain was shown to be significantly impaired in chick caecal colonization compared to the wild-type, indicating the importance of Fur regulation *in vivo* (Palyada *et al.*, 2004).

Iron uptake by siderophores

C. jejuni was long thought to be incapable of synthesizing siderophores (Field *et al.*, 1986; Pickett *et al.*, 1992), and the failure to identify any siderophore biosynthesis genes

Table 1. Cast of characters: iron-uptake system components of *C. jejuni*, followed by energy transduction system and regulation components

Gene	Designation (size, bp)	Protein function/description	Reference
Enterochelin			
<i>cj0755</i>	<i>cfrA</i> (2091)	Outer-membrane receptor	Palyada <i>et al.</i> (2004)
<i>cj1352</i>	<i>ceuB</i> (969)	Permease	
<i>cj1353</i>	<i>ceuC</i> (939)	Permease	
<i>cj1354</i>	<i>ceuD</i> (756)	ATP-binding protein	
<i>cj1355</i>	<i>ceuE</i> (993)	Periplasmic binding protein	
Ferrichrome*			
–	<i>cfhuA</i>	Outer-membrane receptor	Galindo <i>et al.</i> (2001)
–	<i>cfhuB</i>	Permease	
–	<i>cfhuD</i>	Periplasmic binding protein	
Rhodotorulic acid			
<i>cj1658</i>	– (2091)	Permease	http://xbase.bham.ac.uk/ Janvier <i>et al.</i> (1998)
<i>cj1659</i>	<i>p19</i> (540)	Periplasmic binding protein	
<i>cj1660</i>	– (1404)	Integral membrane protein	
<i>cj1661</i>	– (1293)	Permease	
<i>cj1662</i>	– (1119)	Integral membrane protein	
<i>cj1663</i>	– (654)	ATP-binding protein	http://xbase.bham.ac.uk/
Haem			
<i>cj1613c</i>	<i>chuZ</i> (756)	Haem oxygenase	Ridley <i>et al.</i> (2006)
<i>cj1614</i>	<i>chuA</i> (2130)	Outer-membrane receptor	
<i>cj1615</i>	<i>chuB</i> (987)	Permease	
<i>cj1616</i>	<i>chuC</i> (777)	ATP-binding protein	
<i>cj1617</i>	<i>chuD</i> (807)	Periplasmic binding protein	
Transferrins			
<i>cj0173c</i>	<i>cfbpC</i> (909)	Putative ATP-binding protein	van Vliet <i>et al.</i> (2002)
<i>cj0174c</i>	<i>cfbpB</i> (1617)	Putative permease	
<i>cj0175c</i>	<i>cfbpA</i> (1005)	Putative periplasmic binding protein	
Transferrins			
<i>cj0176c</i>	– (132)	Putative lipoprotein	http://xbase.bham.ac.uk/
<i>cj0177</i>	– (852)	Putative iron-transport lipoprotein	
<i>cj0178</i>	<i>ctuA</i> (2268)	Putative transferrin-bound iron utilization outer-membrane receptor	Miller <i>et al.</i> (2008)
Ferrous iron			
<i>cj1397</i>	<i>feoA</i> (225)	Putative ferrous iron-transport protein	Naikare <i>et al.</i> (2006)
<i>cj1398</i>	<i>feoB</i> (1842)	Ferrous iron-transport protein	
Additional receptor			
<i>cj0444</i>	– (2115)†	Non-functional putative TonB-dependent outer-membrane receptor (NCTC 11168, RM1221), functional in strains 81-176, 81116	http://xbase.bham.ac.uk/
Energy-transduction systems			
<i>cj0179</i>	<i>exbB1</i> (762)	Biopolymer-transport protein	http://xbase.bham.ac.uk/
<i>cj0180</i>	<i>exbD1</i> (411)	Biopolymer-transport protein	
<i>cj0181</i>	<i>tonB1</i> (747)	TonB transport protein	
<i>cj1628</i>	<i>exbB2</i> (426)	Biopolymer-transport protein	
<i>cj1629</i>	<i>exbD2</i> (390)	Biopolymer-transport protein	
<i>cj1630</i>	<i>tonB2</i> (684)	TonB transport protein	
<i>cj0109</i>	<i>exbB3</i> (555)	Putative MotA/TolQ/ExbB proton channel family protein	
<i>cj0110</i>	<i>exbD3</i> (390)	Putative ExbD/TolR family transport protein	
<i>cj0753c</i>	<i>tonB3</i> (684)	TonB transport protein	
Regulators			
<i>cj0400</i>	<i>fur</i> (474)	Ferric uptake regulator protein	van Vliet <i>et al.</i> (1998)
<i>cj0322</i>	<i>perR</i> (411)	Peroxide stress regulator protein	van Vliet <i>et al.</i> (1999)

*The ferrichrome uptake system of *C. jejuni* is hypothetical.†Deduced size of *cj0444* from strain 81-176; when non-functional in strain NCTC 11168 size is 2072 bp.

within the genome sequences of *C. jejuni* NCTC 11168, RM1221 and 81-176 supports this view (Parkhill *et al.*, 2000; Fouts *et al.*, 2005; Hofreuter *et al.*, 2006). The organism is, however, capable of utilizing exogenous siderophores as sources of iron; one early study indicated that the *Escherichia coli* siderophore enterochelin and the fungal siderophore ferrichrome were able to promote growth in iron-restricted conditions (Field *et al.*, 1986).

Enterochelin

C. jejuni acquires iron from ferri-enterochelin using the receptor CfrA, which shows homology with other outer-membrane ferric siderophore receptor proteins (Palyada *et al.*, 2004), and the binding-protein-dependent inner-membrane ABC transporter system encoded by the *ceuBCDE* genes (Parkhill *et al.*, 2000). Genes encoding parts of the enterochelin transport system were first identified in *Campylobacter coli* (Richardson & Park, 1995). A *cfrA* mutant of *C. jejuni* NCTC 11168 was unable to grow when supplied with ferri-enterochelin as the only iron source (Palyada *et al.*, 2004), although, inexplicably, a site-specific *cfrA* mutant of *C. coli* was apparently as capable of utilizing ferri-enterochelin as were wild-type cells (Guerry *et al.*, 1997). The crystal structure of CfrA indicates that it is a classical β -barrel ligand-gated porin, but homology modelling indicates that some regions thought to be important for ligand binding differ significantly from the corresponding regions of the *E. coli* enterochelin receptor FepA (Carswell *et al.*, 2008).

The *ceuBCDE* operon is very similar in *C. jejuni* and *C. coli*; *ceuBC* encode the inner-membrane permease proteins, *ceuD* an ATPase and *ceuE* the periplasmic binding lipoprotein (Parkhill *et al.*, 2000; Palyada *et al.*, 2004). A signature sequence that is conserved in siderophore-binding periplasmic proteins was identified at amino acids 137–152 of CeuE (Park & Richardson, 1995). A *ceuE* mutant of *C. jejuni* was slightly impaired in its ability to grow when provided with ferri-enterochelin, indicating that CeuE is involved in, but not essential for, utilizing iron from ferri-enterochelin; this suggests redundancy among inner-membrane transport systems, an observation supported by other studies described below. The potential significance of ferri-enterochelin uptake *in vivo* is highlighted by the fact that *cfrA* or *ceuE* mutants were markedly impaired in colonization of the avian gastrointestinal tract (Palyada *et al.*, 2004). In addition, the growth increase seen when NCTC 11168 was incubated under iron-limited conditions in the presence of noradrenaline was lost in a *cfrA* mutant strain, suggesting that noradrenaline increases growth by enhancing CfrA-dependent iron uptake (Haigh *et al.*, 2008).

Ferrichrome

C. jejuni strain M129 carries the *cfhuABD* operon encoding proteins homologous with the ferrichrome-uptake systems of *E. coli* and *Pseudomonas aeruginosa* (Galindo *et al.*,

2001). This operon, which has not been found in any of the sequenced strains of *C. jejuni* (Parkhill *et al.*, 2000; Fouts *et al.*, 2005; Hofreuter *et al.*, 2006; Pearson *et al.*, 2007), is surprisingly GC rich – 65 mol%, compared with the average 30–35 % of the *C. jejuni* genome – but there was no suggestion that the region is a genomic island. CFhuA is an 80 kDa receptor, which is 33 % identical to the *E. coli* ferrichrome receptor FhuA and is expressed under low-iron conditions. Southern blot analysis demonstrated that only six of eleven *C. jejuni* isolates tested had a *fhuA* homologue (Galindo *et al.*, 2001). CFhuB and CFhuD demonstrate homology with the permease FhuB (26 % identity) and the periplasmic binding protein FhuD (25 % identity), respectively, of the *E. coli* ferrichrome-uptake system. Of all iron sources tested, ferrichrome most effectively promoted growth of strain M129, but experimental confirmation of direct roles for CFhuA in the uptake of ferrichrome and for *cfhuABD* in *C. jejuni* pathogenesis are still required.

Rhodotorulic acid

Recent data indicate a role for the *cj1658–cj1663* locus of the *C. jejuni* NCTC 11168 genome in the uptake of iron from the fungal hydroxamate siderophore ferri-rhodotorulic acid. A 19 kDa protein designated P19, first purified from *C. jejuni* 81-176 by anion-exchange chromatography (Janvier *et al.*, 1998), is a periplasmic binding protein. Gene *cj1658*, which is located immediately upstream of the *p19* gene, encodes a membrane-associated protein that does not resemble a classical TonB-dependent receptor and may be a permease; *cj1661–cj1663* encode proteins that resemble an inner-membrane ABC transporter system (Parkhill *et al.*, 2000). The potential involvement of this region in iron uptake was inferred from the observation that *cj1658* and *p19* homologues are found on a *Yersinia pestis* iron-uptake pathogenicity island (Carniel, 2001). *C. jejuni* NCTC 11168 *cj1658* or *p19* mutants were unable to utilize iron supplied as ferri-rhodotorulic acid for growth (Stintzi *et al.*, 2008); the ability to acquire a siderophore that may not commonly be encountered during transmission either may not be physiologically relevant or may reflect an association with a relevant siderophore with a similar structure.

Iron uptake from host sources

Haem

C. jejuni can use iron derived from haem-containing compounds for growth, including haemin, haemoglobin, haemin-haemopexin and haemoglobin-haptoglobin (Pickett *et al.*, 1992). Mutations in an iron-regulated gene named *chuA*, which encodes a 70 kDa receptor with similarity to other iron-regulated bacterial receptors (van Vliet *et al.*, 1998), resulted in failure of cells to grow when supplied with haemin or haem-containing compounds as sole iron sources (Pickett *et al.*, 1992). In *C. jejuni* NCTC 11168, *chuA* is part of an operon, *chuABCD*. ChuB is

predicted to be a permease, ChuC the ATPase and ChuD the periplasmic binding protein of the inner-membrane ABC transporter system (Parkhill *et al.*, 2000; Ridley *et al.*, 2006). A *chuA* mutant strain grew significantly less well than the wild-type with haemin or haemoglobin as iron sources, implying an important role for ChuA in haem uptake. A slight, but statistically significant, reduction in growth of *chuB*, *chuC* or *chuD* mutant strains was also observed, implying that the ABC transport system is involved in, but not essential for, haem uptake, suggesting redundancy among the inner-membrane transport systems. Upstream of *chuA*, and separated by a region containing divergent promoters, is *chuZ*, which encodes an iron-responsive cellular haem oxygenase (Ridley *et al.*, 2006), a homologue of the haem-uptake protein HugZ in *Plesiomonas shigelloides* (Henderson *et al.*, 2001). Expression of *chu* is not essential for colonization of the caecal lumen model, but the *chu* genes are upregulated in both the rabbit ileal loop model and the chick caecum (Palyada *et al.*, 2004; Stintzi *et al.*, 2005).

Transferrin proteins

C. jejuni NCTC 11168 cells can utilize iron bound to the iron-transport glycoproteins human ferri-transferrin (Tf), human ferri-lactoferrin (Lf), and ferri-ovotransferrin for growth (Miller *et al.*, 2008). The association of human ferri-Lf with the *C. jejuni* cell surface appears to be iron-responsive, with the majority (>90%) of released iron found in the cell cytoplasm and periplasm. Partitioning of *C. jejuni* from the iron source by dialysis membrane reduced levels of growth significantly, and also reduced the levels of internalized iron; internalization of iron was saturable. The uptake of iron from the transferrin family was recently attributed to genes in the *cj0173c*–*cj0178* region of the *C. jejuni* NCTC 11168 genome (Miller *et al.*, 2008). Organized as two operons separated by divergent promoters, *cj0176c*–*cj0173c* and *cj0177*–*cj0178* demonstrate homology with known siderophore-dependent iron-uptake systems (Parkhill *et al.*, 2000). Genes *cj0175c*–*cj0173c* were originally identified as a Fe^{3+} -transporting inner-membrane system similar to one described for *Serratia marcescens* (Zimmermann *et al.*, 1989). The operon, named *cfbpABC*, demonstrates homology with *hitABC* of *Haemophilus influenzae* and *Y. pestis* (van Vliet *et al.*, 2002). CfbpC (*Cj0173c*) shows similarity to an ATPase, with CfbpB (*Cj0174c*) annotated as the permease; CfbpA (*Cj0175c*) is the periplasmic binding protein. The crystal structure of CfbpA has been solved and shown to preferentially bind free Fe^{2+} (which is then oxidized to Fe^{3+}) or Fe^{3+} chelated to oxalate (Tom-Yew *et al.*, 2005). Requirement for a synergistic anion, as seen with other ferric-transport proteins, was not observed, indicating that this is a novel type of binding protein. CfbpA was proposed to be similar to neisserial FbpA, a component of the FbpABC transport system thought to transport Tf-derived iron into the cytoplasm (Tom-Yew *et al.*, 2005). Upstream of *cj0175c* and transcribed in the same orientation is gene

cj0176c, which encodes an uncharacterized lipoprotein (Parkhill *et al.*, 2000; van Vliet *et al.*, 2002).

Cj0177 and *Cj0178* have been suggested to function as a haem-uptake system in addition to ChuABCDZ (Chan *et al.*, 2006); the authors propose that multiple haem-uptake systems may be required since available haem sources are diverse. The lipoprotein *Cj0177* is homologous with the inner-membrane protein PhuW from *P. aeruginosa*, which is co-expressed with a haem-uptake ABC transporter system and is necessary, but not essential, for fully efficient haem uptake (Ochsner *et al.*, 2000). The crystal structure of *Cj0177* showed two bound cofacial haem groups in a pocket formed by the *Cj0177* dimer (Chan *et al.*, 2006). *Cj0178* shows homology with the haemin receptor PhuR of *P. aeruginosa* (Ochsner *et al.*, 2000), but experimental evidence for such a role in *C. jejuni* is lacking. Interestingly, *Cj0178* is required for both chick and rabbit ileal loop colonization, suggesting the importance of this receptor *in vivo* (Palyada *et al.*, 2004; Stintzi *et al.*, 2005). More recently, mutation of *cj0178* was shown to cause decreased binding and acquisition of iron from Tfs, and reduced growth in the presence of human ferri-Lf compared to wild-type (Miller *et al.*, 2008). Moreover, a *cj0178* mutant was not affected in either haem acquisition (Miller *et al.*, 2008) or ferri-enterochelin utilization (Palyada *et al.*, 2004). A decrease in growth was also seen when a *cfrA* mutant strain was incubated with ferri-Lf, but the difference was not as marked as that seen with the loss of *cj0178*; mutation of *chuA* had no effect on iron acquisition from Tfs (Miller *et al.*, 2008). Deletion of the gene for the permease *Cj0174c* resulted in a small but significant decrease in growth with ferri-Lf (Miller *et al.*, 2008).

In addition to these major iron receptors, the *C. jejuni* NCTC 11168 genome also contains the pseudogene *cj0444*. Interestingly, in strains where *cj0444* is a pseudogene, genes *cfrA* and *cj0178* are functional (NCTC 11168, RM1221), while in *C. jejuni* strains 81-176 and 81116, which contain a functional copy of *cj0444*, the receptors CfrA and *Cj0178* are absent (Parkhill *et al.*, 2000; Fouts *et al.*, 2005; Hofreuter *et al.*, 2006; Pearson *et al.*, 2007). *Cj0444*, annotated as CJJ81176-0471 in strain 81-176, shares 34%, 32% and 24% identity with CfrA, ChuA and *Cj0178* from NCTC 11168, respectively (<http://xbase.bham.ac.uk/campydb/blast.pl>), which may suggest a role for *Cj0444* in ferri-enterochelin uptake. As yet there are no experimental data to confirm that functional *Cj0444* substitutes for CfrA or *Cj0178*, but immune cross-reactivity has been observed between *Cj0444* and CfrA (Zeng & Lin, 2008).

ExbBD-TonB energy transduction systems

Located downstream of *cj0178* are *cj0179*–*cj0181*, which encode ExbB, ExbD and TonB homologues (Parkhill *et al.*, 2000). There are two additional ExbBD-TonB systems, encoded by *cj1628*–*30* and by *cj0109*–*10* and *cj0753c* in the *C. jejuni* NCTC 11168 genome (Parkhill *et al.*, 2000). Note that in two of the ExbBD-TonB systems, the *exbBD* genes

are transcriptionally coupled to a *tonB* gene, while *cj0109–10* are coupled to the ATP synthase operon (Parkhill *et al.*, 2000; van Vliet *et al.*, 2002). The TonB proteins may function with specific uptake systems; for example *cj0753c* is found adjacent to (but divergently transcribed from) *cfrA*, and *cj0181* is immediately downstream of *cj0173c–cj0178*. Alternatively, the different TonB proteins may function with any of the uptake systems (Parkhill *et al.*, 2000; van Vliet *et al.*, 2002).

Uptake of ferrous iron

The *C. jejuni* NCTC 11168 genome contains a homologue of the *E. coli* *feoB* gene (*cj1398*, 29 % identity) adjacent to a smaller gene (*cj1397*) with limited identity (16 %) to the *feoA* gene of *E. coli*, but lacks a *feoC* homologue (Parkhill *et al.*, 2000). The putative *feoB* and *feoA* orthologues form an operon (Naikare *et al.*, 2006). Previous work with *C. jejuni* strains M129 and F38011 suggested that *feoB* mutants did not differ from the wild-type in the amount of Fe^{2+} taken up, and the authors therefore concluded that FeoB is not required for Fe^{2+} uptake in *C. jejuni* (Raphael & Joens, 2003). However, more recently it was shown that mutation of *feoB* in NCTC 11168 reduced Fe^{2+} transport by half, and the Fe^{2+} that was taken up remained in the periplasm (Naikare *et al.*, 2006). Moreover, wild-type cells out-competed the *feoB* mutant in survival and colonization studies in the rabbit ileal loop model. Colonization of the chick caecum was also compromised in a *feoB* mutant, and wild-type cells out-competed the mutant in an intestinal infection model using colostrum-deprived piglets (Naikare *et al.*, 2006). The *feoB* gene is functional in NCTC 11168 and potentially so in 81116 (Parkhill *et al.*, 2000; Pearson *et al.*, 2007), but non-functional in 81-176 and RM1221 due to frameshift mutations (Fouts *et al.*, 2005; Hofreuter *et al.*, 2006); identification of candidate genes for Fe^{2+} uptake in addition to *feoB* in the latter strains is therefore required along with experimental confirmation of their role, if any, in Fe^{2+} transport. The full genome sequences of strains M129 and F38011 are not yet known; they might contain additional *feoB* homologues or genes for alternative Fe^{2+} uptake systems.

Concluding remarks

The acquisition of iron by *Campylobacter* spp. relies upon outer-membrane receptor proteins that appear almost exclusively specific for particular iron sources. The outer-membrane receptors, some of which are essential for colonization, appear to be non-redundant, and so it is interesting that only a subset of these receptors may be present or functional in some strains. The combinations of receptors in different strains presumably enable exploitation of iron sources characteristic of different host environments, but no epidemiological studies have yet been performed. The requirement for FeoB, CfrA and Cj0178 for successful host colonization demonstrated by multiple animal models supports the suggestion that

several iron-uptake systems are essential for survival and colonization in the host by *Campylobacter*. The importance of these systems for host colonization highlights the need for the detailed molecular characterization of each, but the high level of strain conservation found among the iron-uptake proteins of the Chu (Ridley *et al.*, 2006) and P19 systems, which currently do not appear to be essential for host colonization, seems counterintuitive and also requires clarification. The role of Cj0444 needs elucidating; identification of the iron source this receptor binds and transports will assist in this process. In contrast to the specificity of the outer-membrane receptors, the inner-membrane ABC transporter systems show redundancy, with a single system apparently capable of transporting iron derived from a number of sources.

Over recent years there have been major advances in our understanding of the methods by which *C. jejuni* acquires iron. The requirement for many sources, lack of redundancy in the outer-membrane proteins and requirement for components of the major iron-uptake systems for colonization indicate the importance of iron in the progression of disease in the human host. A link has been shown between the production of stress hormones, iron availability and an increase in pathogenicity of other organisms (Freestone *et al.*, 2008). The stress-hormone-induced increases in *C. jejuni* pathogenesis (Cogan *et al.*, 2006) impact not only on colonization and disease in humans, but also on colonization of chickens (Humphrey, 2006). A reduction in intensive poultry production coupled with an increase in awareness of poultry stress and welfare may reduce stress-hormone-enhanced *Campylobacter* colonization of chickens, and in turn reduce the risk of human infection (Humphrey, 2006).

Further characterization of a number of the iron-uptake systems, comparisons between strains with different system content and comparative analysis of regulation of the systems are all required for complete understanding of the role of iron during colonization and infection by campylobacters. Characterization of how *Campylobacter* improves its physique by pumping iron (including hormonal enhancement?) will build a powerful body of knowledge to aid our understanding of intestinal colonization and disease.

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