A combination of unusual transcription factors binds cooperatively to control *Myxococcus xanthus* developmental gene expression

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*Myxococcus xanthus* is a bacterium that undergoes multicellular development requiring coordinate regulation of multiple signaling pathways. One pathway governs aggregation and sporulation of some cells in a starving population and requires C-signaling, whereas another pathway causes programmed cell death and requires the MazF toxin. In response to starvation, the levels of the bifunctional transcription factor/antitoxin MrpC and its related proteolytic fragment MrpC2 are increased, inhibiting the cell death pathway via direct interaction of MrpC with MazF. Herein, we demonstrate that MrpC2 plays a direct role in the transcriptional response to C-signaling. We show that MrpC2 binds to sequences upstream of the C-signal-dependent *fmgA* promoter. These sequences are present in other C-signal-dependent promoter regions, indicating a general role for MrpC2 in developmental gene regulation. Association of MrpC and/or MrpC2 with the *fmgA* promoter region in vivo requires FruA, a protein that is similar to response regulators of 2-component signal transduction systems, but may not be phosphorylated. DNA binding studies showed that this association likely involves an unusual mechanism for a response regulator in which FruA and MrpC2 bind cooperatively to adjacent sites upstream of the *fmgA* promoter. We propose that this unusual mechanism of combinatorial control allows coordination of morphogenetic C-signaling with starvation signaling and cell death, determining spatiotemporal gene expression and cell fate.

**Understanding how cells integrate many different signals to regulate genes and determine cell fates during multicellular development is a fundamental question.** *Myxococcus xanthus* provides an attractive model to address this question because starvation initiates a relatively simple developmental process (1). Thousands of rod-shaped cells coordinate their movements to build fruiting bodies in which cells differentiate into dormant, spherical spores [supporting information (SI) Fig. S1]. However, not all cells form spores. Alternative fates are programmed cell death (PCD) (2) or persistence outside of fruiting bodies as peripheral rods (3).

Signals act at different times during the *M. xanthus* developmental process to control gene expression, coordinate cell movements, and determine cell fates. Starvation triggers the stringent response, which involves production of the second messenger (p)ppGpp (Fig. S1), and leads to activation of early developmental genes and secretion of protease activity that generates a mixture of peptides and amino acids known as A-signal (4). Extracellular A-signaling governs expression of many additional genes, including the *mrp* operon (Fig. S1) (5). Later, when cells begin to aggregate, C-signaling takes over. The C-signal appears to be a proteolytic cleavage product of CsgA that is associated with the cell surface (6–8). Because C-signaling requires cell alignment (9) and possibly end-to-end contact, it is paracrine or short-range signaling, which is common in eukaryotes but rare among bacteria (10). The short-range nature of C-signaling and its effects on cell movement and gene expression can explain its critical role in coordinating aggregation with sporulation (4, 11, 12). Cell alignment within a nascent fruiting body has been proposed to allow a high level of C-signaling and activation of genes required for sporulation.

FruA plays an important role in regulating genes important for aggregation and sporulation (Fig. S1). FruA is similar to response regulators of 2-component signal transduction systems (13). The N-terminal domain of FruA was proposed to be phosphorylated in response to C-signal (14), but the domain lacks 2 aspartate residues that are normally important for phosphorylation of a third aspartate residue, and the putative histidine protein kinase (HPK) has not been identified despite considerable effort. The C-terminal domain of FruA has been shown to bind to sites in the promoter regions of developmentally regulated genes (15–17). FruA positively regulates expression of these genes, but in the case of *fmgA* (formerly referred to as the Ω4400 locus), mutational analysis of the promoter region implied that an additional transcriptional activator is required (18).

Here, we report identification of the activator as MrpC2 and we name the gene at the Ω4400 locus *fmgA* (FruA- and MrpC2-regulated gene A). MrpC2 lacks the N-terminal 25 residues of MrpC and might be generated by proteolytic activity of a developmentally regulated protease (LonD) (Fig. S1) (19). MrpC is similar to transcription factors in the cAMP receptor protein (CRP) family (20), but a nucleotide effector has not been identified. Recently, MrpC was shown to interact with the toxin MazF, which mediates PCD during development (2). In addition to identifying MrpC2 as an activator of *fmgA* transcription, we show that FruA is required for association of MrpC and/or MrpC2 with the *fmgA* promoter region in vivo and that FruA and MrpC2 bind cooperatively to *fmgA* promoter region DNA in vitro. Cooperative binding of a response regulator and an independent transcription factor is an unusual mechanism of gene regulation. Preliminary results indicate that this mechanism is shared by other C-signal-dependent genes (see Discussion). We propose that cooperative binding of FruA and MrpC2 facilitates integration of positional information (via short-range C-signaling) with nutritional status and PCD, governing gene expression and cell fate, analogous to combinatorial control during development of multicellular eukaryotes.

**Results**

**MrpC2 Binds to a cis-Regulatory Sequence in the *fmgA* Promoter Region.** Transcription from the *fmgA* promoter is important for development because aggregation of *M. xanthus* DK4292 containing Tn5 lac Ω4400 was delayed by ~6 h compared to wild-type DK1622 (SI Text). Mutational analysis identified cis-regulatory sequences at −86 to −77 and −63 to −46 upstream of the *fmgA* promoter region DNA (Fig. S2). Cooperative binding of FruA and MrpC2 facilitated integration of positional information (via short-range C-signaling) with nutritional status and PCD, governing gene expression and cell fate, analogous to combinatorial control during development of multicellular eukaryotes.

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promoter (Fig. 1A) (18), and subsequent analysis showed that the FruA DNA-binding domain (FruA-DBD) binds to the sequence between −86 and −77 (17). The sequence between −63 and −46 contains 2 elements found in other C-signal-dependent promoter regions; a 5-bp element (consensus GAACA) and a C box (consensus CAYYCCY; Y means C or T) (21–23). Mutations in this region nearly abolish fmgA promoter activity (Fig. 1A) (18), suggesting that a transcriptional activator binds to this region and perhaps to similar sequences in other C-signal-dependent promoter regions. To identify the putative activator, DNA-binding proteins were partially purified as described previously (24) from M. xanthus that had undergone 12 h of development, a time when fmgA is expressed (18). Proteins in the AS fraction were incubated with a 32P-labeled DNA fragment (−101 to +25) spanning the fmgA promoter region, and electrophoretic mobility shift assays (EMSA) revealed a single shifted complex (Fig. 1A). EMSAs with DNA probes having a mutation between −63 and −46 eliminated or greatly reduced formation of the shifted complex, with the exception of the single base-pair change at −53, which also had a smaller effect on promoter activity in vivo (Fig. 1A) (18). The shifted complex appeared to be formed by a protein in the AS fraction that binds to sequences between −63 and −46 upstream of the fmgA promoter.

To purify the putative activator protein from the AS fraction, DNA-affinity chromatography was performed with the fmgA promoter region (−101 to +25). The major protein species after purification had an apparent molecular weight of 30 kDa (Fig. 1B). The affinity-purified protein (APP) generated a shifted complex indistinguishable from that observed with the AS fraction (Fig. 1C). Also, like the AS fraction (Fig. 1A), APP failed to generate a shifted complex with mutant (−63 to −60) fmgA promoter region DNA (Fig. 1C). Therefore, APP was subjected to mass spectrometry analysis after protease digestion. Peptide sequences matching MrpC were the only significant matches to M. xanthus proteins predicted from the genome sequence. MrpC is similar to CRP-family transcription factors and was shown previously to be essential for development (20). A form of MrpC lacking the N-terminal 25 residues, called MrpC2, was identified previously in an AS fraction based on binding to the fmgA promoter region (24). Our results suggested that MrpC2 in the AS fraction binds to the fmgA promoter region at a site (−63 to −46) important for promoter activity.

To test the idea that MrpC2 in the AS fraction was responsible for the shifted complex (Fig. 1A), antibodies against MrpC were added after the complex had been allowed to form. EMSAs revealed the formation of super-shifted complexes and loss of the original-shifted complex (Fig. S2), supporting the idea that MrpC2 in the AS fraction binds to fmgA promoter region DNA.

To confirm that MrpC2 binds to the fmgA promoter region, N-terminally His-tagged MrpC2 (His10-MrpC2) was expressed in E. coli and purified (Fig. S3A). His10-MrpC2 exhibited a similar pattern of binding to wild-type and mutant fmgA promoter-region DNA as seen with the AS fraction (Fig. 1D). The complex produced by His10-MrpC2 migrates more slowly than the complex produced by the AS fraction, presumably because of the 10 His residues plus 8 additional residues present in the His10-MrpC2 fusion protein. Mutations between −63 and −46 eliminated or reduced MrpC2 binding, with the exception of a single base-pair change at −53. These results, taken together with the effects of mutations in this region on fmgA promoter activity (Fig. 1A) (18), imply that MrpC2 binding to this region activates fmgA transcription. Because the region includes a 5-bp element and a C box, which are found in a similar arrangement upstream of other C-signal-dependent promoters (21–23), MrpC2 might directly activate other C-signal-dependent genes (see Discussion). Interestingly, mutations upstream of −63 appeared to enhance (−74 to −70) or reduce (−76 to −75) MrpC2 binding, whereas 2 mutations between −86 and −77, that impair binding of FruA-DBD (17), did not affect MrpC2 binding (Fig. 1D). We conclude that FruA and MrpC2 bind to adjacent, important, cis-regulatory sequences upstream of the fmgA promoter.

We tested nucleotides for an effect on DNA binding by MrpC2 because it is similar to CRP-family transcription factors (20); however, we found no evidence for a nucleotide effector (SI Text).

**FruA Is Required for Association of MrpC and/or MrpC2 with the fmgA Promoter Region in Vivo.** The proximity of the FruA and MrpC2 binding sites in the fmgA promoter region suggested that one protein might recruit the other or that the two proteins might bind cooperatively. Expression of fruA (24) depends on MrpC2 (Fig. S1) (24), so neither transcription factor is expected to accumulate in a mrpC mutant. However, MrpC and MrpC2 accumulate normally in a fruA mutant (19) (data not shown), yet fmgA fails to be expressed (17). Why are MrpC and MrpC2 insufficient to activate fmgA transcription? We hypothesized that MrpC and MrpC2 fail to bind to the fmgA promoter region in the absence of FruA. To test this...
Cooperative Binding of MrpC2 and FruA to the fmgA Promoter Region.

The requirement for FruA for association of MrpC and/or MrpC2 with the fmgA promoter region in vivo is consistent with recruitment or cooperative binding. To distinguish between these models and to test the notion that FruA directly affects binding of MrpC2 and/or cooperative binding. To distinguish between these models and to test the notion that FruA directly affects binding of MrpC2 and MrpC2 with the fmgA promoter region. The PCR analysis revealed that the fmgA promoter region was enriched by ChIP with antibodies against MrpC relative to control antibodies for wild type, but not for the fruA mutant (Fig. 2). Neither strain showed enrichment of rpoC coding region DNA (as a negative control). We conclude that FruA is required for association of MrpC and/or MrpC2 with the fmgA promoter region in vivo.

Interestingly, the C-terminal DNA-binding domain of FruA was insufficient to enhance shifted complex formation in combination with MrpC2. The complexes formed by the combination of proteins were indistinguishable from those produced when FruA-ACTHis6 or His10-MrpC2 alone was incubated with fmgA promoter region DNA (Fig. 3B). We conclude that the N-terminal domain of FruA contains an important determinant for enhanced formation of shifted complex formation in combination with MrpC2 and the fmgA promoter region.

To characterize the enhanced DNA binding observed in the presence of His10-MrpC2 and FruA-His6, DNase I footprinting of complexes in solution was performed. Protected and hypersensitive sites were observed with His10-MrpC2 alone in the region spanning from –84 to –39 (Fig. 4A), a slightly larger region than mapped by EMSAs (Fig. 1). The hypersensitive sites suggest that His10-MrpC2 bends the DNA on binding. Protection and hypersensitivity in this region increased when FruA-His6 was present in combination with His10-MrpC2, but was not observed with FruA-His6 alone, suggesting that His10-MrpC2 binding was increased in the presence of FruA-His6. DNase I footprinting of the other strand with FruA-His6 alone revealed a hypersensitive site near –91 (Fig. 5), slightly upstream of a region that was previously shown to bind FruA-ACTHis6 (17). The intensity of this hypersensitive site increased when His10-MrpC2 was present in combination with FruA-His6, but hypersensitivity was not observed with His10-MrpC2 alone, suggesting that FruA-His6 binding was increased in the presence of His10-MrpC2. As observed with the other strand (Fig. 4A), there were protected and hypersensitive sites downstream of –91 with His10-MrpC2 alone, and these signals increased in the presence of both proteins (Fig. 5). In addition, hypersensitive sites were observed near –74 and –63 when both proteins were present, but not with either protein alone (Fig. 5), suggesting simultaneous binding of MrpC2 and FruA to the same DNA molecule. The DNase I footprinting results are summarized in Fig. 4B. These results demonstrate cooperative binding of FruA and MrpC2 to the fmgA promoter region, providing plausible explanations for the observed dependence of MrpC and/or MrpC2 on FruA for association with the fmgA promoter region in vivo (Fig. 2) and for the observed enhancement of shifted complex formation in vitro (Fig. 3A and Fig. S4A).

To determine whether the binding sites for both His10-MrpC2 and FruA-His6 in the fmgA promoter region are important for enhanced formation of shifted complexes, EMSAs were performed with mutant DNA fragments expected to impair binding of one or
of the FruA-His6 binding site for enhanced formation of shifted complexes. Likewise, the His10-MrpC2 binding site is important, because a DNA fragment containing a mutation at −63 to −60, which eliminates detectable His10-MrpC2 binding, also abolished detectable enhancement of shifted complex formation (Fig. 5).

Furthermore, both binding sites must be on the same DNA fragment. No enhancement of shifted complex formation was observed when His10-MrpC2 and FruA-His6 were added to a mixture of 2 DNA fragments (only one of which was 32P-labeled in each of two separate experiments) capable of binding only His10-MrpC2 [the modified (+5 bp) −76 to −41 fragment] or only FruA-His6 (a fragment spanning from −101 to −64) (Fig. S6).

Supershift assays provided further evidence that both His10-MrpC2 and FruA-His6 are responsible for enhanced formation of shifted complexes with fmgA promoter region DNA (Fig. S7). These results support the interpretation that enhancement of shifted complex formation involves binding of both His10-MrpC2 and FruA-His6 to adjacent (possibly overlapping) sites upstream of the fmgA promoter, and, together with our footprinting and ChIP results, support a model in which FruA and MrpC2 bind cooperatively to regulate fmgA transcription during M. xanthus development.

Discussion

We have discovered that a crucial cis-regulatory element in the fmgA promoter region is bound by MrpC2 and that association of MrpC and/or MrpC2 with the fmgA promoter region in vivo requires FruA. Our DNA binding studies revealed cooperative binding of FruA and MrpC2 to adjacent (possibly overlapping) sites upstream of the fmgA promoter. Our preliminary results, described below, indicate that several other C-signal-dependent promoter regions are cooperatively bound by FruA and MrpC2.

Cooperative binding of a response regulator and an independent transcription factor is an unusual mechanism of gene regulation that has not been reported previously. Although FruA is similar to response regulators, it may be active without phosphorylation, and MrpC2 is a proteolytic fragment of MrpC, which functions not only as a transcription factor but also as an antitoxin in the regulation of PCD (2). Therefore, our evidence supports a model in which cooperative binding of 2 unusual transcription factors facilitates the coordination of multiple signaling pathways to ensure proper control of gene expression and cell fate during M. xanthus development.

Preliminary studies indicate that cooperative binding of MrpC2 and FruA is a conserved mechanism of gene regulation in response to C-signaling during M. xanthus development. The cis-regulatory element to which MrpC2 binds in the fmgA promoter region includes a 5-bp element and a C-box. These 2 sequences are similarly arranged upstream of other C-signal-dependent promoters and are important for promoter activity (21–23), suggesting that MrpC2 may bind to these sites. Indeed, in the promoter region of the operon identified by Tn5 lac O4499 (22), MrpC2 binds near a 5-bp element, and in combination with FruA, formation of shifted complexes in EMSAs is greatly enhanced (unpublished data). In the promoter region of the dev operon (23), whose products are important for sporulation, MrpC2 binds to a region that includes a 5-bp element and 2 C-box-like sequences, and addition of FruA greatly enhances complex formation in EMSAs (S.M., P. Viswanathan, and L.K., unpublished data). In the promoter region of the gene identified by Tn5 lac O4403 (21), MrpC2 binds to a region that includes 2 5-bp elements in inverted orientation, and enhancement of shifted complex formation in combination with FruA is likewise observed (J. Lee, S.M., and L.K., unpublished data).

Our preliminary studies, taken together with the evidence presented here for fmgA, indicate that cooperative binding of MrpC2 and FruA is a conserved mechanism of C-signal-dependent gene regulation.

Cooperative binding of MrpC2 and FruA to promoter regions of C-signal-dependent genes is an unusual mechanism of gene regu-
Our discovery of potent cooperative binding by recombinant proteins suggests that FruA may be phosphorylated, which would not increase DNA binding of FruA (Fig. S4). Phospho-donors, which activate many response regulators (25), did not enhance DNA binding of FruA (Fig. S4). The detailed mechanism of cooperative binding of MrpC2 and FruA to the fmgA promoter region remains to be explored. The binding sites of the 2 proteins may partially overlap, because a 2-apart mutation at −83 to −77 impairs FruA-DBD-His6 binding (17) and DNA upstream of −76 is required for His6-MrpC2 binding (data not shown). The 2 proteins may interact with opposite faces of the DNA in a region of overlap, analogous to certain homeodomain proteins, which bind DNA cooperatively (30). As for some homeoprotein–DNA complexes, cooperativity might depend not only on protein–protein interactions, but on bending of the DNA by one or both proteins. Binding of either MrpC2 or FruA alone to the fmgA promoter region produced DNAse 1 hypersensitivity indicative of DNA bending, and the combination of proteins increased the intensity and number of hypersensitive sites (Fig. 4 and Fig. S5), demonstrating cooperative binding. Likewise, EMSAs showed that the combination of proteins enhances formation of shifted complexes (Fig. 3A and Fig. S4A) and that this depends on sequence important for binding of each protein (Fig. S5) consistent with cooperative binding. The shifted complexes that were observed also depended on the percentage of polyacrylamide in gels used in the EMSAs, with 8% gels facilitating detection of FruA binding and detection of UC that presumably represents binding of FruA and MrpC2. The gel matrix influences stability of protein–DNA complexes during EMSAs (31). The enhancement of shifted complex formation by MrpC2 and FruA requires the N-terminal domain of FruA (Fig. 3B), suggesting that this domain interacts directly with MrpC2, but further studies will be needed to elucidate the detailed mechanism of cooperativity.

Several lines of evidence suggest that the N-terminal domain of FruA, which is similar to the receiver domain of response regulators that is typically phosphorylated by an HPK, might function without phosphorylation. First, the N-terminal domain of FruA lacks 2 aspartate residues that are highly conserved in receiver domains and normally play an important role in phosphorylation of a third aspartate residue (14, 25). Second, extensive efforts have failed to identify a cognate HPK. Third, FruA is not phosphorylated in vitro by homologous HPKs. EnvZ/HPr from E. coli and A. anabaena, respectively (R. Zhou and L.K., unpublished data). Fourth, a phosphomimetic D59E substitution in FruA did not increase DNA binding or enhancement of shifted complex formation in combination with MrpC2 (Fig. S4D). Fifth, treatment with small molecule phosphodonoros, which activates many response regulators (25), did not increase DNA binding of FruA (Fig. S4E–G). Although these results do not rule out the possibility that FruA is phosphorylated, our discovery of potent cooperative binding by recombinant (presumably unphosphorylated) FruA and MrpC2, which depends on the N-terminal domain of FruA, reveals an unusual function of a receiver domain that may not be phosphorylated. Receiver domains that cannot or need not be phosphorylated in order for the pseudoresponsive regulator protein to function have been described in bacterial DNA-binding proteins (32–34) and in proteins that regulate circadian rhythms in bacteria (35, 36) and plants (37). If C-signaling does not lead to phosphorylation of FruA, then what is the mechanism of signal transduction? Cooperative binding of FruA and MrpC2 to the fmgA promoter region and other promoter regions, together with activation of fruA transcription by MrpC2 (24), represents a coherent feed-forward regulatory loop design found commonly in regulatory networks because of its beneficial characteristics (38). C-signaling could affect production of MrpC2 and/or activity of FruA (Fig. S1). If there is an effect of C-signaling on FruA, it is likely posttranslational because a mutant defective in C-signaling accumulates FruA normally during development (14).

The complex regulation of MrpC2 production and the recent finding that MrpC plays a role in PCD make this unusual transcription factor/antitoxin an attractive target for regulation by C-signaling, which has not been examined. Mutants defective in C-signaling are defective in PCD (39). MrpC is phosphorylated by a cascade of Ser/Thr protein kinases (STPKs), presumably in response to an unknown signal during growth, inhibiting accumulation of MrpC and MrpC2 (19). Starvation conditions may remove the signal (Fig. S1), allowing MrpC and MrpC2 to accumulate. Recently, it was shown that the Espa signal transduction pathway influences the MrpC and MrpC2 concentrations (40), presumably providing another link to starvation (Fig. S1). Also, MrpC was shown to interact with the toxin MazF, inhibiting PCD (2). On the other hand, MrpC appears to directly activate mazF transcription. It is important to test whether MrpC2 differs from MrpC in either of these activities. The concentrations of MrpC, its phosphorylated or cleaved forms, and their interactions with MazF and at different promoters, may determine the fate of cells in a developing population of M. xanthus (2).

Commitment to form a spore has been hypothesized to involve induction of genes at the O7536 locus, which in turn depends on induction of the dev operon (41). Because dev appears to be regulated by cooperative binding of MrpC2 and FruA (S.M. P. Viswanathan, and L.K., unpublished data), we propose that commitment to sporulation is governed by this key transcription factor. MrpC is a major hub in the regulatory network, linked extensively to starvation (Fig. S1). Its direct involvement in commitment to sporulation might couple persistent starvation to the decision to form a spore. FruA is likewise a major hub in the regulatory network. Transcription of fruA is highly regulated (41), and it is unclear how much of this regulation feeds through MrpC. Short-range C-signaling contributes positional information (i.e., cell alignment in the nascent fruiting body) to the decision to sporulate (41, 11, 12), and it may do so through MrpC and/or a posttranslational effect on FruA, as discussed above. Commitment to sporulation may also be governed by a third activator of dev transcription, LadA, which likely responds to a signal and acts positively from a site downstream of the promoter (42). Combinatorial regulation of dev by 3 transcription factors that bind upstream and downstream of the promoter resembles regulation of developmental genes in multicellular eukaryotes.

Materials and Methods

**Bacterial Strains and Plasmids.** Strains and plasmids used in this study are listed in Table S1.

**Growth and Development.** E. coli containing plasmids were grown at 37 °C in Luria–Bertani medium (43) containing 200-μg/ml ampicillin. Growth and development of M. xanthus was as described (21).
Preparation of fmgA DNA Fragments. The preparation of 32P-labeled DNA fragments for EMSAs and DNase I footprinting is described in SI Text.

EMSAs and DNase I Footprinting. EMSAs were performed as described (17), except the binding reaction mixtures were incubated at 25 °C for 15 min. For footprinting, 0.2 units of DNase I (Promega) was added to the binding reaction mixture (20 μl) for 2 min at 25 °C. The mixture was the same as for EMSAs, except it contained 5 mM MgCl2, 0.5 mM CaCl2, 0.025 μg/μl double-stranded poly(dI-dC), and no glycerol. Reactions were stopped by adding 100 μl of solution containing 300 mM sodium acetate, 20 mM EDTA, 0.2% SDS, 0.02 μg/μl proteinase K, and 100 μg/ml yeast RNA, and incubating at 52 °C for 15 min. After extraction with 10 μl of phenol (twice), DNA was precipitated with ethanol. The DNA was resuspended in formamide loading buffer (43), boiled for 3 min, subjected to electrophoresis on an 8% polyacrylamide gel containing 8 M urea, and visualized by autoradiography.

Sequencing ladders were generated by using the SequiTether EXCEL II DNA Sequencing Kit protocol (Epipcent Biotechnologies).

DNA Affinity Chromatography. A fmgA DNA fragment (−101 to +25) was synthesized by PCR with a 5′-biotin label at −101, bound to streptavidin beads, and DNA-afinity chromatography was performed with the AS fraction as described (42).

Preparation of MrpC2 and Frua. Histidine–MrpC2 (19) and Frua-DBD-His2 (17) were purified as described previously from E. coli strains SMHmsMrpC2 and EDFYFrua, respectively. Frua-His2 was purified from E. coli SMFUrahIs as described in SI Text.

ChIP. M. xanthus strains MDY4400.DZF1 and MDY4400.FA were used for ChIP as described (17) with the following modifications: Anti-MrpC antibodies (500 ng) (19) or control IgG (500 ng) (Santa Cruz Biotechnology) were used for immunoprecipitation, 2-fold serial dilutions were made of the input DNA samples, and the primers used for PCR of the fmgA promoter region were the one for +25, described in SI Text, and one upstream (yielding a product of ~180 bp) in the vector used for ectopic integration (5′-CTGGCAGAATTTGGGATC-3′).

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