CHEMOTAXIS IN *CAMPYLOBACTER JEJUNI*

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Chemotaxis is the common way of flagellated bacteria to direct their locomotion to sites of most favourable living conditions, that are sites with the highest concentrations of energy sources and the lowest amounts of bacteriotoxic substances. The general prerequisites for chemotaxis are chemoreceptors, a chemosensory signal-transduction system and the flagellar apparatus.

Epsilonproteobacteria like *Campylobacter* sp. show specific variations of the common chemotaxis components. CheV, a CheW-like linking-protein with an additional response regulator (RR) domain, was identified as commonly used coupling scaffold protein of *Campylobacter jejuni*. It attaches the histidine autokinase (CheAY), which also has an additional RR-domain, to the chemoreceptors signalling domains. Theses additional RR-domains seem to play an important role in the regulation of the CheAY-phosphorylation state and thereby in sensory adaptation.

The *Campylobacter*-chemoreceptors are arranged into the three groups A, B, and C. Group A contains membrane-anchored receptors sensing periplasmic signals, group B consists only of one receptor with two cytoplasmic ligand-proteins representing a bipartite energy taxis system that senses pyruvate and fumarate, and group C receptors are cytoplasmic signalling domains with mostly unknown cytoplasmic ligand-binding proteins as sensory constituents. Recent findings demonstrating different alleles of the TLP7 chemoreceptor, specific for formic acid, led to an amendment of this grouping.

Keywords: *Campylobacter jejuni,* chemotaxis, receptors, Tlp groups, signal transduction, *Epsilonproteobacteria,* review

Introduction

The *Epsilonproteobacterium Campylobacter jejuni* is, due to recent epidemiological data, the most common germ causing bacterial foodborne enteritis worldwide [1–3]. *Campylobacter* enteritis can lead to various post-infectious complications like the Guillain–Barré syndrome (GBS), reactive arthritis, reactive myositis and idiopathic peripheral neuropathy [4–6].

The bacterium's flagellar motility is an important factor for the intestinal colonization of its avian and mammalian hosts but also for the invasion of intestinal epithelial cells and for overcoming the mucosal barrier [7–12].

Chemotaxis, presumably the most ancient form of signal processing, is the way of archaea and bacteria to direct their flagellar locomotion according to the presence of specific chemoeffectors. This phenomenon was already observed by Engelmann and Pfeffer at the end of the nineteenth century [13–15].

Chemoeffectors attracting bacterial cells to a point of high effector-concentration are called attractors and chemotaxis in the direction of increasing attractor concentrations is defined as "positive". In contrast, chemorepellent-gradients cause bacteria to swim to points of lower chemoeffector concentrations and this is considered as "negative" chemotaxis. Typically, energy sources act as chemoattractors while toxic substances function as chemorepellents. In this way, bacteria are trying to fathom the most favorable niches with relatively high chemoattractor and low chemorepellent concentrations. Temporal sensing is the fundamental principle that underlies this process of decision-making. The overall bacterial motion is principally a series of alternating phases of straight swimming and tumbling. If a bacterium is moving along a chemical gradient of a chemoattractor, the straight swimming phases last longer, but if the bacterium gets "abroad" and swims accidentally in regions of lower attractor-concentration, it starts sooner to tumble and tries to reorientate depending on the chemotaxins concentration [16]. The structural prerequisites for chemotaxis are simply at least one flagellum, a set of different chemoreceptors and a chemosensory signal-transduction system connecting both.

Experimental approaches

There are principally two major techniques to study chemotaxis and flagellar motility: microscopy and different variations of chemotaxis assays.

While conventional light microscopy is not sufficient to document the fast movement of *C. jejuni* or even to visualize the extremely thin flagellar filaments, dark field microscopy [17] and video-enhanced differential interference-contrast microscopy [18] coupled with computer-

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ized image analysis [19, 20] are widely used in the field of bacterial taxis. Fluorescence microscopy is another useful tool especially to study protein-protein-interactions within the chemoreceptor- signalling cascade [21, 22]. A special fluorescence microscopy technique called stimulated emission depletion (STED) microscopy is able to achieve an effective resolution of 15 nm in biological samples. Recently, it has been increasingly used to visualize subcellular structures and advanced into an important tool in this field [23, 24].

Different kinds of chemotaxis assays were established in order to screen for substances with chemoeffector activity with reference to a specific bacterial species [25]. For *Campylobacter* a semiquantitative version, the so-called chemical-in-plug assay, based on opalescence changes of a (semi-)solid agar due to the concentration of bacterial cells is commonly used [26, 27]. Recently, serious concerns for this method were considered as chemotaxis-independent responses were observed using non-chemotactic and non-motile mutants in this assay [28].

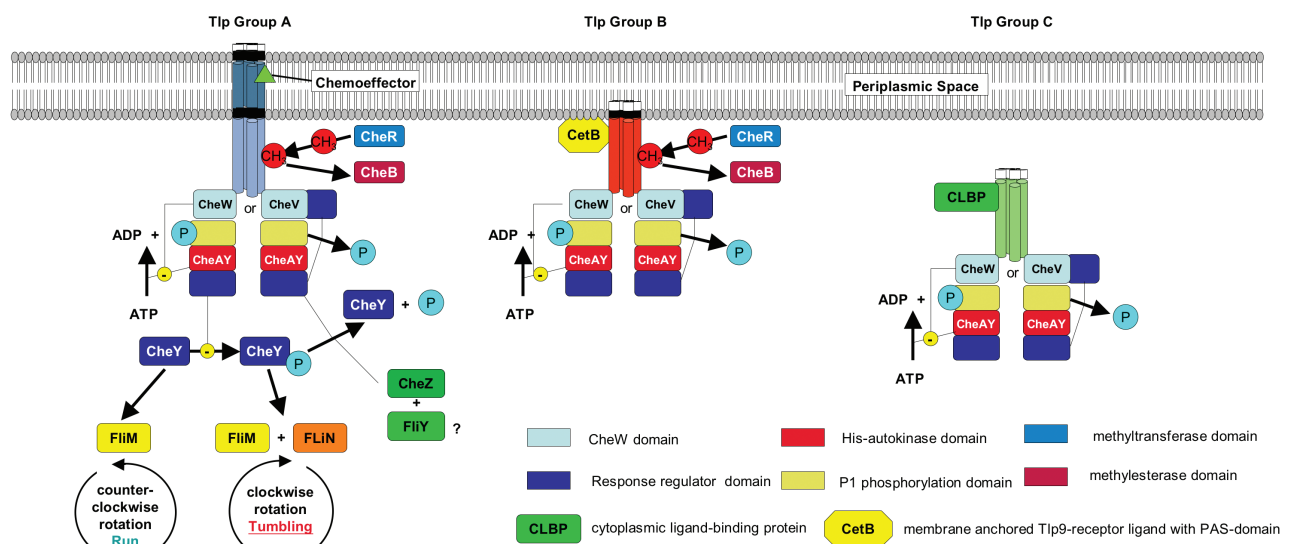*Chemoreceptor signal transduction in* C. jejuni

C. jejuni detects the variations of chemotaxin-concentrations in its environment by a set of different chemoreceptors, that share a common two-component system architecture for signal transduction (see *Fig. 1*) [25].

These two-component systems consist commonly of a membrane-associated histidine autokinase (CheA) and a cytoplasmic RR (CheY) [29].

These chemoreceptors, belonging to the group of methyl-accepting chemotaxis proteins (MCPs), exist as either integral membrane or free drifting cytoplasmic pro-

teins [30]. The first sense the environmental signals via their *N*-terminal periplasmic sensory domain to their *C*-terminal cytoplasmic signalling domain. The second interact with cytoplasmic proteins, which detect chemoeffector signals and sense this intracellular signal likewise to their *C*-terminal cytoplasmic signalling domain. The MCP-monomers have an average molecular mass of 60 kDa. They arrange to very stable homodimers, which usually form groups of three [29]. A coupling scaffold protein called CheW interacts with the MCPs cytoplasmic signalling domains. When CheW attaches the histidine kinase, CheA the final ternary signalling complex is compiled [25, 31–34]. Thus CheA autophosphorylation, which primarily occurs at the P1 phosphorylation domain, is inhibited. Hence, the phosphorylation of the CheY RR-domain is reduced.

Most members of the *Epsilonproteobacteria* like *Campylobacter* sp. show specific modifications of this general scheme [35]. The preferentially and parallel to CheW involved coupling scaffold protein of *C. jejuni* is CheV. CheV is a CheW-like linking-protein with an additional RR-domain that may play a role in mediating adaptation to attractants [36, 37]. A second modification is an additional RR-domain (homologue to CheY) at the *C*-terminus of the CheA-protein, which is termed CheAY [30, 38]. The characteristic module of these RR-domains is the CheY-like phosphoacceptor domain commonly designated as receiver (REC) domain [39]. Typically, CheA proteins lacking a (secondary) P2 phosphorylation domain (besides the P1 phosphorylation domain) are CheAY proteins [40]. It is not finally clear, which role this additional RR-domain plays in the function of CheAY. CheAY is able to phosphorylate its RR-domain but CheY seems to be the preferred substrate [41]. Otherwise, CheY can phos-

Fig. 1. Chemotaxis signal transduction cascades for the three Tlp-chemoreceptor groups: The signalling domains forming trimers of dimers are depicted in steel blue (group A), Carnelian red (group B) and light green (group C); CheW and the CheW-homologue domain of CheV are depicted in light blue; CheV owns an additional response regulator (RR) domain, which is also part of CheAY and CheY. In contrast to *Helicobacter pylori*, CheA is composed of not only a His-autokinase domain but also a P1 phosphorylation domain. It has an additional RR-domain and that is why it is named CheAY in *C. jejuni*. For further details, see text.

phorylate CheAY at its RR-domain [41]. Thus, it was assumed that CheAY might remove phosphoryl groups from CheY and functions in that way as a so-called phosphate sink [35].

However, increasing ligand occupancy results in higher amounts of non-phosphorylated CheY. Non-phosphorylated CheY interacts weakly with the FliM switch proteins of the flagellar motor, which are the final effectors of the sensory transduction chain [42–45]. Because of the only weak interaction between unphosphorylated CheY and FliM, CheY is not able to break up the FliN–FliN interaction within the FliN-dimer via the N-terminal hydrophobic patch [46]. Thus, a CheY–FliN interaction does not take place and a counterclockwise rotation of the flagella is induced leading to a fast straightforward movement of the bacterial cell, a so-called "run". While moving along a gradient of a chemoattractant, the intracellular concentration of phosphorylated CheY inside the bacterial cytoplasm decreases. Therefore, the rate of site directed "runs" along the gradient increases [47].

In the case of decreasing ligand occupancy of the TLP-receptors CheAY autophosphorylation is less inhibited, resulting in a higher amount of CheAY-P. CheAY-P inhibits the dephosphorylation of CheY, which results in higher quantities of phosphorylated CheY and CheB. CheB is a receptor-demethylating enzyme, which is also activated by phosphorylation (see below). CheY-P interacts as well with the *N*-terminal parts of the FliM proteins of the motor switch complex but in a stronger way than non-phosphorylated CheY [43]. This stronger binding tethers CheY-P to FliM so that it is able to break up FliN-dimers to bind to FliN [46]. Thus, it triggers a clockwise flagellar rotation resulting in bacterial "tumbling" [44, 46].

The cytoplasmic phosphatase CheZ dephosphorylates phosphate-activated CheY and quenches thereby the signal for bacterial "tumbling" [48–50]. Additionally, it is assumed that the flagellar switch protein FliY, which has proven phosphatase activity in *Bacillus subtilis* also dephosphorylates CheY-P. In *C. jejuni* FliY the CheY binding domain is missing, but the active site consensus sequence of the CheC phosphatase family is present in FliY [35, 51].

Until the bacterium reaches the place of a balance between the varying chemoeffectors, this chemosensing will result in a stereoscopic "zigzag" path through the three-dimensional spatial gradients of different chemoattractors and chemorepellents [25, 52].

Different mechanisms of sensory adaptation

Sensory adaption in general is defined as the restoration of the prestimulus state in the continual presence of the chemoeffector. *Epsilonproteobacteria* like *Campylobacter* sp. seem to have two different mechanisms to achieve this. The first mechanism bases on the methylation of certain sites of the MCP receptor signalling domains (see *Fig. 1*). The second is based on the modification of the CheAY coupling by CheV.

Adaptation due to methylation/demethylation of methyl-accepting domains of the chemoreceptors signalling domains was recently shown to work at least for Tlp1 and Tlp4/DocC (but not Tlp9/CetA) [53]. The crucial methylation adaptation system proteins are the methyltransferase CheR and the methylesterase CheB. Further, central parts are the cytoplasmic domains of the MCP-receptors that have adjacent to the CheA(Y)- and CheV/CheW-binding sites, methyl-accepting domains with methylable glutamyl-side chains [54].

The methylation of the specific glutamyl-residues on the chemoreceptor signalling domains is a S-adenosyl-methionine-consuming process catalysed by CheR [47]. Methylation is triggered by attracting stimuli. It promotes the autophosphorylation of CheA(Y) favouring clockwise flagellar rotation (tumbling) [55, 56]. It was demonstrated that the signalling domains possess a specific site, which can be methylated by CheR, and a further distinct CheR-binding site that is formed by a pentapeptide, which is specific for high-abundance receptors. CheR is able to methylate the designated sites of the low-abundance receptors while binding to these specific binding sites onto the high-abundance receptors [57, 58].

The methylesterase CheB is CheR's functional antagonist. Firstly, it demethylates the Tlp-signalling domains during adaptation to repelling stimuli. CheB proteins usually consist of a methylesterase domain and a RR-domain that controls, depending on its phosphorylation state, the demethylation activity. In *C. jejuni*, however, this CheB RR-domain is absent [30, 53]. Secondly, CheB possesses an amidase activity. Thus, CheB can catalyse the conversion of glutamine residues into glutamate on the Tlp-receptor signalling domains. The liberation of glutamate residues inhibits the autophosphorylation of CheA(Y), which favours a counterclockwise flagellar rotation [59]. Thirdly, CheB is regulated by CheA(Y)-mediated phosphorylation. Phospho-CheB has a significantly increased methylesterase activity, while the unphosphorylated enzyme has less methylesterase activity [60, 61]. Fourthly, the CheA(Y) binding sites for CheY and CheB are identical. Thus, CheB and CheY compete with each other in this regard [62].

Finally, a high methylation rate of the signalling domains decreases the Tlp-receptors affinity to chemoactive ligands [63, 64].

The second sensory adaptation mechanism involves the CheW-like coupling protein CheV. CheV and CheW are expressed in parallel in *C. jejuni* and other *Epsilonproteobacteria*. The noticeable differences between both proteins are the higher affinity to the Tlp-receptors signalling domains (at least for these of Tlp1) [65] and the additional RR-domain of CheV [30].

The varying binding strength of CheV and CheW to different chemoreceptor signalling domains suggests that both proteins may out-compete each other depending on the specific receptor and the protein concentration [33, 66]. Thus, CheW preferentially binds Tlp4, whereas CheV demonstrates a high affinity to Tlp1, Tlp4, Tlp6 as well as

Tlp8 [65, 66]. It is possible that this competition is part of a kind of sensory adaptation. It could also be that alternative pathways are controlled by either protein [33].

For *B. subtilis*, an adaptation mechanism was demonstrated requiring CheV phosphorylation [37]. It is assumable that a similar mechanism could also exist in *C. jejuni*, but the phosphorylation status of *Campylobacter*-CheV is not finally clear [37]. CheV catalyses the dephosphorylation of CheA(Y), but the halftime of phosphorylated CheV in *Epsilonproteobacteria* seems to be remarkably short [41, 67].

It was also suggested that a delayed activation of CheZ that results in an enhanced CheY dephosphorylation, plays a role in sensory adaptation to chemoeffector stimulation [68].

All these parallel-acting adaptational mechanisms determine the extent of reaction to a fireworks of various attracting and repelling chemoeffectors and help to maintain the bacterial cells orientation in a steady-state of conflicting and cumulative signals [69].

Specific Campylobacter *chemoreceptors*

The genome of *C. jejuni* encodes for altogether ten chemoreceptors, designated as "Tlps" for transducer-like proteins. These chemoreceptors can be categorized into three different groups (A–C) with respect to structural homologies. With regard to their individual molecular composition, they are either integral membrane proteins or proteins that reside in the cytoplasm as shown in *Fig.* 2 [30]. Thereby, group A receptors possess the similar composition as the MCPs of *Escherichia coli* and as the family A transducers of *Halobacterium salinarium* [70]. In general, two amino-terminal located transmembrane domains and a periplasmic ligand-binding domain is followed by a highly conserved carboxy-terminal signalling domain [71]. In addition, a structurally conserved HAMP-domain

(a linker domain present in histidine-kinases, adenyl-cyclases, methyl-accepting-proteins and phosphatases) connects the transmembrane helices with the signalling domain. Thereby, the HAMP-domain, which is conserved in histidine kinases, adenylyl cyclases, MCPs and phosphatases, converts ligand-induced conformational changes into kinase-controlling signals [72–75]. Finally, within the signalling domain reside glutamyl-residues for the reversible methylation or demethylation of the chemoreceptor by CheR and CheB, respectively (see above) [76].

The group A receptors of *C. jejuni* are Tlp1, Tlp2, Tlp3, Tlp4 and Tlp10, whereby the signalling domains of the Tlps 2, 3 and 4 are identical [38]. The situation for chemoreceptor Tlp7 is inconsistent. Tlp7 belongs to the group A receptors if encoded by one single gene (*cjj81176-0975*) as in the reference strains 81-176 and 81116. But in the *C. jejuni* strains NCTC 11168 and B2, the sections for periplasmic binding and transmembrane localization are encoded on gene *cj0952c*, whereas the signalling domain is encoded by the adjacent gene *cj0951c* [30, 77]. Thus, the membrane-associated partial receptor encoded by *cj0952* (termed $Tlp7_m$) belongs like the *cjj81176-0975*-encoded receptor, consisting of the membrane-associated and the cytoplasmatic domains (termed $Tlp7_{mc}$), to group A, whereas the cytoplasmic part, encoded by *cj0951c* ($Tlp7_c$), should be considered as group C chemoreceptor. However, up to now it was not shown that $Tlp7_m$ and $Tlp7_c$ sense together. It is assumable that $Tlp7_c$ could also interact and sense with other ligands than $Tlp7_m$. Additionally, the two-gene receptor variant could be significantly more often detected in *C. jejuni* isolates of bovine origin as well as of the multilocus sequence types ST-21 and ST-61 [78]. The most extensive studies about the function of these chemoreceptors and how signals are transduced have been carried out in *E. coli* [79, 80]. The chemoreceptors are as mentioned above composed as transmembrane homodimers that form trimers of dimers and extended clusters. The development of nanodiscs, small particles of
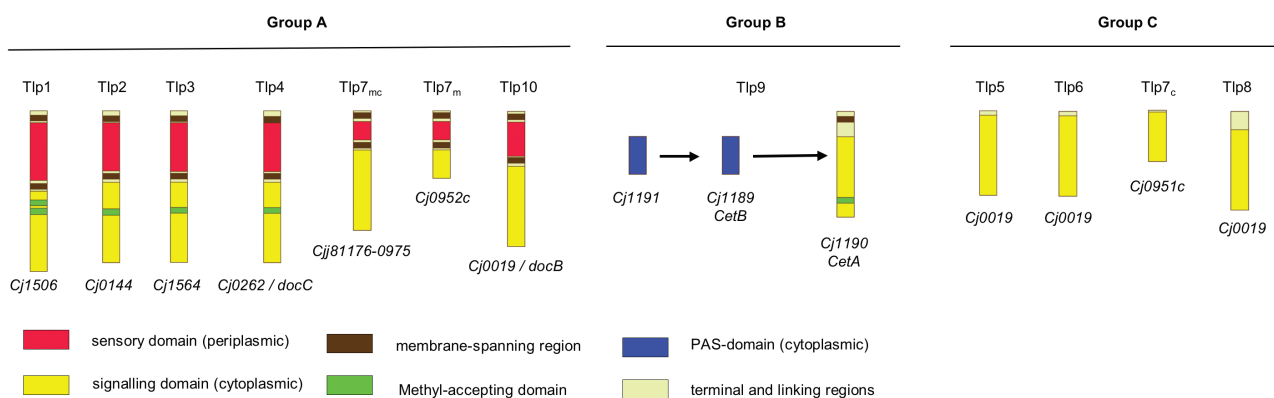
Fig. 2. Domain organization of the three different *C. jejuni* Tlp-chemoreceptor groups: group A receptors are anchored by membrane-spanning regions in the inner and obviously also in the outer membrane, have a periplasmic sensory and a cytoplasmic signalling domain; the only group B receptor Tlp9 (CetA), anchored in the inner membrane, interacts with CetB triggering pyruvate and fumarate signals [84]; group C chemoreceptors consist of a single cytoplasmic signalling domain; the cytoplasmic Tlp7c (encoded by *cj0951c*) receptor is assigned to group C, while it was shown that there is no read-through mechanism [77], whereas the membrane associated forms $Tlp7_m$ (encoded by *cj0952c*) and $Tlp7_{mc}$ (encoded by *cjj81176-0975* according to the nomenclature of the 81-176 sequence) are assigned to group A (depiction modified after Marchant et al. [30])

lipid bilayers containing a defined number of chemoreceptors, allowed to characterize the interaction of chemoreceptors with regard to ligand binding, transmembrane signalling and activation of the chemotaxis histidine kinase CheA(Y). Thereby, it has been demonstrated that dimers of receptors are capable of binding ligands and to perform transmembrane signalling. Maximal activation of the histidine kinase was in turn preferentially observed in nanodiscs that contained trimers of dimers [81]. Transmembrane chemoreceptors together with CheW/CheV and CheA(Y) aggregate in patches predominantly at the cell poles, whereby clustering depends not solely on the chemoreceptor, but also on the presence of the associated CheW/CheV and CheA(Y) proteins [82]. Moreover, it can be speculated that these patches are assembled by different trimers of different homodimers in a way that allows the regulation of CheA(Y) proteins via interactions with the chemoreceptors [83].

In addition to the MCPs of chemoreceptor group A, the genome of *C. jejuni* encodes for four chemoreceptors Tlp5, Tlp6, Tlp7c and Tlp8, which represent the receptor group C [30]. In contrast to group A receptors, these molecules contain neither a periplasmic binding domain nor transmembrane regions and, thus, are believed to reside in the cytosol. However, despite their homology to signalling domains of other chemoreceptors, nothing is known about their biological function, but the estimated cytosolic localisation may be a hint that they sense intracellular signals in *C. jejuni*.

Chemoreceptor group B is comprised only by Tlp9 (CetA), which together with CetB, possesses structural similarities to the energy taxis receptor Aer of *E. coli*. CetB has a PAS (Per, ARNT and Sim) domain while CetA includes two transmembrane helices, a HAMP-domain and a highly conserved domain (HCD). Both proteins together represent a bipartite energy taxis system; whereas dimers of CetB are only peripherally associated with the membrane, CetA is membrane-anchored by its transmembrane domains. As in the case of *E. coli* energy taxis receptor Aer, experimental data suggest that CetB is a signal sensing protein that conveys the signals to CetA. CetA in turn transmits the energy taxis signals to core signal transduction proteins of the chemotactic system CheW/CheV, CheA(Y) and, thus, to CheY [84, 85]. Based on genome sequence analysis data, there is a potential second cytoplasmic ligand-binding protein for Tlp9 encoded by *cj1191* [30, 38].

The chemotactical behaviour of *C. jejuni* was initially reported by Hugdahl and co-workers [26]. They described the chemotactical response of the bacterium with respect to different amino acids, carbohydrates, organic acids and components of mucin and bile. In more recent investigations the panel of substances, which affect the chemotactic competence could be enlarged and it was shown that chemoattractants most notably serve as respiratory electron donors and electron acceptors contributing to the maintenance of energy metabolism [27]. Our current knowledge about the respective receptors of *C. jejuni* that

interact with particular chemoattractants or chemorepellents is still poor. However, it could be shown that Tlp9 (CetA, CetB) triggers the response of *C. jejuni* towards pyruvate and fumarate, whereby the former serves as an electron donor and the latter is known to be a respiratory electron acceptor [84]. In addition, Tlp7 (*cj0952c/cj0951c, cjj81176-0975*) could be linked to the sensing of electron donor formate, and Tlp1 was identified to represent the chemoreceptor for aspartate [65, 77]. Despite the progress that has been achieved to match the chemoreceptors of *C. jejuni* to its particular ligand, the overall picture of receptors and their respective chemoeffectors is still incomplete and awaits further examinations.

The role of chemotaxis in host colonization and the pathogenesis of campylobacteriosis

The direct association of the chemotaxis system with the flagellar apparatus affects apparently the bacterial motility of *C. jejuni*. Motility in turn is an essential factor for the pathogenicity of campylobacteriosis, as non-motile strains possess a decreased capacity to infect host cells [7–9]. Consequently, the loss of chemoreceptor functions can be associated with reduced motility and, thus, reduced infectivity as it has been demonstrated by transposon insertion for the chemoreceptors Tlp3 (*cj1564*) as well as CetB (*cj1189*) and CetA (*cj1190*) representing the bipartite receptor Tlp9 [84, 86]. Vegge and co-workers could add Tlp4 (*cj0262c*) to the pool of chemoreceptors contributing to a complete *C. jejuni*-motility [27]. Recently, it was also shown that a transposon insertion in Tlp 7 (*cj0951c/ cj0952c* or *cjj81176-0975*) is accompanied by reduced motility of *C. jejuni* [77]. Finally, since all signals, which are sensed by the chemoreceptors, are transmitted to flagellum via the chemotaxis core proteins it is unsurprising that the loss of proteins such as CheA and CheY also results in a motility-deficient phenotype [86].

In addition to the motility affecting phenotype mentioned above, some chemoreceptors are also involved in the commensal colonization of the avian intestine by *C. jejuni*. Tlp1 (*cj1506c*), Tlp4 (*cj0262c*) and Tlp10 (*cj0019c*) could be demonstrated, in contrast to Tlp7 (*cj0951c/ cj0952c* or *cjj81176-0975*) and Tlp9 (*cj1189*), to be relevant for chicken intestinal colonization since a mutation within the corresponding genes significantly decreased the number of bacteria in the faeces. Interestingly, a transposon insertion in gene Tlp10 (*cj0019*) did not affect the *in vitro* motility of the bacterium indicating that the lack of a chemoreceptor is not mandatorily linked to the motility of *C. jejuni* [65, 87]. The *in vivo* examination of the intestinal pathology induced by *C. jejuni* was not feasible for decades since an appropriate vertebrate model was not available. Recently, Bereswill and co-workers established a gnotobiotic mouse model with a complete human intestinal microflora [88]. These intestinal "humanized" mice could be stably colonized by the pathogen, which was accompanied by a pro-inflammatory immune response and,

therefore, are suitable as an infection and inflammation model. Using this model it could be shown that a Tlp7-deficient *C. jejuni*-mutant strain colonizes the "humanized" mice with similar bacterial loads compared to the wild type strain but does not induce immunopathology regarding the number of apoptotic cells and the number of T-lymphocytes within the colon mucosa.

Acknowledgements

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