Protein–protein interactions in the chemotaxis signalling pathway of *Treponema denticola*

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Motile bacteria employ sophisticated chemotaxis signal transduction systems to transform environmental cues into corresponding behavioural responses. The proteins involved in this signalling pathway have been extensively studied on a molecular level in various model organisms, including enterobacteria and *Bacillus subtilis*, and specific protein–protein interactions have been identified. The chemotaxis operon of spirochaetes encodes a novel chemotaxis protein, CheX, in addition to homologues to the central components of established chemotaxis systems. Interestingly, the closest functionally characterized homologue of CheX is CheC of the complex *B. subtilis* chemotaxis pathway. In this study, the yeast two-hybrid system was applied to investigate protein–protein interactions within the chemotaxis signalling pathway of *Treponema denticola*, with special focus on CheX. CheX was found to interact with CheA and with itself. The other chemotaxis proteins exhibited interactions comparable to their homologues in known chemotaxis systems. Based on these findings, a model integrating CheX in the chemotaxis signal transduction pathway of *T. denticola* is proposed.

**INTRODUCTION**

Oral spirochaetes, including *Treponema denticola*, reside in deep periodontal pockets and are typically associated with periodontal diseases (Sela, 2001). These motile bacteria respond chemotactically to certain environmental stimuli, including serum, that are likely to be present in their natural habitat, the inflamed tissue of periodontal pockets (Li et al., 2001). These motile bacteria in deep periodontal pockets and are typically associated with periodontal diseases (Sela, 2001). Recently, chemotaxis and motility were connected to the ability of *T. denticola* to penetrate into tissue, underscoring the importance of these features in the pathogenic process (Lux et al., 2001). Similar associations between directed movement and virulence have been reported for a growing number of organisms (Josenhans & Suerbaum, 2002; Lux & Shi, 2004).

The genome of *T. denticola* contains all the components necessary for flagellated motility and a complete chemotaxis signal transduction chain (Seshadri et al., 2004). Gene inactivation mutagenesis has confirmed the role of some of these proteins in *T. denticola* motility and chemotaxis (Kataoka et al., 1997; Li et al., 1996, 1999; Limberger et al., 1999; Lux et al., 2002). In particular, homologues to methyl-accepting chemotaxis proteins (MCPs), DmcA and DmcB (Kataoka et al., 1997; Li et al., 1999), have been characterized. Recent whole-genome analysis has indicated the presence of an additional 18 putative MCP-encoding ORFs, as well as an operon containing cheR and cheB, which are required for adaptation (Seshadri et al., 2004). Most interestingly, the operon encoding homologues to the key components of the chemotaxis signalling pathway, CheA, CheW and CheY, contains an additional gene, cheX, which is localized between cheW and cheY. Since cheX is part of the cheAWXY transcriptional unit, a potential role in chemotaxis has been assumed (Greene & Stamm, 1999). Similar chemotaxis operons have been discovered in the genomes of *Treponema pallidum* and *Borrelia burgdorferi* (Fraser et al., 1997, 1998), the causative agents of syphilis and Lyme disease, respectively. Phenotypic analysis of *B. burgdorferi* and *T. denticola* mutant strains lacking cheX confirmed the involvement of this novel chemotaxis protein in the directed motility of spirochaetes, thus rendering CheX the most intriguing component of the spirochaete chemotaxis pathway (Charon & Goldstein, 2002).

Homologues to CheX can be found in a number of organisms (Lux et al., 2000), but a direct connection to the chemotaxis pathway is only apparent in spirochaetes and *Bacillus subtilis*. CheC, the CheX homologue in *B. subtilis*, is involved in adaptation and possibly motor control. Interaction of CheC with its antagonist CheD and MCPs (Rosario & Ordal, 1996), as well as CheA and the motor protein FlfiY, has been demonstrated (Kirby et al., 2001). In addition, a role for CheC in dephosphorylation of CheY~P has been suggested (Szurmant et al., 2004). During the review process for this manuscript, a study revealing the crystal
structure of the CheC and CheX homologues in *Thermotoga maritima* demonstrated CheY phosphatase activity for both proteins (Park et al., 2004), further substantiating their role in chemotactic signalling. In addition, this study by Park et al. confirmed that both proteins fold into similar structures, even though their primary sequences do not exhibit a striking homology to each other.

To further elucidate the role of the novel spirochaete chemotaxis protein CheX, the renamed yeast two-hybrid system (Fields & Song, 1989) was used to investigate potential interactions with known proteins in the chemotactic signal transduction pathway. In addition, established interactions between CheA, CheW and the MCPs (Borkovich et al., 1989; Gegner et al., 1992; Liu & Parkinson, 1989; Schuster et al., 1993), as well as interactions of CheY with CheA (Schuster et al., 1993; Swanson et al., 1995) or the motor switch proteins (McEvoy et al., 1999; Welch et al., 1993) were examined.

**METHODS**

**Strains, media and growth conditions.** *T. denticola* strain 35405 (Cheng et al., 1985) was grown anaerobically (85% N₂, 10% H₂ and 5% CO₂) at 37°C in TYGVs (Ohta et al., 1986). *Escherichia coli* strain DH5α and yeast strain p69-4A (James et al., 1996) were grown at 37°C and 30°C, respectively, and manipulated using standard protocols. Selective yeast dropout media lacking leucine and tryptophan (SD – Leu, – Trp), histidine (SD – His), or histidine and adenine (SD – His, – Ade) were purchased from Clontech. All other chemicals were obtained from Sigma. YPDA plates containing 40 μg X-Gal ml⁻¹ were prepared as described elsewhere (Clontech Laboratories, 1996).

DNA manipulation and plasmid construction. Bacterial genomic and plasmid DNA isolation, PCR amplification, cloning and transformation procedures were performed according to standard protocols (Sambrook et al., 2001). Primer pairs that were used to PCR-amplify full-length cheA, cheW, cheY, cheX, fliY, fliG and fliM genes of *T. denticola*, in addition to a dmcB fragment that encodes a truncated DmcB (‘DmcB’), corresponding to a stable cytoplasmic fragment of the *E. coli* chemoreceptor Tsr (Ams & Parkinson, 1994), are listed in Table 1. The indicated external restriction sites were used to clone each of the amplified fragments into both pGAD and pGBD vectors (James et al., 1996) to generate plasmids that encode fusion proteins of the individual chemotaxis components with the GAL4 transcription activation domain (GAL4-AD) and the GAL4 DNA-binding domain (GAL4-BD), respectively.

**Yeast two-hybrid analysis.** All possible combinations of the plasmids generated above, including combinations with the empty pGAD and pGBD, were transformed into yeast strain p69-4A using the lithium acetate method (Clontech Laboratories, 1996) to test the pairwise interaction of the proteins of interest as well as self-activation of the individual constructs. The presence of both plasmids was verified by PCR analysis of the yeast colonies using plasmid- and insert-specific primers in addition to phenotypic screening on ‘low-stringency’ SD – Leu, – Trp. Growth on these plates merely confirms the presence of the pGAD and pGBD plasmid derivatives without selective pressure on interaction of the fusion proteins. The colonies were then transferred for further evaluation onto the ‘medium-stringency’ and ‘high-stringency’ selective dropout plates described above. A qualitative screen for lacZ reporter gene expression was performed on YPDA plates containing X-Gal. The yeast strain p69-4A contains three reporter genes (HIS3, ADE2 and lacZ) under the control of three different promoter elements (GAL1, GAL2 and GAL7) that respond to the same inducer (Gal4) but share little sequence similarity, a feature that greatly reduces promoter-specific false-positive colonies (James et al., 1996). In addition, these promoters display various degrees of stringency of expression, thus allowing qualitative discrimination between more-transient and stronger interactions.

**β-Galactosidase assay.** Yeast transformants carrying the plasmid combination chosen for more detailed analysis were grown overnight at 30°C in liquid selective SD medium. The cultures were then transferred into fresh medium, grown to mid-exponential phase, pelleted and resuspended in 50 μl STE buffer (pH 7–6), 0·5 M NaCl, 0·1% (w/v) SDS, 0·01 M EDTA. Glass beads (0·5 mm, Fisher Scientific) and 20 μl TE buffer (pH 7–6) were added to each tube. This mixture was vortexed vigorously (4 min) at room temperature in a Turbo Mixer (Scientific Industries) and centrifuged at maximum speed for 5 min. The supernatant was transferred into 1·1 ml Z-buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, pH 7–0), supplemented with 50 mM β-mercaptoethanol. One hundred microlitres of this suspension was used to determine the protein concentration of the sample according to Bradford (1976). The remaining sample was used to measure β-galactosidase activity (Miller, 1972) with ONPG (4 mg ml⁻¹ in Z buffer) as a substrate. One unit (U) of enzyme

**Table 1. Primers used in this study**

Underlined are the restriction sites (GGATCC, BamHI; ATCGAT, Clal; GAATTC, EcoRI; CTGCAG, PstI; CCCGGG, Smal; GCATGC, SphI) used to generate gene fusions of GAL4-AD or GAL4-BD encoding genes and the genes of interest listed here.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tbody>
<tr>
<td>cheA</td>
<td>5′-CGCCCGGGATGAGTATTCATCTTCGATATC-3′</td>
<td>5′-CGATCGATCTACAAAAATTTGAAGGCTCGG-3′</td>
</tr>
<tr>
<td>cheW</td>
<td>5′-CGGATATCATGGAAGATAGAAGAAAGCTA-3′</td>
<td>5′-CGTGCACATTATCCGCCCTGACAAATTAC-3′</td>
</tr>
<tr>
<td>cheX</td>
<td>5′-CGGATATCATCGTGATAGATAATTATATC-3′</td>
<td>5′-CGTGCACATTATCTCGGAGAATTG-3′</td>
</tr>
<tr>
<td>cheY</td>
<td>5′-CGGATATCATGGAAGATAGAAGAAAGCTA-3′</td>
<td>5′-CGGATATCATGGAAGATAGAAGAAAGCTA-3′</td>
</tr>
<tr>
<td>‘dmcB’</td>
<td>5′-CGCTGCAGCTAGTACGACCTGCACTCTG-3′</td>
<td>5′-CGGATATCATGGAAGATAGAAGAAAGCTA-3′</td>
</tr>
<tr>
<td>fliY</td>
<td>5′-CGGATATCATGGAAGATAGAAGAAAGCTA-3′</td>
<td>5′-CGGATATCATGGAAGATAGAAGAAAGCTA-3′</td>
</tr>
<tr>
<td>fliG</td>
<td>5′-CGGATATCATGGAAGATAGAAGAAAGCTA-3′</td>
<td>5′-CGGATATCATGGAAGATAGAAGAAAGCTA-3′</td>
</tr>
<tr>
<td>fliM</td>
<td>5′-CGCCCGGGATGAGTATTCATCTTCGAGGAT-3′</td>
<td>5′-CGGATATCATGGAAGATAGAAGAAAGCTA-3′</td>
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</table>
activity corresponds to 1 nmol ONPG hydrolysed min⁻¹ (mg protein)⁻¹. The statistical relevance of the data obtained was determined using the t test.

RESULTS AND DISCUSSION

Qualitative screening for protein–protein interactions

All plasmids encoding Gal4-AD and Gal4-BD protein fusions with the chemotaxis and motor proteins CheA, CheW, CheY, DmcB, FliG, FliM and FliY were transformed into pJ69-4A and tested for self-activation before being subjected to further testing. Unfortunately, all three motor components tested here (FliG, FliM and FliY) turned out to be highly self-activating by being able to grow on medium- or even high-stringency selective dropout plates in the presence of Gal4-AD or Gal4-BD alone. The plasmid constructs encoding those proteins were therefore omitted from further analysis. The phenomenon of self-activation, a problem that appears to affect up to 20% of the proteins in a given genome (Nakayama et al., 2002; Uetz et al., 2000), has been observed previously for Gal4-BD fusions to the motor protein FliN of E. coli (Marykwas et al., 1996). Other motor protein fusions in that study, however, allowed assessment of their respective interactions.

The plasmids encoding protein fusions of CheA, CheW, CheX, CheY and ‘DmcB’ with Gal4-AD or Gal4-BD were then transformed in all possible combinations into the yeast strain pJ69-4A to examine the respective one-on-one interactions. The resulting transformants were tested on plates containing different dropout mixes with increasing degrees of selective stringency allowing qualitative evaluation of the protein–protein interactions tested (Table 2). In this initial screen the novel chemotaxis protein CheX exhibited a strong interaction with itself, as indicated by growth of the yeast strain expressing both the Gal4-AD and Gal4-BD CheX protein fusions on high-stringency dropout plates, suggesting homodimer or higher-order complex formation of the protein. This finding is in very good agreement with a recently published study that demonstrated dimerization of CheX from Th. maritima (Park et al., 2004). The same study suggested that dimerization of CheX is required to form the active centre for CheY dephosphorylation. Since the yeast two-hybrid approach chosen for this study does not enable detection of the complex interactions between a protein dimer and a phosphorylated protein, this could be one of the reasons why an interaction between CheX and CheY was not found.

Interestingly, the combination of both CheX fusion proteins with the respective CheA counterpart resulted in growth on medium-stringency dropout plates but yielded pinkish colonies under high-stringency conditions, implying a weaker interaction between these two proteins. This novel interaction between CheX and CheA, the central kinase of the chemotaxis pathway, is strongly supported by a similar finding in Bacillus subtilis: CheC, the CheX homologue present in B. subtilis, was shown to interact with CheA at a medium-stringency level (Kirby et al., 2001). In contrast to CheC, however, which has been suggested to interact with the MCPs in B. subtilis, CheX did not appear to bind to any of the other proteins (CheW, CheY or the cytoplasmic portion of DmcB) known to be required for chemotactic responses in bacteria (Bren & Eisenbach, 2000).

Furthermore, most of the established interactions between CheA, CheW, MCPs and CheY could be confirmed for the Th. denticola chemotaxis pathway (Table 3). In agreement with previous findings in other bacterial systems (Gegner & Dahlquist, 1991; Gegner et al., 1992; McNally & Matsumura, 1991) demonstrating complex formation of CheA and CheW, both construct combinations yielded growth on high-stringency plates. Binding between CheA and CheY, which is known to be transient, resulted in growth on

Table 2. Qualitative screen of one-on-one protein interactions

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>CheA-AD</th>
<th>CheW-AD</th>
<th>CheX-AD</th>
<th>CheY-AD</th>
<th>‘DmcB’-AD</th>
</tr>
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<tbody>
<tr>
<td>BD</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CheA-BD</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CheW-BD</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>CheX-BD</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CheY-BD</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>‘DmcB’-BD</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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Table 3. Quantification of protein–protein interaction by β-galactosidase activity

<table>
<thead>
<tr>
<th>Protein interaction</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD fusion</td>
<td>BD fusion</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CheW CheA</td>
<td>196 ± 70</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>CheA CheW</td>
<td>99 ± 37</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>CheX CheA</td>
<td>52 ± 18</td>
<td>&lt;0.043</td>
</tr>
<tr>
<td>CheA CheX</td>
<td>50 ± 7</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>CheY CheA</td>
<td>47 ± 15</td>
<td>&lt;0.042</td>
</tr>
<tr>
<td>CheA CheY</td>
<td>49 ± 15</td>
<td>&lt;0.026</td>
</tr>
<tr>
<td>CheX CheX</td>
<td>112 ± 40</td>
<td>&lt;0.029</td>
</tr>
</tbody>
</table>

One-on-one interactions between chemotaxis proteins fused to the GAL4 activating domain (AD fusion) and the GAL4 DNA-binding domain (BD fusion) were quantified by measuring β-galactosidase activity. Unfused (–), AD and BD domains served as a negative control. Mean values and SD of specific β-galactosidase activity units (U) are expressed as nanomoles of ONPG hydrolyzed per minute per milligram of protein. Only interactions with P values <0.05 were considered significant and are listed here.

Since the interactions identified on medium-stringency dropout plates resulted only in an approximately twofold increase above background levels of β-galactosidase activity, a statistical analysis (t test) was performed to evaluate the significance of the apparent protein–protein interactions. All interactions shown in Table 3 were considered significant as they displayed P values of <0.05. Interactions of CheX with Gal4-AD/BD fusion proteins of CheW or CheY were also tested using the quantitative β-galactosidase assay. Even though values above background level were obtained, statistical analysis revealed that they were not significant (data not shown). In conclusion, the interactions revealed in the initial qualitative screen using selective dropout medium plates with different stringencies were confirmed by the quantitative measurements.

CheX was found to bind CheA at a level comparable to the well-established CheA/CheY interactions (McNally & Matsumura, 1991; Schuster et al., 1993), implying a more transient nature of CheA and CheX recognition. CheY that alternates between CheA and the flagellar motor switch changes its affinity for CheA upon phosphorylation of CheY, resulting in its release from CheA (Schuster et al., 1993; Swanson et al., 1995). In a similar manner to CheC, its counterpart present in B. subtilis, CheX, does not contain any apparent conserved phosphorylation or other modification sites, leaving the nature of CheX interaction with CheA to be elucidated. Since the novel chemotaxis protein CheX demonstrated a strong interaction with itself, dimerization or even the formation of higher-order complexes may be an important part of CheX function. The CheX dimer/oligomer could comprise the ‘active’ state of the protein that is controlled by CheA or other proteins in the signal transduction chain. The recent study by Park et al. (2004) demonstrated that CheX of Th. maritima dimerizes to form a putative active centre similar to the one found in a single molecule of the larger CheC protein.

Quantitative assessment of protein–protein interactions

The yeast strain pJ69-4A contains a lacZ gene under the control of the Gal4-binding GAL7 promoter, allowing quantitative assessment of the strength of protein–protein interactions (James et al., 1996). All plasmid combinations subjected to the initial qualitative screen on selective dropout medium plates, especially the interesting new interactions found for CheX, were further analysed by determining in vitro β-galactosidase activity. The protein interactions detected in the initial qualitative screen (Table 2) were confirmed in the quantitative measurement (Table 3), and the protein fusions that did not result in growth on the selective medium dropout plates did not exhibit any significant increase in β-galactosidase activities (data not shown). Fusion protein combinations that enabled growth on high-stringency dropout plates displayed β-galactosidase activities greater than 100 U, whereas interactions revealed on medium-stringency dropout plates corresponded to values of approximately 50 U. Yeast strain pJ69-4A exhibited background levels of β-galactosidase activity of 9 ± 2 U. When transformed with the plasmid pair expressing only Gal4-AD and Gal4-BD, these background levels, however, increased to about 23 U, even though no significant growth or lacZ-mediated colour development was observed in the qualitative screen (Table 2).

Model for chemotactic signal transduction in T. denticola

The results presented here introduce a new player, CheX, in the chemotaxis signal transduction chain of the oral spirochaete T. denticola. A role for CheX in the directed movement of spirochaetes was already suggested by previous observations that inactivation of cheX resulted in an altered motility and chemotaxis phenotype in T. denticola as well as Bor. burgdorferi (Charon & Goldstein, 2002).
Most interestingly, CheC, the CheX homologue present in the complex \textit{B. subtilis} chemotaxis system, was shown to employ similar interactions to those found in this study for CheX. In particular, CheC interacted with CheA, the central kinase in the chemotactic signal transduction pathway, but not CheY, strongly supporting the novel interactions described here for CheX. CheC was also suggested to interact with the putative motor switch protein of \textit{B. subtilis}, FliY, a combination that could not be tested for CheX of \textit{T. denticola} due to self-activation of the FliY constructs. Since previous studies grouped CheC and CheX together with the motor proteins FliY and FliM, to form the CYX protein family (Kirby \textit{et al.}, 2001; Szurmant \textit{et al.}, 2004) and homologous domains are known to mediate protein–protein interactions (Bilwes \textit{et al.}, 1999; Mathews \textit{et al.}, 1998), interaction of CheX with FliY remains a possibility. Furthermore, CheC of \textit{B. subtilis} was recently found to enhance CheY dephosphorylation in a similar manner to FliY (Szurmant \textit{et al.}, 2004), even though direct binding to CheY was not found. Since it is a member of the CYX family, which includes CheC and FliY, the authors suggested a putative similar function for CheX. This assumed ability of CheX to dephosphorylate CheY has recently been confirmed for CheX of \textit{Th. maritima}.

Since \textit{T. denticola} does not contain a homologue to CheD, the antagonist of CheC function in receptor modulation and adaptation (Rosario \\& Ordal, 1996; Rosario \textit{et al.}, 1995), an involvement of CheX in similar functions appears unlikely. In addition, no interaction between CheX and a cytoplasmic fragment of the chemoreceptor DmcB was observed in this study.

\textit{T. denticola} appears to follow the well-characterized basic general pathway present in most motile bacteria. In addition to the established chemotaxis proteins MCPs, CheA, CheW and CheY, whose interactions have been studied extensively on a molecular basis, the chemotaxis system of spirochaetes contains an extra player, CheX. Here, based on the interactions revealed in this study, we propose a model for the chemotaxis signal transduction pathway of \textit{T. denticola} (Fig. 1) integrating CheX. These results are supported by similar findings for the CheX homologue CheC, a component of the far more complex chemotaxis system of \textit{B. subtilis}, in addition to those of a novel study revealing structural and biochemical features of CheX and CheC of \textit{Th. maritima}.

The incoming signal is likely to be perceived by a ternary signalling complex composed of CheA, CheW and MCPs that has been studied extensively, especially in enterobacteria. CheA modulates CheY phosphorylation levels according to the incoming environmental signal. Depending on its phosphorylation status, CheY would then bind to the motor to change the direction of flagellar rotation. In \textit{E. coli}, CheY ~ P binding to the motor triggers clockwise rotation, which results in tumbling of the bacterium (Welch \textit{et al.}, 1993), whereas in \textit{B. subtilis}, an opposite response has been observed (Bischoff \textit{et al.}, 1993). For spirochaetes, it is not clear which form of motor response is triggered by CheY ~ P binding to the motor switch. In functional analogy to CheZ, the enzyme that enhances CheY dephosphorylation in \(\beta\)- and \(\gamma\)-Proteobacteria (Szurmant \\& Ordal, 2004), CheY phosphatase activity has been demonstrated for CheX and CheC. Interestingly, both CheX and CheC appear to communicate with CheA, a feature that has also been described for CheZ (Wang \\& Matsumura, 1996). In the case of CheX, CheA could be involved in the regulation of CheX dimerization that appears to be necessary to form the active centre, thereby enabling CheY dephosphorylation (Park \textit{et al.}, 2004).

Furthermore, in analogy to the observations reported for \textit{B. subtilis} CheC, CheX could receive a signal from CheA and potentially transmit this signal to the motor switch protein FliY, which is encoded by the genomes of most spirochaetes that have been sequenced so far. The possibility that CheX acts as an additional messenger between the signalling complex and the flagella motor is especially intriguing, since spirochaetes require a mechanism allowing coordination of flagellar rotation at both cell poles in response to chemotactic stimuli. This ‘coordination problem’ is distinctive for

\textbf{Fig. 1.} Model for the \textit{T. denticola} chemotaxis signalling pathway. The proteins involved in the chemotactic signal transduction pathway of \textit{T. denticola}, CheA, CheW, a chemoreceptor (MCP), CheY and CheX, as well as the motor proteins FliM and FliY, are shown. Solid lines indicate established reactions and interactions, whereas dashed lines represent novel interactions found in this study that are confirmed by findings in \textit{B. subtilis} and \textit{Th. maritima}. For details see text.
spirochaetes. Hence a unique chemotaxis protein such as CheX could play a role in this process, as suggested earlier by Charon & Goldstein (2002).

We are currently constructing a comprehensive yeast two-hybrid library of *T. denticola* to identify other potentially CheX interacting proteins. This approach, in addition to a detailed phenotypic analysis of mutant strains lacking CheX and/or CheY, will provide novel insights into the function of CheX in *T. denticola* chemotaxis and motility.

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