Identification of a Gene Involved in Polysaccharide Export as a Transcription Target of FruA, an Essential Factor for Myxococcus xanthus Development*

Received for publication, July 1, 2005 Published, JBC Papers in Press, July 22, 2005, DOI 10.1074/jbc.M507191200

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Fruiting body development in Myxococcus xanthus is a multicellular event that is coordinated by exchanging intercellular signals. FruA is a transcription factor essential for fruiting body development and is thought to play a key role in the C-signal pathway. Here we present the first identification of a gene regulated by FruA. The gene was isolated from a genomic library via in vitro selection in a DNA binding assay by using the DNA-binding domain of FruA tagged with His$_6$ at the C-terminal end (FruA-DBD-H$_6$). The gene, named fdgA (FruA-dependent gene A), encodes a protein homologous to the outer-membrane auxiliary family protein involved in the polysaccharide export system. FruA-DBD-H$_6$ bound the upstream promoter region of the fdgA gene from nucleotide $-$89 to nucleotide $-$64 with respect to the transcription initiation site, which was required for the induction of fdgA expression during development. fdgA mRNA induced during development was absent in a fra deletion strain. The deletion of fdgA resulted in defective fruiting body formation and reduced sporulation efficiency (1% that of the parent strain). Moreover, FruA was required for the developmental expression of sasA, which is also involved in the biosynthesis of the lipopolysaccharide O-antigen and is required for fruiting body development. Furthermore, the expression of both fdgA and sasA was partially dependent on the C-signal. These findings expand our understanding of the signal transduction pathway mediated by FruA during development in M. xanthus.

Developmental programs in living organisms are guided by a number of different signal transduction systems involved in the integration of environmental and cellular signals. These systems regulate the temporal and spatial expression of genes specific to distinctive stages of development. The developmental progression of a soil bacterium, Myxococcus xanthus, is also controlled by a number of signal transduction systems (1, 2). Upon nutrient limitation, M. xanthus cells begin to coordinate migration toward aggregation centers by gliding on a solid surface to form a fruiting body that holds $10^5$ cells. Inside fruiting bodies only 10% of the starved cells differentiate to spores. The developmental process is achieved by a series of sophisticated intercellular signaling pathways that regulate the expression of a specific set of genes. Five intercellular signals, the A-, B-, C-, D-, and E-signals, are known to be involved and each is essential for fruiting body development. The C-signal is a cell-surface associated morphogen required for rippling, aggregation, and sporulation, and its transmission occurs by a contact-dependent mechanism that involves end-to-end contact between cells (3–5). Recently, it has been reported that the C-signal (p17) is a proteolytic product of CsgA (p25) and corresponds to the C-terminal part of p25 (6).

The fraA gene encodes a putative transcription factor essential for development and is a member of the FixI response regulator subfamily of the two-component His-Asp phosphorelay system (7, 8). FruA is required for the execution of aggregation, fruiting body formation, and subsequent sporulation. The expression of fraA is developmentally regulated, dependent on A- and E-signals, and initiates $6$ h after the onset of development. Recently, a fraA promoter-binding protein was isolated from developmental cell extracts by using a DNA binding assay with the DNA fragment containing the upstream region of the fraA promoter (9). Based upon its partial sequence, this fraA promoter-binding protein was found to be identical to MrpC, which belongs to the cyclic AMP receptor protein family of transcriptional regulators (9). MrpC activates the expression of fraA by binding cis-acting elements in the upstream region of the fraA promoter. MrpC and MrpAB had previously been identified via transposon insertion mutagenesis as essential loci for cellular aggregation and sporulation (10). The fraA and mrpB genes encode a histidine kinase and a NtrC-like response regulator, respectively, of the two-component His-Asp phosphorelay system. It has been proposed that the expression of MrpC depends on the mrpAB operon and that MrpC autoregulates its own gene expression (11).

Based on the analysis of developmental markers in a fraA::Tc strain, the expression of various developmental genes induced after $5$ h of development was severely hampered (7). In addition, genes known to be expressed before $4$ h of development were found to be normally expressed during early development but were unable to be repressed later in fraA::Tc (7). Furthermore, among six proteins identified by the analysis of protein expression patterns in the wild-type, fraA::Tc, and ΔcsgA strains during development, the expression of five proteins was found to be C-signal-independent and one to be C-signal-dependent (12). These results indicate that developmental genes under the control of FruA can be classified into two groups, C-signal-independent and C-signal-dependent (12). In addition, transposon mutagenesis identified mutants involved in the C-signal pathway that were phenotypically divided into two classes (13). Class I mutants are deficient in aggregation but sporulate and produce C-factor at wild-type levels. Class II mutants have deficiencies in all C-factor responses. Because fraA was also identified genetically as the only class II mutant gene (14), FruA has been proposed to play a key role and to be activated by phosphorylation with a C-signal receptor kinase in the C-signal transduction system (15). However, such a kinase has not been identified to date.

Given that the expression of a number of genes was altered between the wild-type and fraA::Tc strains during development (7, 8, 12), it was important to identify which genes are directly activated or repressed by

* This work was supported by a grant from the Foundation of the University of Medicine and Dentistry of New Jersey. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY648299.

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FruA. To identify genes directly regulated by FruA, we performed in vitro selection for a genomic DNA library by using the DNA-binding domain of FruA tagged with eight histidine residues at the C-terminal end (FruA-DBD-H8). \(^2\) We isolated a DNA fragment containing the promoter region and the N-terminal part of a protein homologous to the outer membrane auxiliary (OMA) family protein, which is involved in the polysaccharide export system. This gene was named \(fda\) (FruA-dependent gene \(A\)). \(cis\)-Acting elements in the \(fda\) promoter were identified, and developmental expression of \(fda\) was not detectable in a \(\Delta\text{fruA}\) strain newly constructed in this study. Moreover, the deletion of \(fda\) resulted in defective fruiting body formation and reduced sporulation efficiency. In addition, the induction of the \(sasA\) locus that is required for the biosynthesis of the lipopolysaccharide O-antigen and development (16, 17) was not detected in the \(\Delta\text{fruA}\) strain during development.

**MATERIALS AND METHODS**

**Bacterial Strains and Growth Conditions—** \(M.\ xanthus\) DZF1 (18) was used as a parent strain and was grown in CYE medium (19) supplemented with kanamycin or streptomycin when necessary. For fruiting body formation, \(M.\ xanthus\) was spotted on clone-fruiting (CF) agar plates (20). \(Escherichia\ coli\) JM83 (21) was used as a recipient strain for transformation unless otherwise mentioned and was grown in Luria-Bertani medium (22) supplemented with antibiotics when necessary.

**Preparation of FruA-DBD-H8**—The DNA fragment encoding FruA-DBD-H8 from Pro to 152 to Leu at 229 tagged with histidine residues at the C-terminal end was amplified by PCR with pMF05 (7) and the oligonucleotide primers 5′-TCCAATAGCGGGGATGACGTCCAAAGC-3′ and 5′-TCTGGATCCCTA(GTG)8GAGGTCCGGCGGCCGGA-3′ (Ndel and BamHI sites are underlined). PCR products were digested with Ndel and BamHI and ligated into pET11a (Novagen). The resultant plasmid, pET11a/FDBD-H8, was transformed into \(E.\ coli\) BL21 (DE3) (23). FruA-DBD-H8 was induced by the addition of isopropyl 1-thio-\(\beta\)-D-galactopyranoside at a final concentration of 1 mM in Luria-Bertani medium supplemented with ampicillin at 37 °C for 2 h. Cells were harvested by centrifugation and washed with 10 mM Tris-\(\text{HCl}\) (pH 7.9). Cells were resuspended in the buffer H (10 mM Tris-\(\text{HCl}\) (pH 7.9), 500 mM NaCl, 1 mM \(\beta\)-mercaptoethanol, and 10 mM imidazole) supplemented with protease inhibitors (Roche Applied Science) and disrupted by sonication. After centrifugation at 100,000 \(\times\) g for 30 min, the supernatant was mixed with Triton X-100 at a final concentration of 0.1% and was applied to nickel-nitritriacetic acid (Qiagen). After incubation for 30 min, the column was washed with the buffer H containing Triton X-100 and then with the buffer H containing 40 mM imidazole. FruA-DBD-H8 was eluted with a buffer containing 10 mM Tris-\(\text{HCl}\) (pH 7.9), 50 mM NaCl, 1 mM \(\beta\)-mercaptoethanol, and 250 mM imidazole. The eluate was dialyzed against a buffer composed of 10 mM Tris-\(\text{HCl}\) (pH 7.9), 50 mM NaCl, 1 mM \(\beta\)-mercaptoethanol, 10 mM \(\text{EDTA}\), and 50% (w/v) glycerol. All procedures for the protein purification were performed either on ice or in a cold room.

**In Vitro Selection for a Genomic DNA Library—** A genomic DNA library was constructed by digesting partially with MspI chromosomal DNA prepared from \(M.\ xanthus\) DZF1. DNA fragments of ~400 base pairs were isolated by PAGE. For in vitro selection a DNA binding assay was performed as described below with the library (200 ng) as a probe and purified FruA-DBD-H8 (50 ng) in a total volume of 50 \(\mu\)l. After the DNA binding reactions, the mixtures were subjected to PAGE. FruA-DBD-H8/DNA complexes were isolated from the gel after PAGE. To isolate the complexes from the gel, the \(\alpha\)\(^{32}\)P-labeled DNA fragment of 350 bp containing FruA-binding sites from the \(dofA\) promoter was used as a probe for reference. \(^3\) The gel pieces containing FruA-DBD-H8/DNA complexes with migration similar to that of FruA-DBD-H8/\(\alpha\)\(^{32}\)P-labeled \(dofA\) promoter complexes were excised. The complexes were electroeluted from the gel pieces and extracted with phenol and chloroform.

The DNA fragments were precipitated with ethanol and resuspended in water. A portion of the recovered DNA fragments was ligated at the CCl site in modified pBluescript SK− (Stratagene), which contains an additional HindIII site between the CCl site and the SalI site (total of two HindIII sites next to the CCl site). The ligation mixtures were transformed, and plasmids were prepared from transformed cells growing on the plates. The plasmids were digested with HindIII, and the DNA fragments were isolated with PAGE. The DNA fragments were used for the next in vitro selection as a probe. The in vitro selection was repeated three times as described above. After the fourth selection, plasmids containing the selected fragments were sequenced.

**DNA Binding Assay and Footprint Analysis—** The fda promoter region from nt −165 to +9 was amplified by PCR using the plasmid containing the 388-bp MspI fragment shown in Fig. 2 and the oligonucleotide primers (a) (5′-TCAAGCGTTCCGGAATGGGAAGCGGGA-3′) and (b) (5′-TCCGGATCCGAAGCGCTTGGATCGCA-3′) (see Fig. 2 for the location; the HindIII and BamHI sites are underlined). An amplified product was digested with HindIII and BamHI and cloned in pBluescript SK− (Stratagene). A DNA fragment for a probe was prepared by digesting the plasmid with HindIII and BamHI and isolating the fragment by PAGE. The isolated DNA fragment was then labeled with \(\alpha\)\(^{32}\)P\(\text{dCTP}\) by a Klenow fragment of DNA polymerase I.

For footprint analysis, a probe corresponding to the region from nt −165 to +41 was prepared as described above with oligonucleotide primers (a) and (c) (5′-TCCGGATCCCGGAATGGGAAGCGGGA-3′). A DNA fragment for a probe was prepared by digesting the plasmid with HindIII and BamHI and isolating the fragment by PAGE. The isolated fragment was labeled with \(\alpha\)\(^{32}\)P\(\text{dCTP}\) by a Klenow fragment of DNA polymerase I. Therefore, only the leading strand shown in Fig. 2 was labeled.

DNA binding reactions were performed at 25 °C for 10 min in 10 \(\mu\)l of the reaction mixtures containing 20 mM Tris acetate (pH 7.9), 50 mM potassium acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, 10 \(\mu\)g/ml bovine serum albumin, 10% glycerol, and 1 \(\mu\)g of poly(dI-dC)poly(dI-dC) (Amersham Biosciences). The binding patterns were analyzed by 5% PAGE followed by autoradiography. For footprint analysis, after DNA binding reactions DNAse I was added to the reaction mixtures, and the mixtures were further incubated at 25 °C for 3 min. The reactions were stopped by adding the solution containing 25 mM EDTA and 0.5 mM ammonium acetate. After extraction of the reaction mixtures with phenol/chloroform/isoamylalcohol (25:24:1), the DNA probes were precipitated with ethanol in the presence of glycerogen. The DNA probes were resuspended in sequence loading buffer and analyzed by sequencing gel followed by autoradiography.

**lacZ Fusion Analysis—** The fda promoter regions from nt −165, −93, −82, −71, and −62 to +162 were fused to lacZ in the transcriptional fusion vector pZKAT (9). \(\beta\)-Galactosidase activity was measured at 0 and 20 h after the initiation of development on CF agar plates as described (24).

**Construction of Mutant Strains—** A \(\Delta\text{fruA}\) strain was constructed by replacing the CCl-PvuII fragment with a streptomycin resistance gene. The CCl and the PvuII sites are located at the positions 11 and 213 from the N-terminal end of FruA, respectively (7).

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\(^2\) The abbreviations used are: FruA-DBD-H8, DNA-binding domain of FruA tagged with eight His residues at the C-terminal end; CF, clone-fruiting; nt, nucleotide(s); OMA, outer membrane auxiliary; ORF, open reading frame.

\(^3\) T. Ueki and S. Inouye, unpublished data.
A Δ\(fgdA\) strain was constructed by replacing the BsaAI-Smal fragment with a kanamycin resistance gene. The BsaAI and Smal sites are located in the promoter region and the open reading frame (ORF) of the \(fgdA\) gene, respectively. Thus, amino acid residues from 1 to 120 of FdgA were deleted. A Δ\(fgdA/\DeltafgdA\) strain was constructed by integrating the plasmid containing the promoter region (−3 kbp) and the entire ORF of the \(fgdA\) gene into the chromosome of the Δ\(fgdA\) strain.

RESULTS

Identification of a Gene Regulated by FruA from a Genomic DNA Library.—To identify a gene directly regulated by FruA, we performed in vitro selection for a genomic DNA library in a DNA binding assay. We used the DNA-binding domain of FruA tagged with eight histidine residues at the C-terminal end. The DNA fragments isolated after each selection were labeled with \(32P\) and were used for a DNA binding assay with FruA-DBD-H8. As the selections were repeated, more fragments were bound by FruA-DBD-H8 (Fig. 1). A DNA fragment in the length of 388 bp isolated after the fourth cycle of the selection was found to contain a putative promoter region and an N-terminal part of an ORF based on a search in the Institute for Genomic Research web site at www.tigr.org (Fig. 2). The DNA fragment encompassing an entire gene was cloned from the genome. The isolated gene was termed FruA-dep.

Expression of \(fgdA\) during Vegetative Growth and Development—\(fgdA\) expression was examined by primer extension analysis with total RNA prepared from D2F1 and \(\DeltafruA\) during vegetative growth (0 h) and fruiting body development (12 and 20 h). Three 5′-ends of \(fgdA\) mRNA were identified. One (P1) of them was detected in both vegetative and developmental cells, and its expression was independent of \(fruA\) (Fig. 3A). In contrast, the other two were detected only in developmental cells. One (P2) of them was observed in early (12 h) and late (20 h) development, and the other (P3) was in late development. These development-specific mRNAs were not detectable in the \(\DeltafruA\) strain. The nucleotides of these 5′-ends were determined by DNA sequence ladders (data not shown) and are shown in Fig. 2. The DNA fragment containing the promoter region from nt −165 to +7 with respect to the transcription initiation site (P1) was found to be bound by FruA-DBD-H8 (Fig. 3B). Footprint analysis showed that the two regions from nt −89 to −74 (region b) and from nt −72 to at least −64 (region a) were protected by FruA-DBD-H8 from DNase I and that the band (C at nt −73) was hypersensitive to DNase I between the two regions (Fig. 3C). As shown in Fig. 3B, because three complexes were observed when an amount of FruA-DBD-H8 was increased, it appears to have three binding sites.

To examine the roles of these regions in \(fgdA\) expression in vivo, various regions of the upstream \(fgdA\) promoter were transcriptionally fused to the lacZ gene and introduced into the attB site. The activity of β-galactosidase was measured at 0 and 20 h of development (Fig. 3D). The deletion of the region from nt −165 to −94 (promoter −93) did not affect \(fgdA\) expression during vegetative growth and development, and the deletion of the region from nt −165 to −83 (promoter −82) slightly reduced \(fgdA\) expression during development. In contrast, the deletion of 11 bases between nt −82 and −72 (promoter −71) from promoter −82 resulted in drastic decrease in \(fgdA\) expression, and region a alone (promoter −62) failed in the activation of the \(fgdA\) promoter. The increase of β-galactosidase activity by promoter −62 during development was most likely due to the increase in basal level expression of \(fgdA\) (P1w) during development (Fig. 3A).

Function of \(fgdA\)—The \(fgdA\) gene encodes a protein homologous to GumB of Xanthomonas campestris that belongs to the OMA family involved in polysaccharide export (25). FdgA has a typical lipoprotein consensus sequence that includes the leader sequence followed by the invariant Cys, circled in Fig. 2, and the signal peptidase II cleavage site (26).

Analysis of DNA sequences revealed that another ORF (named Orf1) is located downstream of \(fgdA\). However, reverse transcription PCR analysis showed that orf1 was transcribed independently of the \(fgdA\) promoter (data not shown), suggesting that \(fgdA\) is monicistronic.

To elucidate the function of the \(fgdA\) gene, a Δ\(fgdA\) strain was constructed. Although the \(fgdA\) gene was expressed during vegetative growth, the Δ\(fgdA\) strain grew in CYE liquid medium (19) similarly as the parent strain (data not shown). In contrast, during development, the Δ\(fgdA\) strain showed defective fruiting body formation on CF agar plates (Fig. 4). The Δ\(fgdA\) strain formed bigger and fewer fruiting bodies, which were not as dark as those of the parent strain. In contrast, under this condition the \(\DeltafruA\) strain formed no apparent fruiting bodies (Fig. 4). A number of viable spores were then examined after the fruiting bodies were harvested and treated with sonication. The efficiency of sporulation of the Δ\(fgdA\) strain was ~1% that of the parent strain after 5 days of development on CF agar plates.

To confirm that the defective development of the Δ\(fgdA\) strain was due to the absence of the \(fgdA\) gene, the DNA fragment containing the promoter region and the coding region of the \(fgdA\) gene was introduced into the Δ\(fgdA\) strain. The Δ\(fgdA/\DeltafgdA\) strain formed fruiting bodies similar to those of the parent strain (Fig. 4). Furthermore, the Δ\(fgdA/\DeltafgdA\) strain formed viable spores in efficiency similar to that of the parent strain. Therefore, the \(fgdA\) gene was essential for fruiting body formation and sporulation during development.

Expression of the \(sasA\) Locus—Genes at the \(sasA\) locus that are involved in lipopolysaccharide O-antigen biosynthesis have been shown
FIGURE 3. The expression of the \( \Delta \text{fga} \) gene. Panel A, \( \Delta \text{fga} \) expression in \( M. \text{xanthus} \) DZF1 and \( \Delta \text{fra} \). Total RNA was prepared from DZF1 and \( \Delta \text{fra} \) during vegetative growth (0 h) and fruiting body development (12 and 20 h) and was analyzed by primer extension. The oligonucleotide 5'-AGCAT-GACCGCCAAAACCCGTC-3' was used as a primer. The positions of markers (\(^{32}P\)-labeled MspI digests of pBR322) are indicated in bases. Panel B, DNA binding assay. The probe contains the promoter region from nt -165 to +9. Lane 1, no FruA-DBD-H\(_8\); lane 2, 0.25 ng/\( \mu \)l FruA-DBD-H\(_8\); lane 3, 0.5 ng/\( \mu \)l FruA-DBD-H\(_8\); lane 4, 1 ng/\( \mu \)l FruA-DBD-H\(_8\); lane 5, 2 ng/\( \mu \)l FruA-DBD-H\(_8\); DNA/FruA-DBD-H\(_8\) complexes are indicated by arrows. Panel C, footprint analysis. The regions that include sequences protected from DNase I are indicated by vertical lines. Lane 1, no FruA-DBD-H\(_8\); lane 2, 0.25 ng/\( \mu \)l FruA-DBD-H\(_8\); lane 3, 0.5 ng/\( \mu \)l FruA-DBD-H\(_8\); lane 4, 1 ng/\( \mu \)l FruA-DBD-H\(_8\); lane 5, 2 ng/\( \mu \)l FruA-DBD-H\(_8\). Lanes A, 1, 2, 3, 4, and 5 represent sequence ladders generated by the primer 5'-GATCCTTAG-CAGCGCGTGTCCGA-3', which was labeled at the 5'-end with \( \gamma ^{32} \)P-ATP by T4 polynucleotide kinase. Panel D, lacZ fusion analysis. The promoter regions up to nt -165, -93, -82, -71, and -62 were fused to lacZ. \( \beta \)-Galactosidase activity was measured at 0 (open bars) and 20 h (closed bars) after the initiation of development on CF agar plates. The activity is shown as a percentage of the activity of the promoter up to nt -165 at 20 h.

FIGURE 4. Analysis of the \( \Delta \text{fga} \) strain during fruiting body development. \( M. \text{xanthus} \) DZF1, \( \Delta \text{fra} \), \( \Delta \text{fga} \), and \( \Delta \text{fga} \) were spotted on CF agar plates. Photographs were taken by a Polaroid camera under a dissecting microscope after a 5-day incubation at 30°C.

FIGURE 5. The expression of the sasA locus. sasA expression in \( M. \text{xanthus} \) DZF1 and \( \Delta \text{fra} \) was analyzed by primer extension as described in Fig. 3A. The oligonucleotide 5'-CTGGACGAGGCTGATGAGCA-3' was used as a primer.

to be essential for development (see "Discussion" for details) (16, 17). Therefore, we next examined whether the sasA locus is regulated by FruA. Primer extension analysis showed that sasA mRNA induced during development (\( P_{D1} \)) was undetectable in the \( \Delta \text{fra} \) strain, but vegetative mRNA (\( P_{V} \)) was expressed in both the parent and the \( \Delta \text{fra} \) strains (Fig. 5). Interestingly, the DNA binding assay revealed that FruA-DBD-H\(_8\) did not bind the sasA promoter region under conditions in which FruA-DBD-H\(_8\) could bind the \( \Delta \text{fga} \) promoter region (data not shown). Therefore, it appears likely that the expression of sasA is indirectly regulated by FruA.

Effect of C-signal on Expression of \( \Delta \text{fga} \) and sasA—Because FruA is proposed to function in the C-signal transduction pathway (14, 15), the expression of \( \Delta \text{fga} \) and sasA was examined in a \( \Delta \text{csgA} \) strain (27). Primer extension analysis revealed that \( \Delta \text{fga} \) mRNA induced during development (\( P_{D2} \)) decreased in the \( \Delta \text{csgA} \) strain (Fig. 6A). In addition, sasA mRNA induced during development also decreased in the \( \Delta \text{csgA} \) strain (Fig. 6B). Therefore, it appears likely that the expression of both \( \Delta \text{fga} \) and sasA is partially dependent on the C-signal.

DISCUSSION

We have identified a gene, named \( \Delta \text{fga} \), encoding an OMA family protein involved in the polysaccharide export system as a transcription target of FruA from the genomic library of \( M. \text{xanthus} \). The expression of \( \Delta \text{fga} \) was differentially regulated during vegetative growth and development. The developmental expression of \( \Delta \text{fga} \) was dependent on FruA. The deletion of \( \Delta \text{fga} \) resulted in defective fruiting body formation and reduced sporulation efficiency during development. \( \Delta \text{fga} \) is the first gene identified as a transcription target of FruA.

To identify genes directly regulated by FruA, we performed in vitro selection for the \( M. \text{xanthus} \) genomic DNA library by using a DNA binding assay with FruA-DBD-H\(_8\). In the in vitro selection described here, a DNA fragment containing a binding site for a specific transcription factor can be isolated from a genomic DNA (28). The recent progress of the genome projects makes it easy to determine whether the binding site identified by in vitro selection is located in the promoter region of a target gene. In contrast, other methods for global analysis of gene expression such as microarray and two-dimensional gels may result in the identification of genes or proteins indirectly regulated by a transcription factor as well as those that are directly regulated.

It appears that FruA activates \( \Delta \text{fga} \) expression during development by binding regions a and b identified by the footprint analysis. The foot-
print analysis indicates that these regions have similar affinity to FruA-DBD-H8 in vitro. However, lacZ fusion analysis shows that region b has the most effect on the activation of the fdgA promoter. Because the region hypersensitive to DNase I is located between regions a and b, it is possible that binding of FruA-DBD-H8 to the fdgA promoter results in structural modification in the fdgA promoter and that this modification is important for the activation of the fdgA promoter. At the early stage of development, fdgA expression is initiated from the P\(_{D1}\) promoter by binding of FruA to regions a and b. When the developmental program is progressed, it is possible that other developmental factors such as a different transcription factor and a \(\sigma\) factor may activate fdgA expression at P\(_{D2}\). The induction of fdgA at P\(_{D2}\) is partially dependent on the C-signal, suggesting that the C-signal may be also involved in the activation of FruA-dependent gene expression during late development.

\(M.\) xanthus produces large amounts of extracellular polysaccharide, often referred to as slime, and excreted slime plays a role in fruiting body development (29). Although similar studies have not been done for sporulation during fruiting body development, the increase in polysaccharide is ~200%, and polysaccharide accounts for 20% of the dry weight of the cell during sporulation induced by glycerol (30). The major monosaccharide components of the hydrolyzed extracellular polysaccharide are glucose, mannose, and rhamnose, whereas the nature of unhydrolyzed polysaccharide remains to be examined. It is not known which polysaccharides are excreted by vegetative cells and which are involved in fruiting body development. Thus, characterization of polysaccharide by using the \(\Delta\)fgA strain may answer those questions, because the fdgA gene encodes an OMA family protein involved in the polysaccharide export system, and fdgA expression is differentially regulated during vegetative growth and development. In addition, at least two homologues of FdgA were identified from the preliminary analysis during vegetative growth and development. In addition, at least two homologues of FdgA were identified from the preliminary

Acknowledgments—Preliminary sequence data were obtained from The Institute for Genomic Research web site at www.tigr.org. We are grateful to L. D. Vales for critical reading of the manuscript. We thank J. Downard for the \(\Delta\)scA strain. We also thank C. Xu for DNA manipulation and H. Nariya for helpful discussions.

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*J. Biol. Chem.* 2005, 280:32279-32284.
doi: 10.1074/jbc.M507191200 originally published online July 22, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M507191200

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