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Mutational Analysis of the *Myxococcus xanthus* Ω4499 Promoter Region Reveals Shared and Unique Properties in Comparison with Other C-Signal-Dependent Promoters

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The bacterium *Myxococcus xanthus* undergoes multicellular development during times of nutritional stress and uses extracellular signals to coordinate cell behavior. C-signal affects gene expression late in development, including that of Ω4499, an operon identified by insertion of Tn*5 lac* into the *M. xanthus* chromosome. The Ω4499 promoter region has several sequences in common with those found previously to be important for expression of other C-signal-dependent promoters. To determine if these sequences are important for Ω4499 promoter activity, the effects of mutations on expression of a downstream reporter gene were tested in *M. xanthus*. Although the promoter resembles those recognized by *Escherichia coli* σ^{54}, mutational analysis implied that a σ^{70}-type σ factor likely recognizes the promoter. A 7-bp sequence known as a C box and a 5-bp element located 6 bp upstream of the C box have been shown to be important for expression of other C-signal-dependent promoters. The Ω4499 promoter region has C boxes centered at −33 and −55 bp, with 5-bp elements located 7 and 8 bp upstream, respectively. A multiple-base-pair mutation in any of these sequences reduced Ω4499 promoter activity more than twofold. Single base-pair mutations in the C box centered at −33 bp yielded a different pattern of effects on expression than similar mutations in other C boxes, indicating that each functions somewhat differently. An element from about −81 to −77 bp exerted a twofold positive effect on expression but did not appear to be responsible for the C-signal dependence of the Ω4499 promoter. Mutations in *sigD* and *sigE*, which are genes that encode σ factors, reduced expression from the Ω4499 promoter. The results provide further insight into the regulation of C-signal-dependent genes, demonstrating both shared and unique properties among the promoter regions so far examined.

Myxococcus xanthus is a gram-negative, rod-shaped bacterium that is found in most soils. It has the ability to undergo multicellular development (21, 23, 45, 48), distinguishing it from most other bacteria. Under starvation conditions on a solid surface, *M. xanthus* cells move in a coordinated fashion called rippling and accumulate at foci. When approximately 10^5 cells have aggregated, mound-shaped structures called fruiting bodies are built, inside which some of the cells differentiate into heat- and desiccation-resistant, spherical myxospores.

The developmental process is believed to be regulated by several extracellular signals (21, 23, 45, 48), including the A- and C-signals, which are the best characterized. A-signaling early in development leads to the production of extracellular proteases, peptides, and amino acids, which are thought to provide a mechanism for cell density sensing (24, 34, 35, 41). C-signaling is the latest acting of the known signals and is required for rippling, aggregation, and sporulation (28, 37, 47). Signaling also leads to changes in gene expression during development (12, 31, 33).

Genes expressed during *M. xanthus* development have been identified by transposition of Tn*5 lac* into the chromosome (30, 32). Tn*5 lac* contains a promoterless *lacZ* gene whose transcription can come under the control of a promoter outside the transposon. Among 2,374 Tn*5 lac* insertions, 29 were shown to be developmentally regulated (32), and 15 of these were shown to depend on C-signaling for full expression (31). The 15 fusions are expressed at various times after 6 h into development. Several were shown to depend absolutely on C-signaling for expression (e.g., Ω4403). Others, such as Ω4400 and Ω4499, were shown to depend partially on C-signaling (i.e., expression was reduced, but not abolished, in the absence of C-signaling).

To gain insight into the differential regulation of C-signal-dependent genes, the promoter regions upstream of Tn*5 lac* insertions Ω4403 (9), Ω4400 (4), and Ω4499 (8) have been identified and searched for conserved sequence elements. Mutational analysis of the Ω4403 (53) and Ω4400 (56) promoter regions has revealed important *cis*-acting DNA elements. In both promoter regions, the identical 7-bp sequence (CATCC CT), which has been called a C box (consensus sequence CAYYCCY, in which Y means pyrimidine), is centered at −49 bp, and a 5-bp element (consensus sequence GAACA) is centered at −61 bp. Both the C boxes and the 5-bp elements were found to be essential for promoter activity. However, single base pair changes in these elements had different effects on promoter activity, suggesting that different transcription factors bind to these regions. Activity of the Ω4403 promoter also required a 10-bp element centered at −74.5 bp. Activity of the Ω4400 promoter required a large region from approximately −63 to −31 bp, which encompasses the 5-bp element, the C box, and adjoining DNA. In addition, a small region from approximately −86 to −81 bp exerted a twofold to fourfold positive effect on expression and was shown to be at least

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partially responsible for the C-signal dependence of the Ω4400 promoter.

Tn*5 lac* Ω4499 is an insertion in the second gene of an operon that is predicted to code for reductase and oxidase components of a cytochrome P-450 system (8). The insertion does not cause a developmental defect, but expression of *lacZ* is strongly induced during development. The timing of expression is similar to that from Tn*5 lac* Ω4400 (32). Expression from both the Ω4499 and Ω4400 promoters was reduced in a *csgA* mutant (31), which fails to produce the CsgA protein involved in C-signaling (29, 36, 39), and expression was restored by codeveloping the *csgA* mutant with wild-type cells, which supplied the C-signal (4, 8). Moreover, expression from both promoters has been shown to correlate closely with the altered levels of CsgA produced in *act* mutants (13, 56). Examination of the Ω4499 promoter region revealed three sequences that match the C box consensus sequence, centered at −55, −33, and −1 bp (8). In addition, centered at −65 bp is a sequence that matches a sequence in the Ω4400 promoter region in eight of nine positions. The sequence is centered at −80 bp in the Ω4400 promoter region and is in the opposite orientation relative to the start site of transcription but, interestingly, it includes the region shown to mediate, at least in part, the response to C-signaling (56).

Here, we report the results of mutational analysis of the Ω4499 promoter region. We found some similarities between the Ω4499 and Ω4400 promoter regions in terms of overall organization, but the effects of single base pair changes were different in many cases from either the Ω4400 (56) or Ω4403 (53) promoter regions, indicating that DNA elements similar in sequence function uniquely to regulate transcription from the three promoters.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains and plasmids that were used in this study are listed in Table 1.

Growth and development. *Escherichia coli* DH5α strains were grown at 37°C in Luria-Bertani medium (44) containing 50 μg of ampicillin per ml. *M. xanthus* strains were grown at 32°C in CTT broth or agar (1.5%) plates (1% Casitone, 10 mM Tris-HCl [pH 8.0], 1 mM KH_2PO_4, 8 mM $MgSO_4$ [final pH = 7.6]) (18). When necessary, 40 μg of kanamycin (Km) per ml was used for selection. Fruiting body development was performed on TPM agar (1.5%) plates (10 mM Tris-HCl [pH 8.0], 1 mM KH_2PO_4-K_2HPO_4, 8 mM $MgSO_4$ [final pH = 7.6]) as described previously (32).

Construction of plasmids. A PCR fragment containing the Ω4499 promoter region from −218 bp to +50 bp relative to the start site of transcription was generated using pMF0051 as the template. The PCR fragment was ligated into XhoI-BamHI-digested pGEM7Zf to form pDY100. Additional deletion constructs were created by PCR using pDY100 and primers designed to produce a product with a XhoI restriction site at the upstream end and a BamHI restriction site at the downstream end. PCR products were then digested with XhoI and BamHI, gel purified, and ligated into pGEM7Zf, and the ligation products were electroporated into *E. coli* DH5α cells. Ampicillin-resistant transformants were selected, and plasmid DNA was sequenced at the Michigan State University Genomics Technology Support Facility to confirm the sequence and end points of the *M. xanthus* DNA insert.

The QuikChange site-directed mutagenesis kit (Stratagene) was used to create mutations in the Ω4499 promoter region that, in most cases, were A↔ C or T↔ G single-base-pair or multiple-base-pair transversion mutations. The plasmid pDY100 described above was used as a template in PCRs with various combinations of mutagenic primers. The *M. xanthus* DNA insert was sequenced to ensure only the proper mutations had been created.

Each mutant derivative of pDY100 was digested with XhoI and BamHI, gel purified, and ligated into pREG1727 previously cut with the same enzymes. The ligated constructs were introduced into *E. coli* DH5α by electroporation, and ampicillin-resistant transformants were selected. A transformant containing the mutant Ω4499 plasmid was identified using colony PCR with primers to ensure proper orientation. The transformants containing the mutated Ω4499 promoter regions were then used to prepare plasmid DNA for introduction into *M. xanthus*.

Construction of *M. xanthus* strains and determination of *lacZ* expression during development. Strains containing pREG1727 derivatives integrated at the Mx8 phage attachment site (designated *attB* in Table 1) were constructed by electroporation (25) of *M. xanthus*, and transformants were selected on CTT-Km plates. Based on previous experience in our laboratory (4, 8, 9), the majority of transformants have a single copy of the plasmid integrated at *attB*. To eliminate colonies with unusual developmental *lacZ* expression, we screened at least 10 transformants on TPM agar plates containing 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside per ml. Any colonies with unusual expression of *lacZ* were discarded and, of the remaining candidates, three independent isolates of each mutant construct were chosen for development. In all cases, the three transformants gave similar results (see Table 2, below) when developmental β-galactosidase activity was measured as described previously (32).

RESULTS

Deletion analysis of the Ω4499 promoter region. Previous analysis of the Ω4499 regulatory region showed that a segment containing from −218 bp to +2.68 kbp relative to the start site of transcription, fused to the *E. coli lacZ* gene and integrated at the Mx8 phage attachment site in the *M. xanthus* chromosome, showed a similar pattern of developmental *lacZ* expression as the *M. xanthus* strain, DK4299, which contains Tn*5 lac* Ω4499 (8). A 5′ deletion to −49 bp with the same 3′ end resulted in a dramatic decrease in expression. To further define the minimal region required for Ω4499 promoter activity, a DNA fragment spanning from −218 to +50 bp of the Ω4499 promoter region was generated by PCR, fused to *lacZ*, and tested for developmental expression (see Materials and Methods). Figure 1A shows that the segment from −218 to +50 bp directed a similar level of β-galactosidase production during development as the segment from −218 bp to +2.68 kbp. This demonstrates that the region between +50 bp and +2.68 kbp is not essential for Ω4499 promoter activity.

To further characterize the upstream boundary of the Ω4499 regulatory region, 5′ deletions were made to −100, −71, and −61 bp in the context of a 3′ end at +50 bp. The segment from −100 to +50 bp showed comparable developmental expression as the segment from −218 to +50 bp (Fig. 1A), indicating that DNA between −218 and −100 bp is not necessary for Ω4499 promoter activity. The deletion to −71 bp led to a 60% decrease in activity compared to the −218 to +50 bp promoter region (Fig. 1B and Table 2), indicating that DNA between −100 and −71 bp is important for Ω4499 activity. Furthermore, the 5′ deletion to −61 bp retained only 4% of wild-type promoter activity (Fig. 1B and Table 2), so DNA between −71 and −61 bp is essential for expression of the Ω4499 promoter.

Effects of mutations in the −25 to −10 bp region of the Ω4499 promoter. The product of the *rpoN* gene, σ^{54} (27), is believed to recognize several promoters in *M. xanthus*, including those for *mbhA* (43), *sdeK* (11), *pilA* (55), *spi* (15, 26), *actABCD* (14), and *asgE* and *orf2* (10). An alignment of these promoter regions with the consensus sequence found in *E. coli* σ^{54}-dependent promoters (49) is shown in Fig. 2A. The Ω4499 promoter matches the consensus sequence at four of seven positions in the −24 region and at three of five positions in the −12 region (Fig. 2A), suggesting that the Ω4499 promoter may be recognized by σ^{54} RNA polymerase. To test this hypothesis, two mutations were created in the context of the Ω4499 pro-

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Source or reference
E. coli DH5α	ϕ80 *lacZ*ΔM15 Δ*lacU169 recA1 endA1 hsdR17 supE44 thi-1 gyrA relA1*	16
M. xanthus		
DK1622	Wild type	22
MDB01	*attB*::pDB01	8
MDY1727	*attB*::pREG1727	56
MDY101	*attB*::pDY101 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY100)[a]	This study
MDY103	*attB*::pDY103 (pREG1727 with 121-bp XhoI-BamHI fragment from pDO2)	This study
MDY104	*attB*::pDY104 (pREG1727 with 111-bp XhoI-BamHI fragment from pDO3)	This study
MDY40	*attB*::pDY40 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY39)	This study
MDY42	*attB*::pDY42 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY41)	This study
MDY44	*attB*::pDY44 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY43)	This study
MDY46	*attB*::pDY46 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY45)	This study
MDY48	*attB*::pDY48 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY47)	This study
MDY50	*attB*::pDY50 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY49)	This study
MDY52	*attB*::pDY52 (pREG1727 with 150-bp XhoI-BamHI fragment from pDY51)	This study
MDY106	*attB*::pDY106 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY105)	This study
MDY108	*attB*::pDY108 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY107)	This study
MDY110	*attB*::pDY110 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY109)	This study
MDY112	*attB*::pDY112 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY111)	This study
MDY114	*attB*::pDY114 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY113)	This study
MDY116	*attB*::pDY116 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY115)	This study
MDY118	*attB*::pDY118 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY117)	This study
MDY120	*attB*::pDY120 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY119)	This study
MDY122	*attB*::pDY122 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY121)	This study
MDY124	*attB*::pDY124 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY123)	This study
MDY126	*attB*::pDY126 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY125)	This study
MDY128	*attB*::pDY128 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY127)	This study
MDY130	*attB*::pDY130 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY129)	This study
MDY132	*attB*::pDY132 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY131)	This study
MDY134	*attB*::pDY134 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY133)	This study
MDY136	*attB*::pDY136 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY135)	This study
MDY138	*attB*::pDY138 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY137)	This study
MDY140	*attB*::pDY140 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY139)	This study
MDY142	*attB*::pDY142 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY141)	This study
MDY144	*attB*::pDY144 (pREG1727 with 268-bp XhoI-BamHI fragment from pDY143)	This study
DK5208	*csgA*::Tn5-132 (Tc^r) Ω205	46
MDY5208-103	*csgA*::Tn5-132 (Tc^r) Ω205 *attB*::pDY103	This study
MDY5208-134	*csgA*::Tn5-132 (Tc^r) Ω205 *attB*::pDY134	This study
ΔsigD	Δ*sigD*	51
MDY4499.SD	*attB*::pDY101	This study
ΔsigE	Δ*sigE*	52
MDY4499.SE	*attB*::pDY101	This study
Plasmids		
pGEM7Zf	Ap^r *lac*α	Promega
pREG1727	Ap^r Km^r P1-*inc attP 'lacZ*	9
pMF0051	pGEM7Zf with 3.2-kb PstI-BamHI fragment from pMF002	8
pDY100	pGEM7Zf with 268-bp fragment from −218 to +50 bp of Ω4499 DNA generated by PCR using pMF0051 as template, inserted as a XhoI-BamHI fragment	This study
pDO2	pGEM7Zf with 121-bp fragment from −71 to +50 bp of Ω4499 DNA generated by PCR using pDY100 as a template, inserted as a XhoI-BamHI fragment	This study
pDO3	pGEM7Zf with 111-bp fragment from −61 to +50 bp of Ω4499 DNA generated by PCR using pDY100 as a template, inserted as a XhoI-BamHI fragment	This study
pDY39	pDY100 with C-to-A mutation at −36 bp	This study
pDY41	pDY100 with C-to-A mutation at −32 bp	This study
pDY43	pDY100 with C-to-A mutation at −31 bp	This study
pDY45	pDY100 with CATTCCT-to-ACGGAAG mutation from −36 to −30 bp	This study
pDY47	pDY100 with GAAC-to-TCCA mutation from −48 to −45 bp	This study
pDY49	pDY100 with TCATTC-to-GACGGA mutation from −59 to −54 bp	This study
pDY51	pGEM7Zf with 150-bp fragment from −100 to +50 bp of Ω4499 DNA generated by PCR using pDY100 as template, inserted as a XhoI-BamHI fragment	This study
pDY105	pDY100 with CGA-to-TAT mutation from −12 to −10 bp	This study
pDY107	pDY100 with T-to-G mutation at −25 bp	This study
pDY109	pDY100 with T-to-G mutation at −30 bp	This study
pDY111	pDY100 with T-to-G mutation at −33 bp	This study
pDY113	pDY100 with T-to-G mutation at −34 bp	This study
pDY115	pDY100 with A-to-C mutation at −35 bp	This study
pDY117	pDY100 with C-to-A mutation at −37 bp	This study
pDY119	pDY100 with T-to-G mutation at −44 bp	This study
pDY121	pDY100 with CCTTC-to-AAGGA mutation from −53 to −49 bp	This study
pDY123	pDY100 with TCA-to-GAC mutation from −59 to −57 bp	This study
pDY125	pDY100 with CCGG-to-AATT mutation from −63 to −60 bp	This study

Continued on following page

TABLE 1—*Continued*

Strain or plasmid	Relevant characteristic(s)	Source or reference
pDY127	pDY100 with ACCA-to-CAAC mutation from −67 to −64 bp	This study
pDY129	pDY100 with GGAC-to-TTCA mutation from −71 to −68 bp	This study
pDY131	pDY100 with TCGCT-to-GATAG mutation from −76 to −72 bp	This study
pDY133	pDY100 with GCCGC-to-TAATA mutation from −81 to −77 bp	This study
pDY135	pDY100 with CATAC-to-ACGCA mutation from −86 to −82 bp	This study
pDY137	pDY100 with GCGTT-to-TATGG mutation from −91 to −87 bp	This study
pDY139	pDY100 with AGATT-to-CTCGG mutation from −96 to −92 bp	This study
pDY141	pDY100 with CGAGG-to-ATCTT mutation from −101 to −97 bp	This study
pDY143	pDY100 with CCC-to-AAA mutation from −29 to −27 bp	This study

[a] Where possible, the plasmid description is given in parentheses after the strain description.

moter region from −218 to +50 bp. One mutation was a T-to-G transversion at position −25 bp, which creates a better match to the *E. coli* σ^{54} consensus sequence (49) in the −24 region. This mutation decreased Ω4499 promoter activity by 50% (Fig. 2B and Table 2). In contrast, a mutation of CGA to TAT at −12 to −10 bp, which changes the highly conserved C in the −12 region to T and creates a perfect match in the −10 region to the consensus sequence recognized by *E. coli* σ^{70} (TATAAT) (38), resulted in a dramatic increase in promoter activity (Fig. 2B and Table 2). These results suggest that a σ factor in the σ^{70} family, rather than σ^{54}, recognizes the Ω4499 promoter.

Effects of mutations in the C box centered at −33 bp and adjacent regions. The Ω4499 promoter region contains three sequences that match the C box consensus sequence (8). Among these, the one centered at −33 bp is 7 bp downstream of a 5-bp sequence (GAACT) that matches the 5-bp element consensus sequence (GAACA) in four of five positions (53). To determine if this C box functions in the same way as any of the C boxes mutated previously, eight mutations were made: a 7-bp change of the entire C box, and seven single-base-pair changes within the C box. These and all subsequent mutations reported here were made in the context of Ω4499 DNA from −218 to +50 bp.

The 7-bp change of the entire C box caused a loss of promoter activity, as did single-base-pair mutations at −34 and −33 bp (Table 2). Single-base-pair mutations at −35, −32, −31, and −30 led to intermediate activity, and the mutation at −36 bp caused a slight increase in expression (Fig. 3A and Table 2).

The pattern of mutational effects observed was different than for any of the C boxes examined previously. Figure 3B compares the effects of single-base-pair mutations in the C box centered at −33 bp in the Ω4499 promoter with the effects of mutations in the C boxes centered at −49 bp in the Ω4400 (56) and Ω4403 (53) promoter regions. These C boxes have the sequence CATCCCT, which differs from the CATTCCT sequence centered at −33 bp in the Ω4499 promoter only at position 4. Except at this position, the single-base-pair changes compared in Fig. 3B are the same. Striking differences between the effects of mutations at positions 1, 4, 5, and 7 on Ω4400 and Ω4403 promoter activity indicated that the C boxes centered at −49 bp function differently. The effects of mutations in the C box centered at −33 bp in the Ω4499 promoter differed markedly from those in the Ω4400 C box at positions 1, 3, 6, and 7, and from those in the Ω4403 C box at positions 3, 5, and 6 (at position 4, a C-to-A change increases activity of the Ω4403

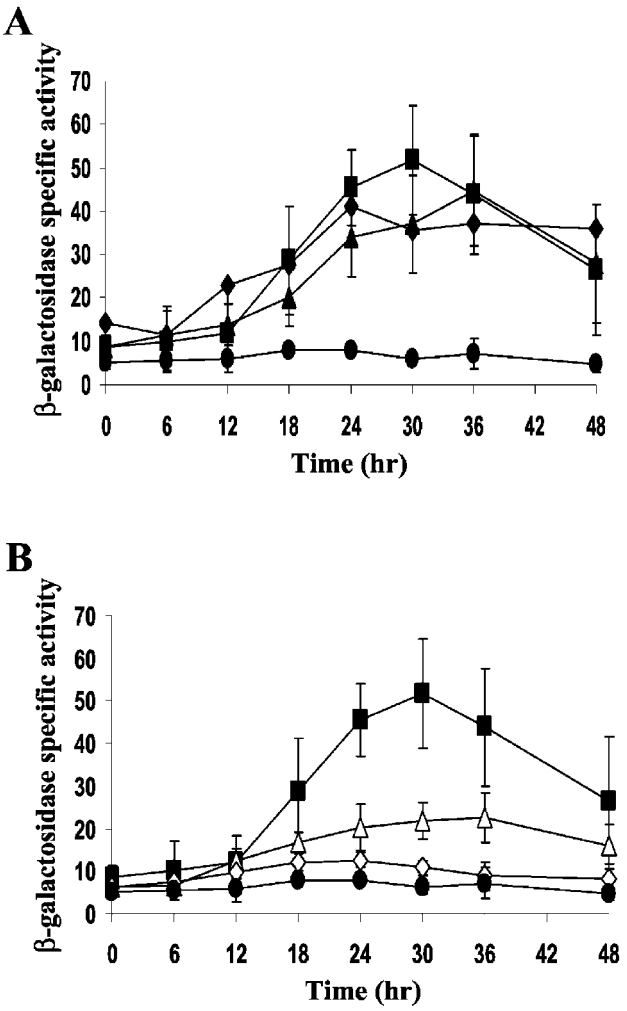


FIG. 1. Deletion analysis of the Ω4499 promoter region. (A) Developmental *lacZ* expression was determined for *M. xanthus* strains bearing integrated plasmids with Ω4499 DNA from −218 bp to +2.68 kbp (◆), −218 to +50 bp (▲), or −100 to +50 bp (■), along with a vector, no-insert control (●). (B) The 5′-deletion constructs contained Ω4499 DNA from −71 to +50 bp (△) or −61 to +50 bp (□). Constructs containing −100 to +50 bp (■) or no insert (●) were included as controls. The data for the −100 to +50 bp construct are the same in both panels and represent six independent determinations made in three separate experiments. The average β-galactosidase activity is expressed as nanomoles of *o*-nitrophenyl phosphate per minute per milligram of protein. Error bars show 1 standard deviation of the data.

TABLE 2. Summary of activities of mutant Ω4499 promoters

Promoter assayed	Avg maximum β-galactosidase sp act during development[a]	% Wild-type activity measured in the same expt[b]
Vector (no insert)	11 ± 4	
Wild-type 4499 promoter	39 ± 18	
Deletions		
−100 to +50 bp	51 ± 10	141 ± 10
−71 to +50 bp	25 ± 5	41 ± 15
−61 to +50 bp	13 ± 2	4 ± 6
Mutations[c]		
CGA −12 to −10 TAT	161 ± 10	877 ± 58
T −25 G	28 ± 5	49 ± 15
CCC −29 to −27 AAA	45 ± 6	178 ± 30
CATTCCT −36 to −30 ACGGAAG	12 ± 1	12 ± 4
T −30 G	27 ± 10	46 ± 28
C −31 A	31 ± 6	53 ± 19
C −32 A	29 ± 6	59 ± 21
T −33 G	7 ± 0.6	0
T −34 G	7 ± 2	0
A −35 C	17 ± 2	61 ± 10
C −36 A	55 ± 9	134 ± 26
C −37 A	11 ± 2	0
T −44 G	17 ± 3	63 ± 15
GAAC −48 to −45 TCCA	19 ± 4	18 ± 13
CCTTC −53 to −49 AAGGA	10 ± 1	0
TCATTC −59 to −54 GACGGA	12 ± 2	10 ± 6
TCA −59 to −57 GAC	9 ± 1	0
CCGG −63 to −60 AATT	7 ± 1	5 ± 6
ACCA −67 to −64 CAAC	10 ± 0.8	0
GGAC −71 to −68 TTCA	7 ± 2	0
TCGCT −76 to −72 GATAG	36 ± 4	170 ± 23
GCCGC −81 to −77 TAATA	19 ± 1	45 ± 6
CATAC −86 to −82 ACGCA	44 ± 8	214 ± 47
GCGTT −91 to −87 TATGG	41 ± 14	155 ± 72
AGATT −96 to −92 CTCGG	48 ± 7	235 ± 40
CGAGG −101 to −97 ATCTT	30 ± 3	99 ± 15

[a] The maximum β-galactosidase specific activity in nanomoles of *o*-nitrophenyl phosphate per minute per milligram of protein (average ± 1 standard deviation) is listed for three independently isolated *M. xanthus* transformants (one determination each) in the case of mutant promoter regions and for one isolate in the case of the wild-type promoter (16 determinations) and vector controls (11 determinations). Samples were assayed at 0, 6, 12, 18, 24, 30, 36, and 48 h during development.

[b] The wild-type promoter and vector-only strains were included in each experiment. The maximum for each mutant promoter region is expressed as a percentage of the maximum observed for the wild-type promoter in the same experiment, after subtracting from both values the maximum observed for vector only in that experiment. The average percentage ± 1 standard deviation is listed. A zero indicates that the expression from the mutant promoter region was equal to or slightly less than that observed for the vector-only control.

[c] For example, mutant CGA −12 to −10 TAT has a mutation changing CGA at positions −12 to −10 bp to TAT, and mutant T −25 G has a mutation changing T at position −25 bp to G.

promoter, as indicated in Fig. 3B, but a C-to-G change abolishes activity [53], as does a T-to-G change in the Ω4499 promoter). A C box centered at −80 bp in the Ω4400 promoter has also been subjected to mutational analysis (56). Single-base-pair changes had less than a twofold effect on expression. We conclude that the Ω4499 C box centered at −33 bp functions differently than the other three C boxes that have been examined.

Two mutations were made in regions adjacent to the C box centered at −33 bp in the Ω4499 promoter. A C-to-A change at −37 bp led to a complete loss of promoter activity, while a CCC-to-AAA mutation from −29 to −27 bp increased activity nearly twofold (Fig. 4 and Table 2).

Effects of mutations in the 5-bp element. The Ω4499 promoter region has a 5-bp element centered at −46 bp with the sequence GAACT, which matches the GAACA consensus sequence at four out of five positions (53). To determine if this 5-bp element is essential for expression, as are the 5-bp elements centered at −61 bp in the Ω4400 (56) and Ω4403 (53) promoters, two mutations were made. A 4-bp mutation, which converted GAAC at −48 to −45 bp to TCCA, resulted in a strong decrease (80%) in activity, demonstrating that this element is important for Ω4499 promoter expression (Fig. 4 and Table 2). A single-base-pair change from T to G at −44 bp retained 60% activity compared to the wild-type promoter (Fig. 4 and Table 2). This result is surprising because mutations at the corresponding position of the 5-bp elements centered at −61 bp in both the Ω4400 (56) and Ω4403 (53) promoter regions caused nearly complete loss of promoter activity. It appears that the 5-bp element, like the C box, functions somewhat differently in the Ω4499 promoter region than in the Ω4400 and Ω4403 promoter regions.

Effects of mutations between −71 and −49 bp. Six mutations were made to investigate the role of DNA upstream of the 5-bp element centered at −46 bp, which our deletion analysis had indicated includes an element between −71 and −61 bp that is

A

-29	TGGCACAACCATTGCT	*actABCD*
-24	TCGCGGAGTCCGGGCA	*orf2*
-28	CGGTGCACAAGGGGCT	*asgE*
-26	CGGCGCTCTCAGCGCG	*sdeK*
-28	GAGCACGCGTCTTGCT	*spi*
-27	TGGCACGCCATCTGCT	*mbhA*
-29	TGGCATGCGTAGTGCT	*pilA*
-26	GTGCGCCTCCCATGCG	*Ω4499*
	TGGYRYR N4 TTGCA	σ^{54} consensus

B

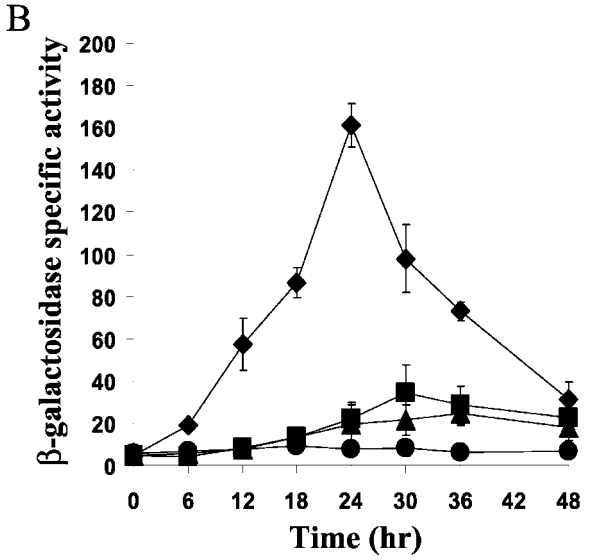
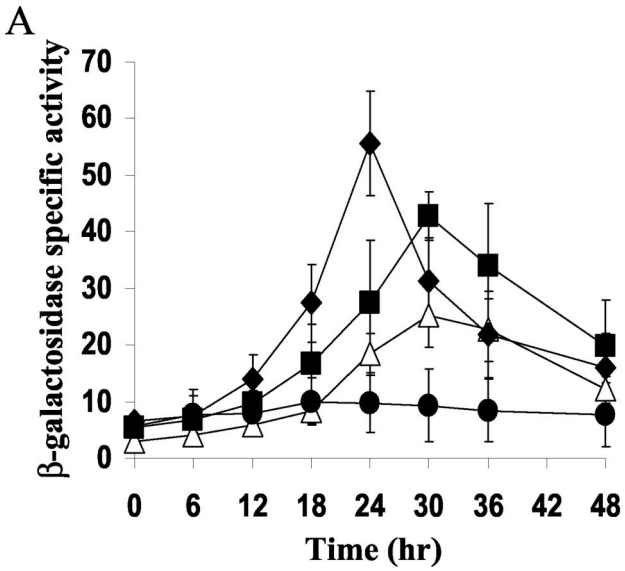


FIG. 2. (A) Comparison of the Ω4499 promoter region to promoters of *M. xanthus* genes believed to be transcribed by σ^{54} RNA polymerase (see text for references). Also shown is the consensus sequence to which *E. coli* σ^{54} binds (49). The numbers to the left indicate the location within the promoter relative to the start site of transcription. The bold nucleotides indicate those that match the consensus sequence, and the underlined nucleotides match those most highly conserved in the consensus sequence. (B) Mutational analysis of the −25 to −10 bp region of the Ω4499 promoter. Developmental *lacZ* expression was determined for *M. xanthus* strains bearing integrated plasmids with a mutation at −12 to −10 bp from CGA to TAT (◆) or a mutation at −25 bp from T to G (▲). The Ω4499 wild-type promoter region from −218 to +50 bp (■) (four determinations in two experiments) and the vector with no promoter insert (●) were included as controls. The meaning of points and error bars is the same as described in the Fig. 1 legend.

essential for Ω4499 promoter activity (Fig. 1B and Table 2). Five of these mutations are shown in Fig. 4. The sixth was a TCA-to-GAC mutation from −59 to −57 bp. All six mutations caused a dramatic decrease or loss of Ω4499 promoter activity. These results show that the entire region from approximately −70 to the 5-bp element centered at −46 bp is required for expression from the Ω4499 promoter.

Effects of mutations between −101 and −72 bp. The region between −101 and −72 bp was divided into 5-bp sections that were mutated to attempt to define the element(s) that led to a 60% decrease in activity upon 5′ deletion to −71 bp (Fig. 1B and Table 2). Only one of the six mutations decreased Ω4499 promoter activity; changing GCCGC to TAATA from −81 to −77 bp lowered activity by 55% (Fig. 4 and Table 2), which is very similar to the decrease observed upon 5′ deletion to −71 bp. This shows that a small region approximately 29 bp up-

A



B

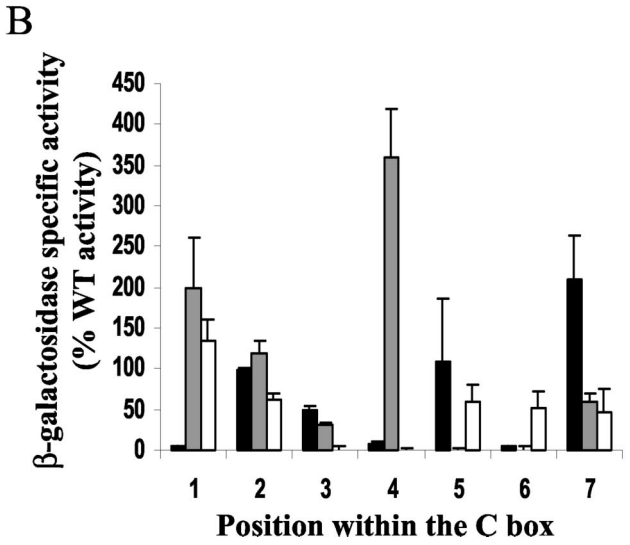


FIG. 3. Mutational analysis of the C box centered at −33 bp in the Ω4499 promoter. (A) Developmental *lacZ* expression was measured for *M. xanthus* strains with a T-to-G change at −30 bp (Δ) or a C-to-A change at −36 bp (◆). The wild-type promoter region from −218 to +50 bp (■) (seven determinations in three experiments) and the vector with no insert (●) were included as controls. The meaning of points and error bars is the same as described in the Fig. 1 legend. (B) Comparison of the effects of single-base-pair mutations in three different C boxes. The *x* axis represents the position in the C box corresponding to the consensus sequence 5′-CAYYCCY-3′, with the A being position 2, etc. The bars represent the average maximum developmental *lacZ* activity expressed as a percentage of the wild-type (WT) promoter activity for the C boxes centered at −49 bp in the Ω4400 (black) (56) and Ω4403 (gray) (53) promoter regions, or centered at −33 bp in the Ω4499 promoter region (white) (Table 2). Error bars show 1 standard deviation of the data.

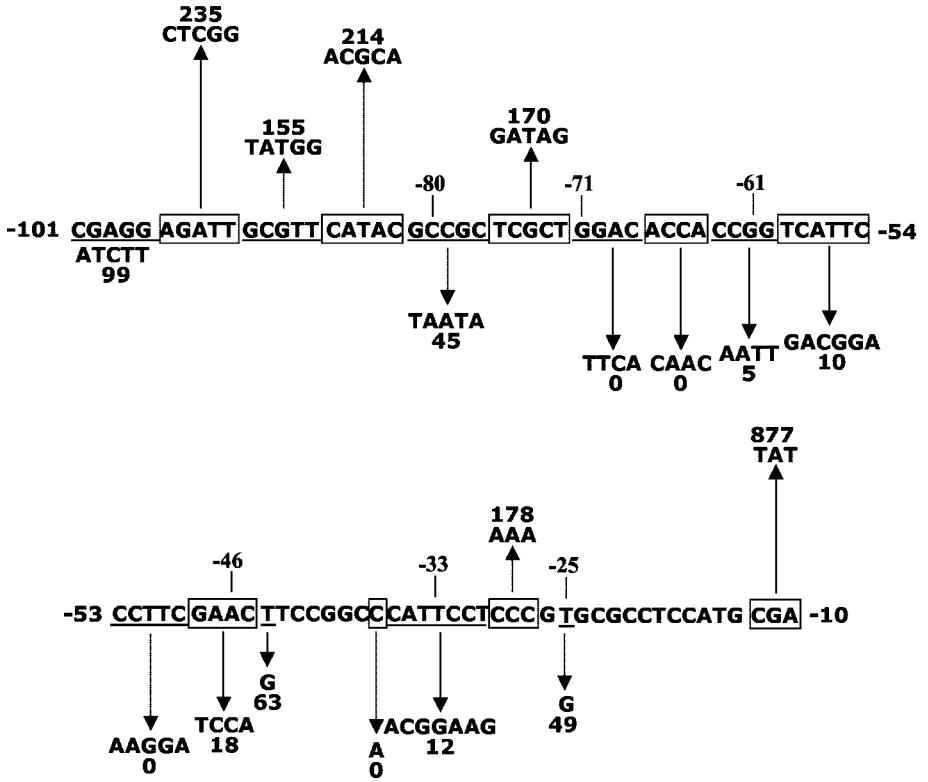


FIG. 4. Summary of the effects of mutations in the Ω4499 promoter region. DNA subjected to mutagenesis is alternately underlined and boxed. Upward and downward arrows indicate that developmental *lacZ* expression was increased or decreased, respectively, by the given change in DNA sequence, and numbers indicate the maximum β-galactosidase specific activity observed for the mutant, expressed as a percentage of wild-type promoter activity measured in the same experiment (Table 2).

stream of the 5-bp element exerts a twofold positive effect on expression from the Ω4499 promoter.

C-signal dependence of the Ω4499 promoter. The Ω4499 promoter is partially dependent on C-signaling for expression (8, 31). In a *csgA* mutant defective in C-signaling, a twofold decrease in Ω4499 promoter activity has been observed. The loss in activity can be restored upon codevelopment with wild-type cells, which provide C-signal. Since a 5′ deletion to −71 bp resulted in about a twofold loss in expression (Fig. 1B and Table 2), we hypothesized that DNA upstream of −71 bp might be responsible for the partial C-signal dependence of the Ω4499 promoter, especially since DNA from −86 to −81 bp was shown previously to mediate, at least in part, the partial C-signal dependence of the Ω4400 promoter (56). We transformed pDY103, containing the Ω4499 promoter region from −71 to +50 bp, into *csgA* mutant *M. xanthus* DK5208 cells and measured developmental *lacZ* expression (Fig. 5). β-Galactosidase specific activity was lower in the *csgA* mutant than in the wild-type background, indicating that the 5′-deleted promoter region remains dependent on *csgA*. Addition of wild-type cells to the *csgA* mutant restored *lacZ* expression during development. This demonstrates that the promoter region remains responsive to extracellular C-signal despite the absence of DNA beyond −71 bp upstream. Similar results were observed for pDY134, which contains the GCCGC-to-TAATA mutation from −81 to −77 bp in the context of the Ω4499 promoter region from −218 to +50 bp (data not shown). Although this mutation causes a twofold decrease in expression in a wild-type background (Fig. 4 and Table 2), the mutant promoter region remains dependent on *csgA* and responsive to extracellular C-signal. We conclude that DNA upstream of −71 bp is not responsible for the partial C-signal dependence of the Ω4499 promoter.

Effects of *sigD* and *sigE* mutations. Our mutational analysis suggests that the Ω4499 promoter is recognized by an σ factor in the σ^{70} family, rather than by σ^{54}. σ^{A} RNA polymerase, the major form in growing cells (3), was unable to produce transcripts from the Ω4499 promoter in vitro (8). Also, a null mutation in *sigB* (encoding σ^{B}) or *sigC* (encoding σ^{C}) had no effect on Ω4499 expression (4). We tested the effect of a null mutation in *sigD* (51) or *sigE* (52) on expression from the wild-type Ω4499 promoter region (−218 to +50 bp). Both mutations led to decreased expression from the Ω4499 promoter, at about 30% of the wild-type level (Fig. 6). These results demonstrate that σ^{D} and σ^{E} directly or indirectly affect the activity of the Ω4499 promoter.

DISCUSSION

Our characterization of the *cis* elements required for activity of the Ω4499 promoter provides further insight into C-signal-dependent gene regulation during *M. xanthus* development, especially when compared with previous mutational analyses of other promoter regions that depend on C-signaling for expres-

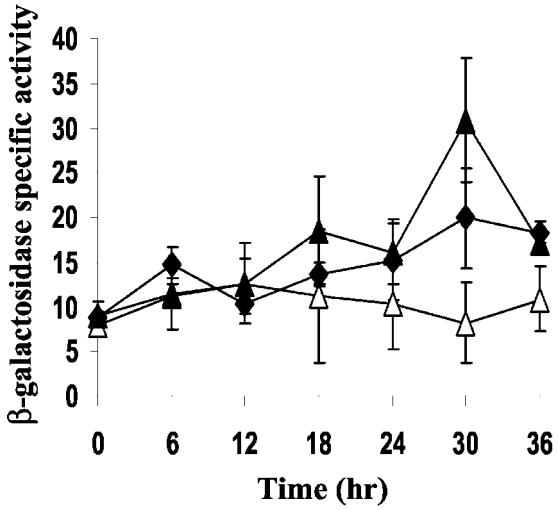


FIG. 5. C-signal dependence of a 5′-deleted Ω4499 promoter region. Developmental *lacZ* expression of pDY103 integrated at *attB* of wild-type DK1622 (◆) or *csgA* mutant DK5208 in the absence (Δ) or presence (▲) of an equal number of wild-type DK1622 cells (lacking *lacZ* but capable of C signaling). The meaning of points and error bars is the same as described in the Fig. 1 legend.

sion (53, 56). The other C-signal-dependent promoters examined so far do not resemble those thought to be recognized by σ^{54} RNA polymerase of *M. xanthus*. Our mutational analysis suggests that the Ω4499 promoter is not recognized by σ^{54} RNA polymerase either. The overall organization of the Ω4499 promoter region is much like that of the Ω4400 promoter region (56). Both include a large region spanning from about −30 to −60 or −70 bp with many sequence elements essential for promoter activity. Both also have a short (5- to 6-bp) region farther upstream (near −81 bp) that exerts a twofold positive effect on expression. Also, expression from both is reduced comparably in a *sigE* mutant. However, our

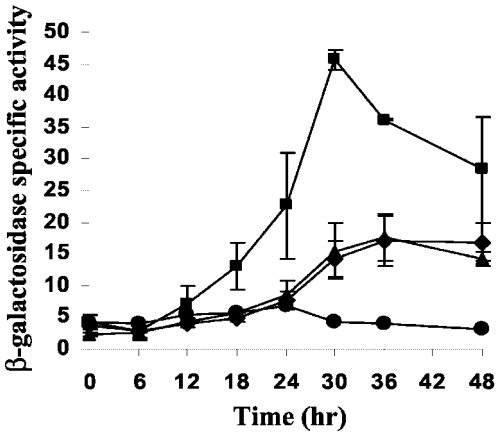


FIG. 6. Effects of *sigD* and *sigE* mutations on expression from the Ω4499 promoter. Developmental β-galactosidase activity was determined for the wild-type Ω4499 promoter region from −218 to +50 bp fused to *lacZ* and integrated into the chromosome of *M. xanthus sigD* (◆), *sigE* (▲), or wild-type DK1622 (■) strains. The vector with no insert (●) served as a negative control. The meaning of points and error bars is the same as that described in the Fig. 1 legend.

results also reveal unique properties of Ω4499 promoter regulation. The effects of mutations in the C boxes are different than has been observed for other C-signal-dependent promoter regions. The short region near −81 bp does not appear to be necessary for C-signal dependence of the Ω4499 promoter, as it is for the Ω4400 promoter. Also, whereas a *sigD* mutation eliminates expression from the Ω4400 promoter, it does not completely abolish Ω4499 expression. We conclude that regulation of the Ω4499 operon exhibits both shared and unique properties in comparison with regulation of other C-signal-dependent genes.

Despite a resemblance between the Ω4499 promoter and *M. xanthus* promoters that are thought to be recognized by σ^{54} RNA polymerase, our mutational analysis did not support the idea that σ^{54} RNA polymerase is responsible for transcription form the Ω4499 promoter. In the alignment shown in Fig. 2A, none of the putative σ^{54}-dependent promoters have a T at the position corresponding to the T at −25 bp in the Ω4499 promoter. Five out of seven have a G at that position, as does the *E. coli* σ^{54} consensus sequence (49). A mutation from T to G at −25 bp was expected to increase, or possibly not change, activity of the Ω4499 promoter, if it were recognized by σ^{54} RNA polymerase. However, the mutation led to a twofold loss in activity (Fig. 2B and Table 2). Conversely, mutating the perfectly conserved C at −12 bp in the Ω4499 promoter was expected to decrease activity. Instead, changing CGA to TAT at −12 to −10 bp led to an eightfold increase in activity (Fig. 2B and Table 2). Taken together, the two results suggest that the Ω4499 promoter is not recognized by σ^{54} RNA polymerase. These findings call into question whether all of the promoters shown in Fig. 2A really are σ^{54}-dependent promoters. Only the *spi* promoter has been subjected to detailed mutational analysis, and the results support the idea that this promoter is recognized by σ^{54} RNA polymerase (26).

Why did the CGA-to-TAT change at −12 to −10 bp increase activity of the Ω4499 promoter? The change creates a perfect match in the −10 region of the mutant promoter to the consensus sequence recognized by *E. coli* σ^{70} RNA polymerase (38). Therefore, the high activity of the mutant promoter could reflect better recognition and/or initiation by RNA polymerase with a σ factor in the σ^{70} family. Its is noteworthy that the mutant promoter was no more active during growth than the wild-type promoter (Fig. 2B). Also, the time of maximum *lacZ* expression from the mutant promoter was similar to that for the wild-type promoter (Fig. 2B). Whether the mutant promoter is transcribed by RNA polymerase(s) with the same σ factor(s) as the wild-type Ω4499 promoter remains an open question.

The Ω4400 and Ω4403 promoters, which are the only other C-signal-dependent promoters so far characterized, do not resemble σ^{54} promoters (4, 9). Neither these promoters (D. Biran and L. Kroos, unpublished data) nor the Ω4499 promoter (8) directed transcription by *M. xanthus* σ^{A} RNA polymerase in vitro. σ^{A} RNA polymerase is the major form of RNA polymerase in growing *M. xanthus* cells (3). It was able to transcribe from the Ω4514 promoter in vitro, but this developmentally regulated promoter does not depend on C-signaling for expression, and its −35 region matches perfectly the consensus sequence (TTGACA) recognized by *E. coli* σ^{70} RNA polymerase (17). In contrast, the −35 regions of the three

C-signal-dependent promoters do not match this consensus sequence (4, 8, 9). One or more transcription factors bound to upstream DNA elements in the C-signal-dependent promoter regions might enable σ^A RNA polymerase to transcribe from these promoters, or a different σ factor might be involved.

In addition to σ^A, six other σ factors in the σ^{70} family have been described in *M. xanthus* (1, 2, 5, 20, 51, 52, 54). Among these, σ^B and σ^C do not appear to be responsible for transcription of Ω4499, Ω4400, or Ω4403, since *sigB* and *sigC* mutants exhibited normal expression of *lacZ* reporters fused to these genes (4). On the other hand, *sigD* and *sigE* mutants showed reduced expression from the Ω4499 promoter (Fig. 6). Since mutations in *sigD* block aggregation (51), the effect on Ω4499 expression might be indirect. Interestingly, in the *sigD* mutant, the Ω4499 promoter retained 30% as much activity as in wild type (Fig. 6), whereas the Ω4400 promoter retained no activity (56). Apparently, one or more transcription factors essential for Ω4400 promoter activity is missing, or its level is insufficient, in the *sigD* mutant, but this does not prevent a low level of transcription from the Ω4499 promoter. Unlike the *sigD* mutant, the *sigE* mutant appears to aggregate normally (52). Yet, Ω4499 expression was reduced in the *sigE* mutant to a similar extent as in the *sigD* mutant (Fig. 6). The reduction in Ω4499 expression in the *sigE* mutant is comparable to that seen previously for expression from the Ω4400 promoter (56). This may imply that σ^E RNA polymerase is partially responsible for transcription from the Ω4499 and Ω4400 promoters. The proposed functional redundancy of σ^E with the highly similar σ^B and σ^C (52) may account for the residual transcription observed in the *sigE* mutant (Fig. 6). Alternatively, the effect of the *sigE* mutation on Ω4499 expression may be indirect.

The Ω4499 promoter region is unique among C-signal-dependent promoters examined thus far in terms of the positions of C boxes and 5-bp elements. It has C boxes centered at −33 and −55 bp (8) with 5-bp elements located 7 and 8 bp upstream, respectively (53). There is also a C box centered at −1 bp (8), but there is no apparent 5-bp element 5 to 10 bp upstream, and we did not test the effects of mutations in this C box. The Ω4400 and Ω4403 promoter regions have the identical C box (CATCCCT) centered at −49 bp, and in each case a 5-bp element is located 6 bp upstream, centered at −61 bp (53). Also, the Ω4400 promoter region has a C box centered at −80 bp, which is in the opposite orientation as the one centered at −49 bp (8), and has no apparent 5-bp element located 5 to 10 bp away in the 5′ direction.

We chose to perform detailed mutational analysis of the C box centered at −33 bp in the Ω4499 promoter region because it matches the C boxes centered at −49 bp in the Ω4400 and Ω4403 promoter regions more closely (six out of seven positions) than does the C box centered at −55 bp (five out of seven positions), and because its distance from the 5-bp element was more similar to that in the Ω4400 and Ω4403 promoter regions (7 bp versus 6 bp) than for the C box centered at −55 bp (8 bp versus 6 bp). However, we found that single-base-pair changes in the Ω4499 C box centered at −33 bp had a very different pattern of effects on *lacZ* expression than did changes in the Ω4400 or Ω4403 C box centered at −49 bp (Fig. 3B), or the Ω4400 C box centered at −80 bp (53, 56). Each C box appears to function somewhat differently. Conceivably, the Ω4499 C box centered at −55 bp might behave in a more similar fashion to one of the other C boxes, but that would be a break from the results observed so far, and it remains to be tested.

In keeping with the observation of different effects of mutations in similar sequences, each 5-bp element examined so far behaves differently with respect to single-base-pair changes, although the mutational analysis is much less complete than for C boxes. In this study, a T-to-G change at −44 bp had relatively little effect on Ω4499 promoter activity (Fig. 4 and Table 2) in comparison to changes at the corresponding position (−59 bp) of the 5-bp elements centered at −61 bp in the Ω4400 and Ω4403 promoter regions (53, 56). In prior studies, the effects of changing C to A at −60 bp in the Ω4400 and Ω4403 promoter regions were shown to be very different (53, 56).

Given that different effects of mutations in similar sequences is observed for both the 5-bp elements and the C boxes and given the similar distance between these *cis*-acting DNA elements in all three C-signal dependent promoters examined so far, we propose that a 5-bp element and a C box together constitute a recognition site for a transcription factor and that different transcription factors bind to these recognition sites in the Ω4499, Ω4400, and Ω4403 promoter regions.

The DNA between the C box and the 5-bp element may be part of a transcription factor recognition site in some cases, but not others. Changing CCGG to AATT between the C box centered at −55 bp in the Ω4499 promoter region and the 5-bp element that lies 8 bp upstream nearly abolished expression (Fig. 4 and Table 2). Likewise, changing the C at −37 bp, which is the first base pair upstream of the Ω4499 C box centered at −33 bp, abolished promoter activity (Fig. 4 and Table 2). A single-base-pair change at the position immediately upstream of the C box centered at −49 bp in the Ω4400 or Ω4403 promoter region also greatly reduced expression, as did a change from GTCCC to TGAAA between the Ω4400 C box centered at −49 bp and the 5-bp element centered at −61 bp (53, 56). On the other hand, changing CCGTC to AATGA at the corresponding position in the Ω4403 promoter region caused a 1.5-fold increase in expression and deleting the CCGTC segment abolished promoter activity (53), suggesting that the segment is an essential spacer between the C box and the 5-bp element but may not be part of a recognition site for a sequence-specific DNA-binding protein.

If our hypothesis that a 5-bp element and a C box (and in some cases the DNA in between) together constitute a recognition site for a transcription factor is correct, it is intriguing that the Ω4499 promoter regions has two such sites in tandem. The more upstream site is located upstream of the region typically occupied by RNA polymerase, while the downstream site overlaps the promoter −35 region. Hence, the upstream and downstream sites are located at positions occupied by the *E. coli* catabolite activator protein (CAP) in class I and class II CAP-dependent promoters (6). The basic features of transcription activation at class I and class II CAP-dependent promoters are understood and appear to be generalizable to other activators. Perhaps one or more transcription factors bind to the putative two sites in the Ω4499 promoter region and activate transcription by contacting RNA polymerase, facilitating formation of closed and open RNA polymerase-promoter complexes, as does CAP. According to this model, the C boxes

centered at −49 bp and 5-bp elements centered at −61 bp in the Ω4400 and Ω4403 promoter regions would each constitute a single site located at a position analogous to that occupied by CAP in class I CAP-dependent promoters. Based on the different effects of mutations in these putative transcription factor recognition sites (Fig. 3B), we speculate that a family of sequence-specific DNA-binding proteins might interact in different ways with similar sequences in the three C-signal-dependent promoter regions. Alternatively, a single protein might bind differently to the putative recognition sites by adopting different conformations, possibly due to different states of posttranslational modification, interactions with other proteins, and/or the influence of DNA adjacent to the putative recognition sites.

The Ω4499 promoter region shares with Ω4400 and Ω4403 promoter regions the requirement for DNA farther upstream, beyond the 5-bp elements, for full promoter activity. In each case, these DNA elements are separated from the 5-bp elements by 5 to 17 bp of DNA in which transversion mutations have little effect on promoter activity (Fig. 4 and Table 2) (53, 56). Both the Ω4499 and Ω4400 promoter regions contain a small element near −81 bp that exerts a twofold to fourfold positive effect on expression. The boundaries of these elements are not well defined. In the Ω4499 promoter region, the element is defined by a mutation that changes GCCGC to TAATA at −81 to −77 bp, resulting in a twofold decrease in promoter activity (Fig. 4 and Table 2). In the Ω4400 promoter region, mutations that change GTC to TGA at −86 to −84 bp, and G to T at −81 bp, result in a fourfold and a twofold decrease in activity, respectively, defining an element with the sequence GTCGGG (56). This sequence is not strikingly similar to the GCCGC sequence in the Ω4499 promoter region. Both are GC rich, but such sequences are common in the *M. xanthus* genome with its high (near 70%) G+C content. In the Ω4403 promoter region, the sequence GGCATGTTCA from −79 to −70 bp has been called a 10-bp element (53). Single-base-pair transversions at any position in this element decrease expression more than twofold, and many abolish expression completely.

The element from −86 to −81 bp in the Ω4400 promoter region was shown to be responsible, at least in part, for the partial dependence of the promoter on C-signaling (56). It was attractive to think that the element from −81 to −77 bp in the Ω4499 promoter region might play the same role, since activity of this promoter also depends partially on C-signaling (8, 31). However, this does not appear to be the case. A segment lacking Ω4499 DNA upstream of −71 bp was still C-signal dependent (Fig. 5). Another candidate sequence to mediate C-signal dependence of the Ω4499 promoter was a 9-bp sequence centered at −65 bp, which matches a 9-bp sequence centered at −80 bp in the Ω4400 promoter region (8). However, transversion mutations at −80 to −76 bp had little effect on Ω4400 promoter activity (56) and, in contrast, mutations at −67 to −60 bp nearly abolished Ω4499 promoter activity (Fig. 4 and Table 2), and so despite their similarity, the 9-bp sequences function differently. Further studies of the Ω4499 promoter region will aim to identify and characterize the *trans*-acting factors that bind to the important *cis*-acting DNA elements defined by our mutational analysis. There do not appear to be binding sites in the Ω4499 promoter for an NtrC-like activator (42) such as ActB (14), or for the CAP-like activator MrpC (50), or for protein X (19). Of the putative *M. xanthus* transcription factors, FruA (7, 40) is the best candidate for a protein that binds to the Ω4499 regulatory region; however, FruA has not yet been reported to bind DNA.

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REFERENCES

1. **Apelian, D., and S. Inouye.** 1990. Development-specific σ-factor essential for late-stage differentiation of *Myxococcus xanthus.* Genes Dev. **4:**1396–1403.
2. **Apelian, D., and S. Inouye.** 1993. A new putative sigma factor of *Myxococcus xanthus.* J. Bacteriol. **175:**3335–3342.
3. **Biran, D., and L. Kroos.**1997. In vitro transcription of *Myxococcus xanthus* genes with RNA polymerase containing σ^A, the major sigma factor in growing cells. Mol. Microbiol. **25:**463–472.
4. **Brandner, J. P., and L. Kroos.** 1998. Identification of the Ω4400 regulatory region, a developmental promoter of *Myxococcus xanthus.* J. Bacteriol. **180:** 1995–2004.
5. **Browning, D. F., D. E. Whitworth, and D. A. Hodgson.** 2003. Light-induced carotenogenesis in *Myxococcus xanthus*: functional characterization of the ECF sigma factor CarQ and antisigma factor CarR. Mol. Microbiol. **48:**237–251.
6. **Busby, S., and R. H. Ebright.** 1999. Transcription activation by catabolite activator protein (CAP). J. Mol. Biol. **293:**199–213.
7. **Ellehauge, E., M. Norregaard-Madsen, and L. Sogaard-Andersen.** 1998. The FruA signal transduction protein provides a checkpoint for the temporal co-ordination of intercellular signals in *Myxococcus xanthus* development. Mol. Microbiol. **30:**807–817.
8. **Fisseha, M., D. Biran, and L. Kroos.** 1999. Identification of the Ω4499 regulatory region controlling developmental expression of a *Myxococcus xanthus* cytochrome P-450 system. J. Bacteriol. **181:**5467–5475.
9. **Fisseha, M., M. Gloudemans, R. Gill, and L. Kroos.** 1996. Characterization of the regulatory region of a cell interaction-dependent gene in *Myxococcus xanthus.* J. Bacteriol. **178:**2539–2550.
10. **Garza, A. G., B. Z. Harris, B. M. Greenberg, and M. Singer.** 2000. Control of *asgE* expression during growth and development of *Myxococcus xanthus.* J. Bacteriol. **182:**6622–6629.
11. **Garza, A. G., J. S. Pollack, B. Z. Harris, A. Lee, I. M. Keseler, E. F. Licking, and M. Singer.** 1998. SdeK is required for early fruiting body development in *Myxococcus xanthus.* J. Bacteriol. **180:**4628–4637.
12. **Gill, R. E., and M. G. Cull.** 1986. Control of developmental gene expression by cell-to-cell interactions in *Myxococcus xanthus.* J. Bacteriol. **168:**341–347.
13. **Gronewold, T. M., and D. Kaiser.** 2002. *act* operon control of developmental gene expression in *Myxococcus xanthus.* J. Bacteriol. **184:**1172–1179.
14. **Gronewold, T. M., and D. Kaiser.** 2001. The *act* operon controls the level and time of C-signal production for *Myxococcus xanthus* development. Mol. Microbiol. **40:**744–756.
15. **Gulati, P., D. Xu, and H. Kaplan.** 1995. Identification of the minimum regulatory region of a *Myxococcus xanthus* A-signal-dependent developmental gene. J. Bacteriol. **177:**4645–4651.
16. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
17. **Hao, T., D. Biran, G. J. Velicer, and L. Kroos.** 2002. Identification of the Ω4514 regulatory region, a developmental promoter of *Myxococcus xanthus* that is transcribed in vitro by the major vegetative RNA polymerase. J. Bacteriol. **184:**3348–3359.
18. **Hodgkin, J., and D. Kaiser.** 1977. Cell-to-cell stimulation of motility in nonmotile mutants of *Myxococcus.* Proc. Natl. Acad. Sci. USA **74:**2938–2942.
19. **Horiuchi, T., T. Akiyama, S. Inouye, and T. Komano.** 2003. Regulation of FruA expression during vegetative growth and development of *Myxococcus xanthus.* J. Mol. Microbiol. Biotechnol. **5:**87–96.
20. **Inouye, S.** 1990. Cloning and DNA sequence of the gene coding for the major sigma factor from *Myxococcus xanthus.* J. Bacteriol. **172:**80–85.
21. **Kaiser, D.** 2003. Coupling cell movement to multicellular development in myxobacteria. Nat. Rev. Microbiol. **1:**45–54.
22. **Kaiser, D.** 1979. Social gliding is correlated with the presence of pili in *Myxococcus xanthus.* Proc. Natl. Acad. Sci. USA **76:**5952–5956.
23. **Kaplan, H.** 2003. Multicellular development and gliding motility in *Myxococcus xanthus.* Curr. Opin. Microbiol. **6:**572–577.
24. **Kaplan, H. B., and L. Plamann.** 1996. A *Myxococcus xanthus* cell density-sensing system required for multicellular development. FEMS Microbiol. Lett. **139:**89–95.
25. **Kashefi, K., and P. Hartzell.** 1995. Genetic suppression and phenotypic masking of a *Myxococcus xanthux* frzF$^-$ defect. Mol. Microbiol. **15:**483–494.

26. **Keseler, I., and D. Kaiser.** 1995. An early A-signal-dependent gene in *Myxococcus xanthus* has a σ^{54}-like promoter. J. Bacteriol. **177:**4638–4644.
27. **Keseler, I., and D. Kaiser.** 1997. σ^{54}, a vital protein for *Myxococcus xanthus.* Proc. Natl. Acad. Sci. USA **94:**1979–1984.
28. **Kim, S. K., and D. Kaiser.** 1991. C-factor has distinct aggregation and sporulation thresholds during *Myxococcus* development. J. Bacteriol. **173:** 1722–1728.
29. **Kim, S. K., and D. Kaiser.** 1990. C-factor: a cell-cell signaling protein required for fruiting body morphogenesis of *M. xanthus.* Cell **61:**19–26.
30. **Kroos, L., and D. Kaiser.** 1984. Construction of Tn*5* *lac*, a transposon that fuses *lacZ* expression to exogenous promoters, and its introduction into *Myxococcus xanthus.* Proc. Natl. Acad. Sci. USA **81:**5816–5820.
31. **Kroos, L., and D. Kaiser.** 1987. Expression of many developmentally regulated genes in *Myxococcus* depends on a sequence of cell interactions. Genes Dev. **1:**840–854.
32. **Kroos, L., A. Kuspa, and D. Kaiser.** 1986. A global analysis of developmentally regulated genes in *Myxococcus xanthus.* Dev. Biol. **117:**252–266.
33. **Kuspa, A., L. Kroos, and D. Kaiser.** 1986. Intercellular signaling is required for developmental gene expression in *Myxococcus xanthus.* Dev. Biol. **117:** 267–276.
34. **Kuspa, A., L. Plamann, and D. Kaiser.** 1992. Identification of heat-stable A-factor from *Myxococcus xanthus.* J. Bacteriol. **174:**3319–3326.
35. **Kuspa, A., L. Plamann, and D. Kaiser.** 1992. A-signalling and the cell density requirement for *Myxococcus xanthus* development. J. Bacteriol. **174:**7360–7369.
36. **Lee, B.-U., K. Lee, J. Mendez, and L. Shimkets.** 1995. A tactile sensory system of *Myxococcus xanthus* involves an extracellular NAD(P)$^+$-containing protein. Genes Dev. **9:**2964–2973.
37. **Li, S.-F., B. Lee, and L. J. Shimkets.** 1992. *csgA* expression entrains *Myxococcus xanthus* development. Genes Dev. **6:**401–410.
38. **Lisser, S., and H. Margalit.** 1993. Compilation of *E. coli* mRNA promoter sequences. Nucleic Acids Res. **21:**1507–1516.
39. **Lobedanz, S., and L. Sogaard-Andersen.** 2003. Identification of the C-signal, a contact-dependent morphogen coordinating multiple developmental responses in *Myxococcus xanthus.* Genes Dev. **17:**2151–2161.
40. **Ogawa, M., S. Fujitani, X. Mao, S. Inouye, and T. Komano.** 1996. FruA, a putative transcription factor essential for the development of *Myxococcus xanthus.* Mol. Microbiol. **22:**757–767.
41. **Plamann, L., A. Kuspa, and D. Kaiser.** 1992. Proteins that rescue A-signal-defective mutants of *Myxococcus xanthus.* J. Bacteriol. **174:**3311–3318.
42. **Reitzer, L. J., and B. Magasanik.** 1986. Transcription of *glnA* in *E. coli* is stimulated by activator bound to sites far from the promoter. Cell **45:**785–792.
43. **Romeo, J. M., and D. R. Zusman.** 1991. Transcription of the myxobacterial hemagglutinin gene is mediated by a F^{54}-like promoter and a *cis*-acting upstream regulatory region of DNA. J. Bacteriol. **173:**2969–2976.
44. **Sambrook, J., E. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
45. **Shimkets, L. J.** 1999. Intercellular signaling during fruiting-body development of *Myxococcus xanthus.* Annu. Rev. Microbiol. **53:**525–549.
46. **Shimkets, L. J., and S. J. Asher.** 1988. Use of recombination techniques to examine the structure of the *csg* locus of *Myxococcus xanthus.* Mol. Gen. Genet. **211:**63–71.
47. **Shimkets, L. J., R. E. Gill, and D. Kaiser.** 1983. Developmental cell interactions in *Myxococcus xanthus* and the *spoC* locus. Proc. Natl. Acad. Sci. USA **80:**1406–1410.
48. **Sogaard-Andersen, L., M. Overgaard, S. Lobedanz, E. Ellehauge, L. Jelsbak, and A. A. Rasmussen.** 2003. Coupling gene expression and multicellular morphogenesis during fruiting body formation in *Myxococcus xanthus.* Mol. Microbiol. **48:**1–8.
49. **Thony, B., and H. Hennecke.** 1989. The −24/−12 promoter comes of age. FEMS Microbiol. Rev. **5:**341–357.
50. **Ueki, T., and S. Inouye.** 2003. Identification of an activator protein required for the induction of *fruA*, a gene essential for fruiting body development in *Myxococcus xanthus.* Proc. Natl. Acad. Sci. USA **100:**8782–8787.
51. **Ueki, T., and S. Inouye.** 1998. A new sigma factor, SigD, essential for stationary phase is also required for multicellular differentiation in *Myxococcus xanthus.* Genes Cells **3:**371–385.
52. **Ueki, T., and S. Inouye.** 2001. SigB, SigC, and SigE from *Myxococcus xanthus* homologous to σ^{32} are not required for heat shock response but for multicellular differentiation. J. Mol. Microbiol. Biotechnol. **3:**287–293.
53. **Viswanathan, P., and L. Kroos.** 2003. *cis* elements necessary for developmental expression of a *Myxococcus xanthus* gene that depends on C signaling. J. Bacteriol. **185:**1405–1414.
54. **Ward, M., H. Lew, A. Treuner-Lange, and D. Zusman.** 1998. Regulation of motility behavior in *Myxococcus xanthus* may require an extracytoplasmic-function sigma factor. J. Bacteriol. **180:**5668–5675.
55. **Wu, S. S., and D. Kaiser.** 1997. Regulation of expression of the *pilA* gene in *Myxococcus xanthus.* J. Bacteriol. **179:**7748–7758.
56. **Yoder, D., and L. Kroos.** 2004. Mutational analysis of the *Myxococcus xanthus* Ω4400 promoter region provides insight into developmental gene regulation by C signaling. J. Bacteriol. **186:**661–671.