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## Demonstration of interactions among *Myxococcus xanthus* Dif chemotaxis-like proteins by the yeast two-hybrid system

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**Abstract** The *Myxococcus xanthus dif* locus encodes several bacterial chemotaxis homologues that are crucial for fibril exopolysaccharide (EPS) production, social gliding motility, and fruiting body development. In primary sequence, DifA is homologous to methyl-accepting chemotaxis protein, DifC to CheW, DifD to CheY, DifE to CheA, and DifG to CheC. In this study, the interactions among the Dif chemotaxis-like proteins were investigated using the yeast two-hybrid (Y2H) system. DifC was found to interact with both DifA and DifE. Using a modified Y2H or a “three-hybrid” system, it was demonstrated that DifC is capable of mediating the formation of DifA, DifC, and DifE ternary protein complexes. The conserved domains of DifE, based on sequence analysis, likely reflect functional conservations of CheA-type kinases, because its P2 domain interacts with DifD, P5 with DifC, and the P3 domain appears to dimerize. Similarly, C-terminal regions of DifA appear to dimerize as well. In addition, DifG was found to interact with DifD, which is consistent with the hypothesis that DifG is a phosphatase of DifD-phosphate. These findings support the models in which Dif proteins constitute a unique chemotaxis-like signal transduction pathway with central functions in regulating EPS production in *M. xanthus*.

**Keywords** *Myxococcus xanthus* · Fibril exopolysaccharide · Dif · Chemotaxis · Yeast two-hybrid · Protein interaction

### Introduction

*Myxococcus xanthus* is a rod-shaped, gram-negative soil bacterium with a complex life cycle (Dworkin 2000; Shimkets 2000). Under growth permissive conditions, *M. xanthus* grows vegetatively like other bacteria. Under nutrient limitation, *M. xanthus* can initiate a well-tuned developmental process and form multicellular structures known as fruiting bodies. During the developmental cycle, hundreds of thousands of cells aggregate on solid surfaces to form the mound-like fruiting bodies. Within the fruiting body, rod-shaped vegetative cells eventually differentiate into spherical myxospores, which are metabolically dormant and environmentally resistant to stress such as heat and desiccation (Dworkin 2000; Shimkets 2000).

The aggregation of *M. xanthus* cells during fruiting body formation is achieved through their surface gliding motility, which is governed by two genetically defined systems (Spormann 1999). The adventurous motility enables the movement of well-isolated cells whereas the social (S) motility refers to the movement of multicellular groups (Hodgkin and Kaiser 1979a, 1979b). S motility is more crucial to development, because all known S-motility mutants are defective in fruiting body formation to various extents (Hodgkin and Kaiser 1979a; MacNeil et al. 1994). *M. xanthus* S motility requires at least two types of cell surface components, the polarly localized type IV pili (Kaiser 1979; Wu and Kaiser 1995) and the fibril exopolysaccharide (EPS) (Shimkets 1986; Arnold and Shimkets 1988; Weimer et al. 1998; Yang et al. 2000; Bellenger et al. 2002). It has been proposed that *M. xanthus* S motility may be powered by the retraction of type IV pili as is the twitching motility of *Pseudomonas* and *Neisseria* (Kaiser 2000; Merz et al. 2000; Sun et al. 2000; Skerker and Berg 2001). Recent studies suggested that fibril EPS might interact with type IV pili and mediate pilus retraction in *M. xanthus* (Li et al. 2003).

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Fibril EPS production in *M. xanthus* is regulated by a few genetic loci including the *dif* defective in fruiting/fibrils locus (Dana and Shimkets 1993; Weimer et al. 1998, 1998; Yang et al. 2000; Bellenger et al. 2002; Black and Yang 2004; Lu et al. 2005). Sequence analysis showed that the *dif* locus encodes several homologues of bacterial chemotaxis proteins (Yang et al. 1998; Black and Yang 2004). DifA is homologous to methyl-accepting chemotaxis protein (MCP), DifC to CheW, DifD to CheY, and DifE to CheA. DifG shares sequence similarity with the *Bacillus subtilis* chemotaxis protein CheC (Rosario et al. 1995; Kirby et al. 2001; Black and Yang 2004; Szurmant et al. 2004). It has been shown previously that *difA*, *difC*, and *difE* mutants produce no detectable levels of fibril EPS, whereas *difD* and *difG* mutants overproduce EPS (Yang et al. 1998; Yang et al. 2000; Bellenger et al. 2002; Black and Yang 2004). Based on the homology of deduced *dif* gene products to chemotaxis proteins and the phenotypes of *dif* mutants, it was proposed that Dif proteins define a signaling pathway that regulates EPS production in *M. xanthus* (Yang et al. 1998, 2000; Bellenger et al. 2002; Black and Yang 2004), and they may interact with one another similarly as bacterial chemotaxis proteins (Black and Yang 2004).

Here, we report the examination of physical interactions among Dif proteins using the yeast two-hybrid (Y2H) system. Both full-length Dif proteins and different domains or segments of DifE and DifA were used in our studies. The results suggested a pattern of interactions among Dif proteins similar to those in bacterial chemotaxis pathways. DifA interacted with DifC, and DifE with both DifC and DifD. Using a “three-hybrid” system, we also showed that DifC could mediate the interactions between DifA and DifE to likely form ternary protein complexes. The results in this report are consistent with a model in which Dif proteins interact with one another to form a chemotaxis-like pathway to regulate fibril EPS production in *M. xanthus*.

## Materials and methods

### Strains, media, and growth conditions

*Escherichia coli* stain XL1-Blue (Bullock 1987) and standard molecular cloning procedures (Sambrook and Russell 2001) were used for routine plasmid constructions. Luria-Bertani (LB) (Sambrook and Russell 2001) liquid medium and LB plates (1.5% agar) were used for the growth and maintenance of *E. coli*. Ampicillin was supplemented at 100 µg/ml to LB media for the selection of plasmids when applicable. The *Saccharomyces cerevisiae* strain used in Y2H studies was PJ69-4A (*MATa trp1-901, leu2-3, 112 ura3-52 his3-200 gal4Δ-gal80ΔLYS2:: GAL1-HIS3 GAL2-ADE2 met2:: GAL7-lacZ*), which has three reporter genes for a positive Y2H interaction: *HIS3* driven by *GAL1* promoter, *ADE2* by the *GAL2* promoter, and *lacZ* by the *GAL7* promoter (James et al. 1996). Growth media for *S. cerevisiae* were

YEPD (1% yeast extract, 2% peptone, 2% dextrose, pH 6.0) and synthetic complete (SC) liquid medium and plates (1.5% agar) (Rose et al. 1990). Certain components were omitted or dropped out from SC media when indicated. All the SC histidine (SC-His) dropout plates used in this study were supplemented with 2 mM 3-amino-1,2,4-triazole (3-AT), a competitive inhibitor of *S. cerevisiae* His3p enzymatic activity (Struhl and Davis 1977). *E. coli* was grown at 37°C and *S. cerevisiae* at 30°C.

### Sequence alignment and domain analysis

Sequence alignment was performed using Clustal X and Clustal W (Thompson et al. 1994; Jeanmougin et al. 1998) from the BCM Search Launcher (Smith et al. 1996). Domain analysis was performed using the Conserved Domain Architecture Retrieval Tool (Geer et al. 2002) and searches of the CDD database (Marchler-Bauer et al. 2003) using the basic local alignment search tool (Altschul et al. 1990). Because *Thermotoga maritima* CheA was used to determine the crystal structure for the bulk of a CheA kinase (Bilwes et al. 1999), it was used as the primary template for the analysis of DifE domain structure.

### Construction of plasmids

Plasmids used in this study are listed in Table 1. The pGAD-C1, 2, 3 and the pGBD-C1, 2, 3 series of plasmids used as Y2H vectors contain the GAL4 transcription activation domain (GAL4AD) and GAL4 DNA-binding domain (GAL4BD), respectively (James et al. 1996). In all of the Y2H constructs, GAL4AD and GAL4BD were fused at their C-terminus to full-length or truncated Dif proteins. Restriction enzymes, T4 DNA polymerase, and T4 DNA ligase used for plasmid construction were from New England Biolabs (Beverly, Mass., USA). For the construction involving polymerase chain reaction (PCR), *Pfu* Ultra DNA polymerase (Stratagene, La Jolla, Calif., USA) with 3′–5′ nuclease activity for proofreading was used. The primers used for PCR amplification for plasmid constructions are listed in Table 2. Plasmids with various DNA fragments from the *dif* locus were used as the templates for PCR reactions. The sequence of PCR fragments in all the plasmid constructs were verified by DNA sequencing.

There are five plasmids (pLZ211, pLZ214, pLZ215, pLZ218, and pLZ219) with different portions of the *difA* gene in Y2H vectors (Table 1, Fig. 1a). For the construction of pLZ211, a PCR fragment amplified using the indicated primers (Table 2) was digested with *ClaI* and ligated into pGAD-C1 digested with *SmaI* and *ClaI*. For the construction of pLZ214 and pLZ215, the relevant PCR fragment (Table 2) was digested with *ClaI* and ligated into pGBD-C3 and pGAD-C3 digested with *SmaI* and *ClaI*. pLZ218 and pLZ219 were constructed

**Table 1** Plasmids used in this study

Plasmids	Features/description <sup>aa</sup>	References
pGBD-C1, 2, 3	GAL4BD vectors; <i>TRP1</i>	(James et al. (1996)
pGAD-C1, 2, 3	GAL4AD vectors; <i>LEU2</i>	(James et al. (1996)
p426GPD	Yeast expression vector; <i>GPD</i> promoter; <i>URA3</i>	(Mumberg et al. (1995)
pDF100	GAL4AD-DifD	This study
pDF101	GAL4BD-DifD	This study
pLZ170	GAL4BD-DifG	This study
pLZ180	GAL4AD-DifG	This study
pLZ201	GAL4BD-DifE	This study
pLZ211	GAL4AD-DifA <sup>b</sup> <sub>54-413</sub>	This study
pLZ214	GAL4BD-DifA <sup>b</sup> <sub>137-360</sub>	This study
pLZ215	GAL4AD-DifA <sup>b</sup> <sub>137-360</sub>	This study
pLZ218	GAL4BD-DifA <sup>b</sup> <sub>256-413</sub>	This study
pLZ219	GAL4AD-DifA <sup>b</sup> <sub>256-413</sub>	This study
pLZ221	GAL4AD-DifC	This study
pLZ222	GAL4BD-DifC	This study
pLZ224	GAL4BD-DifE <sup>b</sup> <sub>321-470</sub>	This study
pLZ228	GAL4BD-DifE <sup>b</sup> <sub>472-540</sub>	This study
pLZ229	GAL4AD-DifE <sup>b</sup> <sub>472-540</sub>	This study
pLZ230	GAL4BD-DifE <sup>b</sup> <sub>722-855</sub>	This study
pLZ232	DifC in p426GPD	This study
pLZ401	GAL4AD-DifE	This study
pMY100	6-His-tagged DifD	This study

<sup>a</sup>All plasmids confer ampicillin resistance

<sup>b</sup>Numbers in *subscript* indicate the amino acid residues contained in the construct

by digesting the PCR fragment amplified with the indicated primers (Table 2) with *EcoRI* and *ClaI* and cloning it into similarly restricted pGBD-C3 and pGAD-C3, respectively.

Six plasmids with either full-length (pLZ201 and pLZ401) or part (pLZ224, pLZ228/229 and pLZ230) of *difE* in Y2H vectors were used in this study (Table 1; Fig. 1b). For pLZ201 and pLZ401, full-length *difE* was amplified by PCR (Table 2), digested with *ClaI* and *PstI*, and ligated into the same sites in pGBD-C3 and pGAD-C3, respectively. pLZ224 was constructed by digesting a PCR fragment (Table 2) with *BamHI* and *ClaI* and ligating it into the corresponding sites in pGBD-C1. To generate pLZ228 and pLZ229, the rele-

vant PCR fragment of *difE* (Table 2) was digested with *ClaI* and *Bg/III* and ligated into the same sites of pGBD-C3 and pGAD-C3. For the construction of pLZ230, a *difE* PCR fragment (Table 2) was digested with *EcoRI* and *PstI* and ligated into the corresponding sites in pGBD-C1.

Full-length constructs were made for DifC (pLZ221/222), DifD (pDF100/101), and DifG (pLZ170/180) in Y2H vectors (Table 1) for this study. To construct pLZ221 and pLZ222, *difC* was PCR-amplified (Table 2), digested with *ClaI* and *PstI*, and ligated into pGAD-C1 and pGBD-C1 digested with the same enzymes. pDF100 and pDF101 were constructed by cloning a *difD*-containing fragment from pMY100 into

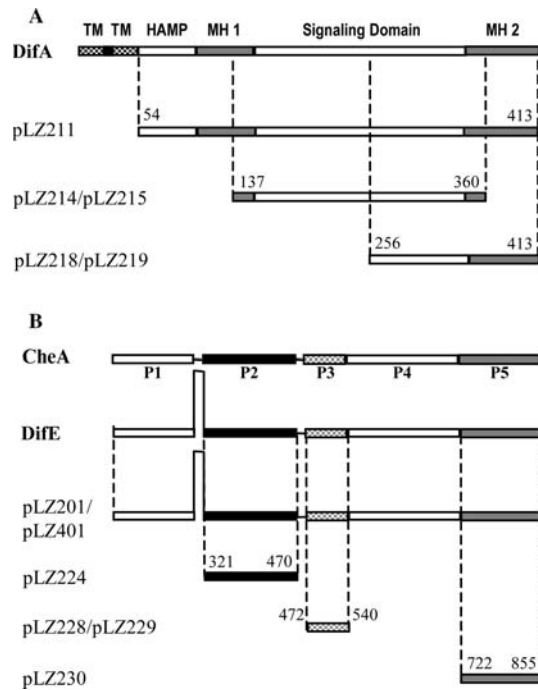
**Table 2** Primers used in this study

Plasmid	Primers <sup>a</sup>	Enzyme <sup>b</sup>
pLZ170/pLZ180	GTGT GGATCCTGGACGTGCC CCCGTCTAG GTCGACACGGG	<i>BamHI</i> <i>SalI</i>
pLZ201/pLZ401	GC ATCGATGACGATGGACATGTCCCCG CT CTGCAGGGGCTGGCTCACGCGGAC	<i>ClaI</i> <i>PstI</i>
pLZ211	GTCACCCGCGTGAAGGTGCTCAGC CAGCTTCGC ATCGATGTTGTCCGA	N/A <i>ClaI</i>
pLZ214/pLZ215	CGGCTCGTCGATGGAGAAGATT CGCCACCAG ATCGATTTCTCGAT	N/A <i>ClaI</i>
pLZ218/pLZ219	CGCGCGGT GAATTCGGCCGCGGC CAGCTTCGC ATCGATGTTGTCCGA	<i>EcoRI</i> <i>ClaI</i>
pLZ221/pLZ222	AGTGGATAG ATCGATACTCCTGTG AATCAC CTGCAGGAGCGCGGACAC	<i>ClaI</i> <i>PstI</i>
pLZ224	ACCGACGCC GGATCCGCGGCTCCA GACGCCGTG ATCGATGACGCTCGG	<i>BamHI</i> <i>ClaI</i>
pLZ228/pLZ229	CCGAGCGTC ATCGATCACGGCGTC TGATGAGGG AGATCTGCGTCATGC	<i>ClaI</i> <i>Bg/III</i>
pLZ230	TCACCCTGC GAATTCCTACTGACGG CT CTGCAGGGGCTGGCTCACGCGGAC	<i>EcoRI</i> <i>PstI</i>
pLZ232 <sup>c</sup>	TAG AAGCTTACTCCTATGCGGCAC CCAGGG GTCGACTCACTTGAATG	<i>HindIII</i> <i>SalI</i>
pMY100	GG AGTACTTCACATGGCTAAGCGGGT GG AAGCTTGGACAGCCCCGCGCCAGG	<i>ScaI</i> <i>HindIII</i>

<sup>a</sup>Sequences of primers are written from 5' to 3'. Restriction sites in primers are *underlined*. Start and stop codons for relevant genes are in *boldface* when applicable

<sup>b</sup>Restriction enzymes for which recognition sites were introduced in primers. N/A Not applicable

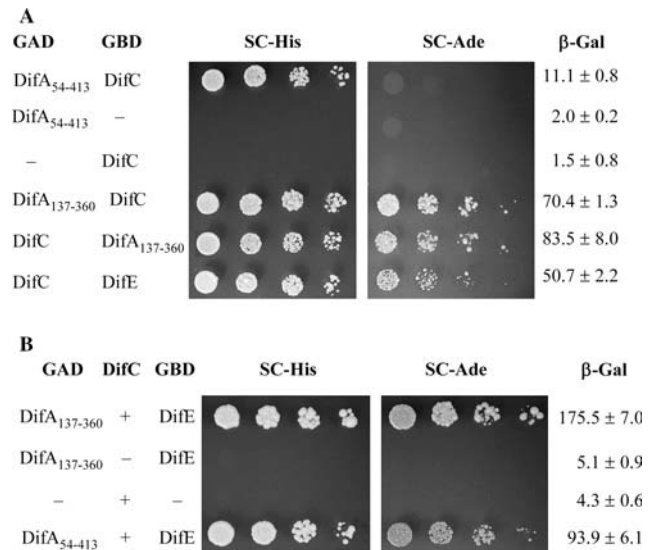
<sup>c</sup>The *difC* start codon GTG was changed to ATG for expression in *Saccharomyces cerevisiae*



**Fig. 1** Domains of DifA and DifE proteins and their yeast two-hybrid (Y2H) constructs. Predicted domains of DifA and DifE are indicated by different *patterned boxes*. DifA and DifE are predicted to contain 413 and 855 amino acids, respectively. The lengths of the domains are drawn approximately to scale relative to the sizes of the open reading frames. Indicated on the *left* are the Y2H plasmids containing various domains of DifA and DifE. The initial and final Dif residues in each plasmid are indicated *above* each fragment. **a** DifA domains and DifA-containing Y2H constructs. *TM* Transmembrane domain, *HAMP* linker region, *MH* methylation helix. **b** DifE domains and DifE-containing Y2H constructs. DifE was aligned with *Thermotoga maritima* CheA with the five domains (P1 through P5) indicated. *Lines* between the P1 and the P2 domains indicate an insertion from residues 155-340 in DifE that shares no homology with any known proteins

pGAD-C1 and pGBD-C1. Full-length *difD* was PCR-amplified (Table 2), digested with *ScaI* and *HindIII*, and cloned into the *SmaI* and *HindIII* sites of pQE30 vector (Qiagen, Valencia, Calif., USA) to generate pMY100 first. pMY100 was then digested with *HindIII*, treated with T4 DNA polymerase, and followed by *BamHI* digestion. The resulting *difD*-containing fragment was cloned into Y2H vectors digested with *PstI*, treated with T4 DNA polymerase, and further digested with *BamHI* to generate pDF101 and pDF100. For the construction of pLZ170 and pLZ180, a PCR fragment (Table 2) with full-length *difG* was digested with *BamHI* and *SalI* and ligated into pGBD-C3 and pGAD-C3 digested with the same enzymes.

pLZ232 was constructed to constitutively express DifC in *S. cerevisiae*. Full-length *difC* was first amplified using primers that converted the original GTG start codon of *difC* to ATG (Table 2). This PCR fragment was digested with *HindIII* and *SalI* and ligated into the corresponding sites in p426GPD, which provides the strong and constitutively active GPD promoter (Mumberg et al. 1995).



**Fig. 2** Examination of interactions among DifA, DifC, and DifE. PJ69-4A transformants were analyzed for  $\beta$ -galactosidase ( $\beta$ -Gal) activity and by spot assays (see “Materials and methods”) on histidine dropout synthetic complete (SC-His) plates with 3-amino-1,2,4-triazole (3-AT) and SC plates lacking adenine (SC-Ade) plates. For the spot assays, the total numbers of yeast cell initially placed in the four columns from left to right are  $2 \times 10^4$ ,  $4 \times 10^3$ ,  $8 \times 10^2$ , and  $1.6 \times 10^2$ , respectively. **a** Pairwise interactions between DifA and DifC and between DifC and DifE. The *columns labeled GAD* and *GBD* show fusions of either GAL4AD or GAL4BD with indicated Dif proteins or their fragments. The *minus signs* indicate vector (pGAD-C1 or pGBD-C1) with no insert. As shown in the *last two rows*, transformants with pairs of DifA<sub>137-360</sub> and DifC in reciprocal vector sets showed similar results. For the other pairs, similar results were also observed when the constructs were made in the reciprocal set of Y2H plasmids (data not shown). **b** Ternary complex formation by DifA, DifC, and DifE. Experimental conditions and labeling are as described for **a**, except that the histidine and adenine dropout plates used here lacked uracil to maintain the DifC expression plasmid (pLZ232) or the corresponding cloning vector (p426GPD). For the *column labeled DifC*, plus signs indicate the presence of pLZ232, and *minus signs* indicate the presence of p426GPD. Similar results were observed with DifA and DifE in the reciprocal set of Y2H vectors

Examination of protein-protein interactions with the Y2H system

For examination of interactions using the Y2H system, the yeast strain PJ69-4A was co-transformed with appropriate GAL4BD and GAL4AD fusion plasmids using the LiAc-PEG method (Gietz and Woods 2002) and plated onto SC plates lacking leucine and tryptophan. Purified transformants were examined for growth on SC plates lacking leucine, tryptophan, and histidine with 2 mM 3-AT and SC plates lacking leucine, tryptophan, and adenine (SC-Ade). Growth on plates was examined daily and documented after 4 days of incubation at 30°C. In the case of examining ternary complex formation by DifA, DifC, and DifE, yeast transformants with three plasmids were selected on SC plates without leucine, tryptophan, and uracil. Transformants were then examined for growth on SC-His and on SC-Ade without uracil. Growth on histidine and

adenine dropout plates was examined either by streak plates or by spotting plates with serial dilutions of cell suspension (Ursic et al. 2004). For the spot assays, cells in exponential growth were harvested and resuspended at  $4 \times 10^6$  cells per milliliter. A volume of 5  $\mu$ l of this cell suspension and its 5 $\times$  serial dilutions were spotted on plates with decreasing concentrations from left to right.  $\beta$ -Galactosidase activity of yeast transformants was examined using the method described by Kippert (1995) and expressed in Miller units (Miller 1992).

## Results

### MCP homologue DifA interacts with CheW homologue DifC

In classical enteric chemotaxis, MCPs directly interact with CheW to transmit signals to the CheA kinase (Falke et al. 1997; Armitage 1999; Bren and Eisenbach 2000; Bourret and Stock 2002). The *M. xanthus* MCP homologue DifA is predicted to be a multi-domain protein as shown in Fig. 1a. Starting from the N-terminus, there are two predicted transmembrane domains encompassing a short periplasmic stretch, followed by a linker or HAMP domain. The highly conserved cytoplasmic MCP signaling domain is flanked by two predicted methylation helices (MHs). To examine the interactions between DifA and DifC, we initially constructed pLZ211 (Fig. 1a), which contains amino acids 54–413 of DifA (DifA<sub>54-413</sub>) fused to the C-terminus of GAL4AD. This construct essentially included the entire predicted cytoplasmic domains of DifA: the HAMP domain, the MHs, and the signaling domain. Plasmids with full-length DifC fused to GAL4 (pLZ221 and 222) were also constructed as described in “Materials and methods.” These DifA and DifC constructs were transformed into *S. cerevisiae* Y2H reporter strain PJ69-4A to examine interactions by growth on SC-His and SC-Ade plates and by analysis of  $\beta$ -galactosidase activity. As indicated in “Materials and methods,” all the histidine dropout plates used in this study were supplemented with 2 mM 3-AT, a His3p inhibitor (Struhl and Davis 1977), because it was discovered early on during this study that growth on histidine dropout plates without 3-AT is not a reliable indicator of positive interactions with PJ69-4A. We observed that most of our negative controls grew on SC-His dropout plates, but supplementation of 2 mM 3-AT was sufficient to eliminate these false positives. The leakiness of the *HIS3* reporter gene in the Y2H system was also observed previously (Martinez-Argudo et al. 2001).

Transformants containing the GAL4AD-DifA<sub>54-413</sub> and the GAL4BD-DifC constructs showed growth on SC-His but not on SC-Ade plates (Fig. 2a). This perhaps is not surprising, because it is known that growth on SC-Ade is a more stringent indicator of interactions in the Y2H system than growth on SC-His (James et al. 1996). Consistent with the growth phenotype, the third reporter

gene *lacZ* is expressed at relatively low levels in these transformants as indicated by  $\beta$ -galactosidase activity (Fig. 2a). Yeast transformants with either of these two constructs and an empty Y2H vector showed no growth on SC-His or SC-Ade plates (Fig. 2a), nor did those with any of the other GAL4-Dif constructs and a vector (data not shown). These observations suggest that DifA can interact with DifC although the interaction is either weak or unstable in the Y2H system with these constructs.

It has been shown in other bacteria that it is the signaling domain of MCP that is responsible for direct interactions with CheW (Liu and Parkinson 1991; Ames et al. 1996; Surette and Stock 1996; Falke and Kim 2000; Shimizu et al. 2000). To examine whether DifA interacts with DifC in a similar manner, a plasmid (pLZ215) with the signaling domain of DifA (DifA<sub>137-360</sub>) fused to GAL4AD was constructed (Fig. 1a). Yeast transformants containing pLZ215 and the GAL4BD-DifC construct grew on both the SC-His and the SC-Ade plates (Fig. 2a). Similar results were observed with the reciprocal constructs GAL4BD-DifA<sub>137-360</sub> (pLZ214) and GAL4AD-DifC (pLZ221) (Fig. 2a). Consistent with their growth phenotype on both SC-His and SC-Ade plates, these transformants also displayed about six- to sevenfold higher  $\beta$ -galactosidase activity than that with the DifA<sub>54-413</sub> (Fig. 2a). None of these GAL4 constructs alone and an empty Y2H vector enabled yeast transformants to grow on either SC-His or SC-Ade plates. These results suggest that DifA can interact with DifC through its conserved signaling domain and the MHs may somehow inhibit or destabilize such interactions at least in the Y2H system.

### DifC interacts with the CheA homologue DifE

To examine whether the CheA homologue DifE interacts with DifC, DifE was fused to the C-termini of GAL4BD and GAL4AD, respectively (see “Materials and methods”). The GAL4AD-DifC (pLZ221) and GAL4BD-DifE (pLZ201) fusion plasmids (Table 1: Fig. 1b) were co-transformed into the yeast strain PJ69-4A. Yeast transformants containing the DifE and the DifC constructs were able to grow on both the SC-His and the SC-Ade plates (Fig. 2a). None of these constructs with an empty vector enabled growth on these plates. Analysis of *lacZ* expression by  $\beta$ -galactosidase activity confirmed the observation with the plate growth assays (Fig. 2a). These results suggest that DifE can directly interact with DifC.

### DifA, DifC, and DifE form a ternary complex

The interactions of DifC with DifA and DifE suggested that DifC may interact with both proteins simultaneously to form a ternary signaling complex analogous to their counterparts in bacterial chemotaxis signal transduction (Falke et al. 1997; Armitage 1999; Bren and

Eisenbach 2000; Bourret and Stock 2002). To examine whether DifC can mediate the formation of such complexes, pLZ232 was constructed (see “Materials and methods”), which has DifC under the control of the constitutive GPD promoter of *S. cerevisiae* (Mumberg et al. 1995). Negative controls with relevant vectors and Dif constructs showed no growth on SC-His and SC-Ade plates and displayed minimum levels of  $\beta$ -galactosidase activity (Fig. 2b). On the other hand, the transformants containing the DifA<sub>137-360</sub> and the DifE Y2H constructs along with the DifC expression plasmid grew on both histidine and adenine dropout plates. Additionally, the yeast transformant containing GAL4AD-DifA<sub>54-413</sub> and GAL4BD-DifC constructs did not grow on SC-Ade plates (Fig. 2a); yet, yeast transformants with the same DifA<sub>54-413</sub> construct, the DifC expression plasmid, and a GAL4BD-DifE construct grew on both the histidine and the adenine dropout plates (Fig. 2b). Transformants containing DifA, DifC, and DifE constructs all displayed significantly higher  $\beta$ -galactosidase activity (Fig. 2b) than the transformants only with pairwise interacting partners (Fig. 2a). These results strongly suggest that DifA, DifC, and DifE can form ternary complexes, and that the interactions among these proteins in a ternary complex are probably more stable and/or stronger than the pairwise interactions between DifA and DifC and between DifC and DifE.

#### Putative kinase DifE interacts with response regulator DifD

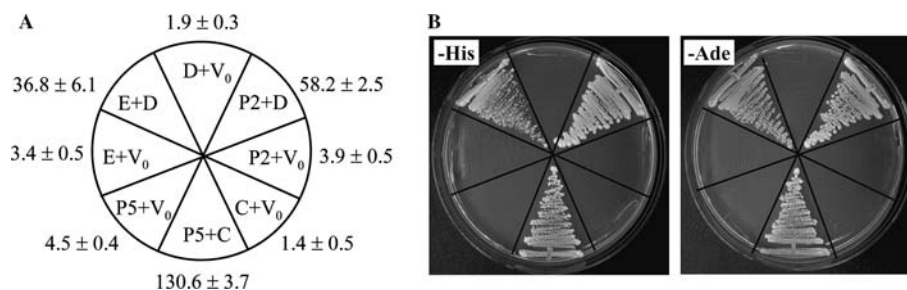
Besides its interactions with CheW, CheA is known to interact with the CheY response regulator (Falke et al. 1997; Armitage 1999; Bren and Eisenbach 2000; Bourret

and Stock 2002). It was proposed that DifE might interact similarly with the CheY homologue DifD (Yang et al. 1998, 2000; Black and Yang 2004). To examine whether DifE can interact with DifD, Y2H constructs with full-length DifD (pDF100 and pDF101) were generated as described in “Materials and methods” and co-transformed with DifE constructs into yeast PJ69-4A. The transformants grew on both the SC-His and the SC-Ade plates, and they expressed *lacZ*, as indicated by  $\beta$ -galactosidase activity (Fig. 3). Transformants with each of these constructs alone and an empty Y2H vector showed no growth on the histidine and adenine dropout plates and negligible levels of  $\beta$ -galactosidase activity. These results suggest that DifE directly interacts with DifD as previously proposed (Black and Yang 2004).

#### DifE interacts with both DifC and DifD through conserved domains

The domains of the CheA-type kinase and their functions have been well-defined (Bilwes et al. 1999). A typical bacterial CheA monomer has five domains, named P1 through P5, from the N-terminus to the C-terminus (Fig. 1b). P1 is the histidine phospho-transfer domain, which is phosphorylated on a conserved histidine residue by the P4 kinase catalytic domain. CheA kinases function as dimeric enzymes, and the dimerization is mediated by the P3 domain. P2 and P5 provide the interacting surfaces for CheY and CheW, respectively. Sequence alignment showed that DifE has similar domain organization as CheA (Fig. 1b; Yang et al. 1998). The only exception is that between the predicted P1 and P2 domains of DifE, there is an insertion of about 185 amino acids (Fig. 1b), which shares no significant homology with any CheA kinases or known proteins. To examine whether DifE interacts with DifD and DifC through the putative P2 and P5 domains respectively, Y2H plasmids with the P2 and the P5 domains of DifE were constructed (Fig. 1b; “Materials and methods”). Yeast transformants containing both the P2 and the DifD constructs grew on both SC-His and SC-Ade plates, as did the transformants containing both the P5 and the DifC constructs (Fig. 3b). The results of  $\beta$ -galactosidase analysis are consistent with the growth phenotypes of these yeast transformants (Fig. 3a). These results suggest that DifE interacts with DifD and DifC via its conserved P2 and P5 domains, respectively.

**Fig. 3** Interactions of conserved DifE domains. Yeast transformants containing Y2H constructs and/or vectors as indicated in **a** were examined for growth by streaking on selective plates as shown in **b**. *V*<sub>0</sub> Y2H vectors without inserts, *C* DifC-containing plasmid pLZ221, *D* DifD-containing pDF100, *E* DifE-containing pLZ201, *P2* DifE P2-containing pLZ224, *P5* DifE P5-containing pLZ230 (see Table 1 and Fig. 1 for more information). The growth of the transformants after 4 days of incubation at 30°C was documented and shown in **b**, with the 3-AT-supplemented SC-His plate on the *left* and the SC-Ade plate on the *right*.  $\beta$ -Galactosidase activity of these transformants was analyzed and indicated in **a** next to each sector with the corresponding transformants. Similar results were observed with Dif proteins fused to GAL4 in the reciprocal set of Y2H vectors



**Table 3** Examination of DifA and DifE dimerization. *SC-His* Synthetic complete-lacking histidine liquid medium plates, *SC-Ade* synthetic complete-lacking adenine liquid medium plates

GAD/GBD <sup>a</sup>	SC-His <sup>b</sup>	SC-Ade <sup>b</sup>	$\beta$ -Galactosidase <sup>c</sup>
DifE-P3/DifE-P3	+	+	210.1 $\pm$ 10.4
DifE-P3/pGBDC1	–	–	4.5 $\pm$ 0.6
pGADC1/DifE-P3	–	–	7.1 $\pm$ 0.9
DifA <sub>256-413</sub> /DifA <sub>256-413</sub>	+	–	27.9 $\pm$ 1.3
DifA <sub>256-413</sub> /pGBDC1	–	–	8.4 $\pm$ 2.1
pGADC1/DifA <sub>256-413</sub>	–	–	5.4 $\pm$ 0.5

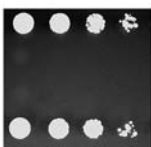
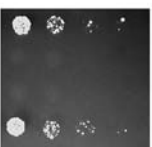
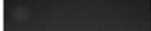

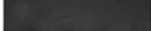



<sup>a</sup> *GAD* and *GBD* indicate fusions with GAL4AD and GAL4BD, respectively

<sup>b</sup> Growth on these plates is indicated by + and no growth by –

<sup>c</sup>  $\beta$ -Galactosidase activity is expressed as Miller units

### Both DifE and DifA may form homodimers

As discussed above, CheA kinases form homodimers through their P3 domains (Bilwes et al. 1999). To test whether the P3 domain of DifE had similar functions, it was fused to both GAL4AD and GAL4BD in Y2H vectors (Fig. 1b). Yeast transformants containing the P3 domain in both vectors grew on SC-His and SC-Ade plates and gave the highest  $\beta$ -galactosidase activity among all the transformants in this study (Table 3; Figs. 2, 3, 4). These results indicate that DifE may form stable homodimers through its P3 domain. MCPs are also known to form homodimers (Falke et al. 1997; Armitage 1999; Bren and Eisenbach 2000; Bourret and Stock 2002). To test whether DifA could form homodimers, various DifA fragments (Fig. 1) were examined for interactions with themselves in Y2H vectors. Among them, only the DifA<sub>256-413</sub> constructs enabled growth of yeast transformants on SC-His plates (Table 3; data not shown). None of the DifA constructs conferred growth to transformants on SC-Ade plates.  $\beta$ -Galactosidase activity ( $\sim$ 28 units) of the transformants with these DifA<sub>256-413</sub> constructs was also lower than the ones capable of growth on SC-Ade (Table 3; Figs. 2, 3, 4). These results suggest that DifA could potentially form homodimers as other MCPs although the interactions among the DifA fragments we examined appear weak or unstable in the Y2H system (Surette and Stock 1996).

GAD	GBD	SC-His	SC-Ade	$\beta$ -Gal
DifG	DifD			66.0 $\pm$ 2.3
DifG	–			2.0 $\pm$ 0.7
–	DifD			3.3 $\pm$ 0.4
DifD	DifG			60.9 $\pm$ 6.2

**Fig. 4** Interactions between DifD and DifG. Transformants of yeast PJ69-4A with the DifD and the DifG Y2H constructs were examined for growth by the spot assay as described for Fig. 2a. Growth on SC-His with 2 mM 3-AT and SC-Ade was documented after 4 days of incubation. Shown on the right is  $\beta$ -galactosidase ( $\beta$ -gal) activity of the transformants. Similar results were observed when reciprocal sets of plasmids were used.  $\beta$ -Galactosidase activities were examined as described in “Materials and methods”

### The CheC homologue DifG interacts with DifD

DifG is another member of the Dif pathway (Black and Yang 2004). It shares approximately 27% sequence identity with the *B. subtilis* chemotaxis protein CheC (Black and Yang 2004). *B. subtilis* CheC was shown to interact with MCP and CheA in Y2H experiments (Kirby et al. 2001). To examine the interactions of DifG with other Dif proteins, GAL4BD-DifG and GAL4AD-DifG fusions were constructed (see “Materials and methods”) and used in Y2H studies. Somewhat surprisingly, yeast transformants containing a DifG fusion construct in combination with any of the available DifA, DifC, and DifE Y2H constructs showed no growth on either the SC-Ade or SC-His plates (Table 1; Fig. 1; data not shown). The only transformants that grew on these plates are the ones with both DifG and DifD constructs (Fig. 4). Analysis of  $\beta$ -galactosidase activity also indicated the expression of the *lacZ* reporter in the transformants with DifD and DifG constructs (Fig. 4). It was reported recently that *B. subtilis* CheC, to which DifG is homologous, might function as a phosphatase of CheY-P (Szurmant et al. 2004). Our results here may also be explained if DifG functions as a phosphatase of DifD-P in *M. xanthus* similarly as CheC in *B. subtilis* (Szurmant et al. 2004).

### DifB shows no interaction with other Dif proteins

DifB, which is downstream of DifA and upstream of DifC, is predicted to be a positively charged protein with some homology to a conserved bacterial protein family with unknown functions (Black and Yang 2004). Previous studies showed that an in-frame deletion of *difB* does not affect EPS production, S motility or development under the conditions examined (Black and Yang 2004). Although the role of DifB remains unclear, the clustering of *difB* with the other *dif* genes may nevertheless suggest interactions between DifB and the other Dif proteins. A GAL4BD-DifB full-length fusion was therefore constructed and co-transformed with other GAL4AD-Dif constructs into the Y2H reporter strain PJ69-4A. None of the resulting yeast transformants showed any growth on either SC-His plates or SC-Ade plates (data not shown). These results suggest that DifB may not interact directly with the other Dif proteins, at least not in a form detectable by the Y2H system.

## Discussion

It was proposed previously that the *M. xanthus* Dif proteins constitute a chemotaxis-like signal transduction pathway with crucial regulatory function in EPS production (Yang et al. 1998, 2000; Bellenger et al. 2002; Black and Yang 2004). In the present study, we provided evidence for direct interactions among the Dif chemotaxis homologues using the Y2H system.

DifC (CheW-like) showed pairwise interactions with both DifA (MCP-like) and DifE (CheA-like) (Fig. 2a). Using a modified Y2H system in which GAL4-DifA and GAL4-DifE fusions were transformed into yeast along with a construct that permits constitutive expression of DifC, we showed that DifC can interact with both DifA and DifE to form a ternary complex (Fig. 2b). We have additionally demonstrated that the interactions between DifC and DifE are mediated through the predicted P5 domain of DifE as expected for chemotaxis-like interactions (Fig. 3). These results support the proposed formation of a ternary signaling complex in which DifA functions as the equivalent of a chemoreceptor and interacts with the CheW homologue DifC, which in turn propagates signals by its interaction with the CheA-like kinase DifE (Black and Yang 2004).

Somewhat surprising was the finding that the reporter gene *lacZ* and *ADE2* were expressed at significantly higher level in the three-hybrid setup (Fig. 2b) than in the two-hybrid experiments with DifA–DifC and DifC–DifE pairwise interactions (Fig. 2a). There are at least two plausible explanations for this observation. It can be argued that the interactions among the three proteins in the three-hybrid system may not be any stronger or more stable than the individual pairwise interactions. The increases in reporter gene expression may merely reflect increased stability or expression of these proteins when co-expressed in *S. cerevisiae*. However, there has been no report of protein stability issues with Y2H systems as far as we are aware, and the *ADH1* promoter (James et al. 1996) for the expression of the fusion proteins is unlikely affected by the co-expression of these three proteins. Alternatively, the increased expression of reporter genes in the three-hybrid setup may truly reflect stronger or more stable interactions in the DifA–DifC–DifE ternary complex. Future studies of the Dif proteins are necessary to fully understand the nature of the interactions among the Dif proteins.

The interaction between DifE and DifD were also demonstrated using the Y2H system. This intermolecular interaction occurs between DifD and the predicted P2 domain of DifE. We also demonstrated the interaction between the P3 domain of DifE with itself, suggesting that DifE may form dimers through this domain. Because it is the P2 domain of CheA that interacts with CheY in bacterial chemotaxis, and because it is the P3 domain that mediates CheA dimerization (Bilwes et al. 1999), these interactions are consistent with DifE being a CheA-type histidine kinase. One distinct feature of DifE is the presence of some extra 185 amino acids inserted between its P1 and P2 domains. Although P2 alone interacted with DifD, the insertion and P2 of DifE together failed to display any interaction with DifD in the Y2H system (data not shown). The biological significance of this insertion in DifE is unknown, and whether the insertion plays a role in regulating the interactions between DifE and DifD in *M. xanthus* remains to be determined.

DifA as an MCP homologue is predicted to be a multi-domain protein. Our results suggest that it is the highly conserved signaling domain of DifA that interacts with DifC, and that the very C-terminus of DifA may interact with itself for DifA dimerization. The interaction of DifA signaling domain with DifC was expected, because it had been well established that MCPs interact with CheW through this domain (Liu and Parkinson 1991; Ames et al. 1996; Surette and Stock 1996; Falke and Kim 2000; Shimizu et al. 2000). Not expected was the finding that the inclusion of the flanking MHs perhaps weakened this interaction. It is known from studies of bacterial chemotaxis that the methylation of chemoreceptors modulates CheA kinase activity to bring about adaptation (Falke et al. 1997; Armitage 1999; Bren and Eisenbach 2000; Bourret and Stock 2002). It has been proposed that the modulation of CheA activity is through conformational changes of MCPs, which are then transmitted through CheW to CheA. However, it is now well-established that the interactions among chemotaxis proteins and among the arrays of chemotaxis signaling complexes are very fluid and dynamic (Djordjevic and Stock 1998; Stock and Da Re 1999; Bourret and Stock 2002; Dahlquist 2002; Gestwicki and Kiessling 2002; Sourjik and Berg 2004). MHs may simply influence the conformation or structure of the DifA signaling domain to modulate the strength of DifA–DifC interactions in the *M. xanthus* Dif system.

DifG is a homologue of *B. subtilis* CheC (Black and Yang 2004). It was reported previously that *B. subtilis* CheC interacted strongly with the *B. subtilis* chemotaxis proteins CheD and the chemoreceptor McpB and weakly with CheA in Y2H systems (Kirby et al. 2001). There is no CheD homologue encoded by the *dif* locus (Yang et al. 1998) or by the *M. xanthus* genome for that matter (<http://www.tigr.org>). We failed to detect any interactions of DifG with either DifA or DifE using the Y2H system (data not shown). Our results showed, however, that DifG does interact with DifD in Y2H systems (Fig. 4). It was reported recently that *B. subtilis* CheC can function as a phosphatase in vitro to hydrolyze CheY-P (Szurmant et al. 2004), indicating direct interactions between *B. subtilis* CheC and CheY. The results here demonstrate that similar interactions also occur between CheC and CheY homologues in gram-negative bacteria.

Although much work is necessary to fully understand the Dif pathway and its regulatory roles in *M. xanthus* EPS production, S motility, and development, the Y2H studies presented here provide important insights into the architecture of the Dif pathway. The proposed formation of chemotaxis-like signaling complexes by DifA, DifC, and DifE (Black and Yang 2004) are supported by the results in this study. The results with DifD and DifG support a prediction by the published report of CheC being a phosphatase of CheY-P in *B. subtilis* (Szurmant et al. 2004). Both DifD and DifG are negative regulators of EPS production in *M. xanthus* (Black and Yang 2004). If DifG functions as a phosphatase of DifD-P, the

current available data could still be explained by the two models in either a linear or a branched pathway (Black and Yang 2004). The differentiation of the two models requires additional experiments. Other unresolved issues include how the Dif pathway regulates both phosphatidylethanolamine taxis (Kearns et al. 2000; Kearns and Shimkets 2001) and EPS production and how the Dif pathway may interact with other chemotaxis or chemotaxis-like pathways in *M. xanthus* (Ward and Zusman 1997; Kirby and Zusman 2003; Vlamakis et al. 2004). Preliminary Y2H experiments detected no interactions between Dif and Frz proteins (Z. Li and Z. Yang, unpublished results).

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## References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410
- Ames P, Yu YA, Parkinson JS (1996) Methylation domain segments are not required for chemotactic signalling by cytoplasmic fragments of Tsr, the methyl-accepting serine chemoreceptor of *Escherichia coli*. *Mol Microbiol* 19:737–746
- Armitage JP (1999) Bacterial tactic responses. *Adv Microb Physiol* 41:229–289
- Arnold JW, Shimkets LJ (1988) Cell surface properties correlated with cohesion in *Myxococcus xanthus*. *J Bacteriol* 170:5771–5777
- Bellenger K, Ma X, Shi W, Yang Z (2002) A CheW homologue is required for *Myxococcus xanthus* fruiting body development, social gliding motility, and fibril biogenesis. *J Bacteriol* 184:5654–5660
- Bilwes AM, Alex LA, Crane BR, Simon MI (1999) Structure of CheA, a signal-transducing histidine kinase. *Cell* 96:131–141
- Black WP, Yang Z (2004) *Myxococcus xanthus* chemotaxis homologs DifD and DifG negatively regulate fibril polysaccharide production. *J Bacteriol* 186:1001–1008
- Bourret RB, Stock AM (2002) Molecular information processing: lessons from bacterial chemotaxis. *J Biol Chem* 277:9625–9628
- Bren A, Eisenbach M (2000) How signals are heard during bacterial chemotaxis: protein–protein interactions in sensory signal propagation. *J Bacteriol* 182:6865–6873
- Bullock WO, Fernandez JM, Short JM (1987) XL1-Blue: a high efficiency plasmid transforming *recA* *Escherichia coli* strain with beta-galactosidase selection. *Biotechniques* 5:376–378
- Dahlquist FW (2002) Amplification of signaling events in bacteria. *Sci STKE* 2002:PE24
- Dana JR, Shimkets LJ (1993) Regulation of cohesion-dependent cell interactions in *Myxococcus xanthus*. *J Bacteriol* 175:3636–3647
- Djordjevic S, Stock AM (1998) Structural analysis of bacterial chemotaxis proteins: components of a dynamic signaling system. *J Struct Biol* 124:189–200
- Dworkin M (2000) Introduction to myxobacteria. In: Shimkets LJ (ed) *Prokaryotic development*. ASM, Washington, DC, pp 221–242
- Falke JJ, Kim SH (2000) Structure of a conserved receptor domain that regulates kinase activity: the cytoplasmic domain of bacterial taxis receptors. *Curr Opin Struct Biol* 10:462–469
- Falke JJ, Bass RB, Butler SL, Chervitz SA, Danielson MA (1997) The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes. *Ann Rev Cell Dev Biol* 13:457–512
- Geer LY, Domrachev M, Lipman DJ, Bryant SH (2002) CDART: protein homology by domain architecture. *Genome Res* 12:1619–1623
- Gestwicki JE, Kiessling LL (2002) Inter-receptor communication through arrays of bacterial chemoreceptors. *Nature* 415:81–84
- Gietz RD, Woods RA (2002) Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. *Methods Enzymol* 350:87–96
- Hodgkin J, Kaiser D (1979a) Genetics of gliding motility in *Myxococcus xanthus*: two genes systems control movement. *Mol Gen Genet* 171:177–191
- Hodgkin J, Kaiser D (1979b) Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): genes controlling movement of single cells. *Mol Gen Genet* 171:167–176
- James P, Halladay J, Craig EA (1996) Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* 144:1425–1436
- Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ (1998) Multiple sequence alignment with Clustal X. *Trends Biochem Sci* 23:403–405
- Kaiser D (1979) Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. *Proc Natl Acad Sci USA* 76:5952–5956
- Kaiser D (2000) Bacterial motility: how do pili pull? *Curr Biol* 10:777–780
- Kearns DB, Shimkets LJ (2001) Lipid chemotaxis and signal transduction in *Myxococcus xanthus*. *Trends Microbiol* 9:126–129
- Kearns DB, Campbell BD, Shimkets LJ (2000) *Myxococcus xanthus* fibril appendages are essential for excitation by a phospholipid attractant. *Proc Natl Acad Sci USA* 97:11505–11510
- Kippert F (1995) A rapid permeabilization procedure for accurate quantitative determination of beta-galactosidase activity in yeast cells. *FEMS Microbiol Lett* 128:201–206
- Kirby JR, Zusman DR (2003) Chemosensory regulation of developmental gene expression in *Myxococcus xanthus*. *Proc Natl Acad Sci USA* 100:2008–2013
- Kirby JR, Kristich CJ, Saulmon MM, Zimmer MA, Garrity LF, Zhulin IB, Ordal GW (2001) CheC is related to the family of flagellar switch proteins and acts independently from CheD to control chemotaxis in *Bacillus subtilis*. *Mol Microbiol* 42:573–585
- Li Y, Sun H, Ma X, Lu A, Lux R, Zusman D, Shi W (2003) Extracellular polysaccharides mediate pilus retraction during social motility of *Myxococcus xanthus*. *Proc Natl Acad Sci USA* 100:5443–5448
- Liu JD, Parkinson JS (1991) Genetic evidence for interaction between the CheW and Tsr proteins during chemoreceptor signaling by *Escherichia coli*. *J Bacteriol* 173:4941–4951
- Lu A, Cho K, Black WP, Duan XY, Lux R, Yang Z, Kaplan HB, Zusman DR, Shi W (2005) Exopolysaccharide biosynthesis genes required for social motility in *Myxococcus xanthus*. *Mol Microbiol* 55:206–220
- MacNeil SD, Mouzeyan A, Hartzell PL (1994) Genes required for both gliding motility and development in *Myxococcus xanthus*. *Mol Microbiol* 14:785–795
- Marchler-Bauer A, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler GH, Mazumder R, Nikolskaya AN, Panchenko AR, Rao BS, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH (2003) CDD: a curated Entrez database of conserved domain alignments. *Nucleic Acids Res* 31:383–387
- Martinez-Argudo I, Martin-Nieto J, Salinas P, Maldonado R, Drummond M, Contreras A (2001) Two-hybrid analysis of domain interactions involving NtrB and NtrC two-component regulators. *Mol Microbiol* 40:169–178

- Merz AJ, So M, Sheetz MP (2000) Pilus retraction powers bacterial twitching motility. *Nature* 407:98–102
- Miller JH (1992) A short course in bacterial genetics: A laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Mumberg D, Muller R, Funk M (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* 156:119–122
- Rosario MM, Kirby JRD, Bochar A, Ordal GW (1995) Chemotactic methylation and behavior in *Bacillus subtilis*: role of two unique proteins, CheC and CheD. *Biochem* 34:3823–3831
- Rose MD, Winston F, Hieter P (1990) Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Shimizu TS, Le Novere N, Levin MD, Beavil AJ, Sutton BJ, Bray D (2000) Molecular model of a lattice of signalling proteins involved in bacterial chemotaxis. *Nat Cell Biol* 2:792–796
- Shimkets LJ (1986) Role of cell cohesion in *Myxococcus xanthus* fruiting body formation. *J Bacteriol* 166:842–848
- Shimkets LJ (2000) Growth, sporulation, and other tough decision. In: Shimkets LJ (ed) Prokaryotic development. ASM, Washington, DC, pp 277–284
- Skerker JM, Berg HC (2001) Direct observation of extension and retraction of type IV pili. *Proc Natl Acad Sci USA* 98:6901–6904
- Smith RF, Wiese BA, Wojzynski MK, Davison DB, Worley KC (1996) BCM Search Launcher—an integrated interface to molecular biology data base search and analysis services available on the world wide web. *Genome Res* 6:454–462
- Sourjik V, Berg HC (2004) Functional interactions between receptors in bacterial chemotaxis. *Nature* 428:437–441
- Spormann AM (1999) Gliding motility in bacteria: insights from studies of *Myxococcus xanthus*. *Microbiol Mol Biol Rev* 63:621–641
- Stock J, Da Re S (1999) A receptor scaffold mediates stimulus-response coupling in bacterial chemotaxis. *Cell Calcium* 26:157–164
- Struhl K, Davis RW (1977) Production of a functional eukaryotic enzyme in *Escherichia coli*: cloning and expression of the yeast structural gene for imidazole-glycerolphosphate dehydratase (*his3*). *Proc Natl Acad Sci USA* 74:5255–5259
- Sun H, Zusman DR, Shi W (2000) Type IV pilus of *Myxococcus xanthus* is a motility apparatus controlled by the *frz* chemosensory system. *Curr Biol* 10:1143–1146
- Surette MG, Stock JB (1996) Role of  $\alpha$ -helical coiled-coil interactions in receptor dimerization, signaling, and adaptation during bacterial chemotaxis. *J Biol Chem* 271:17966–17973
- Szurmant H, Muff TJ, Ordal GW (2004) *Bacillus subtilis* CheC and FliY are members of a novel class of CheY-P-hydrolyzing proteins in the chemotactic signal transduction cascade. *J Biol Chem* 279:21787–21792
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680
- Ursic D, Chinchilla K, Finkel JS, Culbertson MR (2004) Multiple protein/protein and protein/RNA interactions suggest roles for yeast DNA/RNA helicase Sen1p in transcription, transcription-coupled DNA repair and RNA processing. *Nucleic Acids Res* 32:2441–2452
- Vlamakis HC, Kirby JR, Zusman DR (2004) The Che4 pathway of *Myxococcus xanthus* regulates type IV pilus-mediated motility. *Mol Microbiol* 52:1799–1811
- Ward MJ, Zusman DR (1997) Regulation of directed motility in *Myxococcus xanthus*. *Mol Microbiol* 24:885–893
- Weimer RM, Creighton C, Stassinopoulos A, Youderian P, Hartzell PL (1998) A chaperone in the HSP70 family controls production of extracellular fibrils in *Myxococcus xanthus*. *J Bacteriol* 180:5357–5368
- Wu SS, Kaiser D (1995) Genetic and functional evidence that Type IV pili are required for social gliding motility in *Myxococcus xanthus*. *Mol Microbiol* 18:547–558
- Yang Z, Geng Y, Xu D, Kaplan HB, Shi W (1998) A new set of chemotaxis homologues is essential for *Myxococcus xanthus* social motility. *Mol Microbiol* 30:1123–1130
- Yang Z, Ma X, Tong L, Kaplan HB, Shimkets LJ, Shi W (2000) *Myxococcus xanthus dif* genes are required for biogenesis of cell surface fibrils essential for social gliding motility. *J Bacteriol* 182:5793–5798