JOURNAL OF BACTERIOLOGY, Dec. 1988, p. 5552–5556
0021-9193/88/125552-05$02.00/0

Vol. 170, No. 12

Site-Specific Integration and Expression of a Developmental Promoter in *Myxococcus xanthus*

SHENGFENG LI AND LAWRENCE J. SHIMKETS*

Department of Microbiology, University of Georgia, Athens, Georgia 30602

Received 5 July 1988/Accepted 7 September 1988

A series of intercellular signals are involved in the regulation of gene expression during fruiting body formation of *Myxococcus xanthus*. Mutations which block cell interactions, such as *csgA* (formerly known as *spoC*), also prevent expression of certain developmentally regulated promoters. *csgA*$^+$ cells containing Tn*5 lac* ΩDK4435, a developmentally regulated promoter fused to *lacZ*, began synthesizing *lacZ* mRNA 12 to 18 h into the developmental cycle. β-Galactosidase specific activity increased about 12 h later. Neither *lacZ* mRNA nor β-galactosidase activity was detected in a developing *csgA* mutant containing ΩDK4435. The developmental promoter and its fused *lacZ* reporter gene were cloned into a pBR322-derived plasmid vector containing a portion of bacteriophage Mx8. These plasmids preferentially integrated into the *M. xanthus* chromosome by site-specific recombination at the bacteriophage Mx8 attachment site and maintained a copy number of 1 per chromosome. The integrated plasmids were relatively stable, segregating at a frequency of 0.0007% per generation in the absence of selection. The cloned and integrated promoter behaved like the native promoter, expressing β-galactosidase at the proper time during wild-type development and failing to express the enzyme during development of a *csgA* mutant. The overall level of β-galactosidase expression in merodiploid cells containing one native promoter and one promoter fused to *lacZ* was about half that of cells containing a single promoter fused to *lacZ*. These results suggest that the timing of developmentally regulated gene expression is largely independent of the location of this gene within the chromosome. Furthermore, they show that site-specific recombination can be a useful tool for establishing assays for promoter or gene function in *M. xanthus*.

Myxobacteria have one of the most pronounced systems of social behavior among the procaryotes (11). The most complex portion of the life cycle is initiated in response to nutritional stress and results in the formation of a large, multicellular fruiting body. Fruiting body morphogenesis is tightly regulated both temporally and spatially, as tens of thousands of cells travel relatively large distances to assemble this macroscopic structure. Inside the fruiting body, cells differentiate into dormant spores which can survive harsh environmental conditions. Because of the large numbers of cells involved, intercellular communication is necessary to complete the process. While identification and characterization of the signals are important, another point of interest has been the developmental genes that are regulated in response to communication. The construction of the promoter probe Tn*5 lac* provided an important tool for identifying these genes, and these genes may now be isolated and manipulated by recombinant DNA technology.

It is often desirable to place cloned genes back into *Myxococcus xanthus* in a manner that permits their correct developmental expression in order to determine whether the restriction fragment contains all the sequences necessary for correct expression of the gene. An optimal expression system of this sort is expected to meet the following three criteria. First, the recombinant allele should be stably maintained at a low copy number, preferably a single copy per chromosome. Second, it should not undergo recombination with the native allele, which could lead to gene conversion or marker rescue. Third, the allele should maintain its regulated expression. Recent experiments have suggested that a site-specific recombination system from bacteriophage Mx8 can be exploited to fulfill the first two criteria. Myxophage Mx8 is a lysogenic bacteriophage for *M. xanthus* that, by analogy with bacteriophage lambda, contains an AttP site which is used to integrate the phage genome into a chromosomal AttB site by site-specific recombination (15). Stellwag et al. showed that plasmids containing the AttP fragment from Mx8 also integrate into the AttB site (15). When a plasmid contains both a region of homology with the chromosome and an AttP site, the plasmid preferentially integrates by site-specific recombination about 99% of the time (12). Whereas gene conversion and marker rescue are common after homologous integration due to recombination between the two alleles (10, 12, 14), they rarely if ever occur after site-specific integration (12, 16). In this manuscript, it is shown that site-specific recombination can fulfill the third criterion. A cloned, developmentally regulated promoter was inserted into the *M. xanthus* chromosome by site-specific recombination, and the cloned promoter was expressed at the proper time in development.

MATERIALS AND METHODS

Cells and growth conditions. *M. xanthus* was grown vegetatively in CTT broth or on CTT agar (3). *Escherichia coli* was grown in L broth or on L agar with antibiotics as indicated.

Plasmid construction. The site-specific integration vector pLJS60 was constructed with the pBR322-derived vector pREG422 (courtesy of R. Gill). Plasmid pREG422 was digested with *Sal*I, which cuts once in the gene for tetracycline resistance. Plasmid pBR328 containing the 12.6-kilobase-pair (kbp) Mx8 *Eco*RI B fragment (kindly furnished by J. Fink and J. Zissler [15]) was digested with *Xho*I, and the 5.6-kbp fragment containing the Mx8 AttP function was

* Corresponding author.

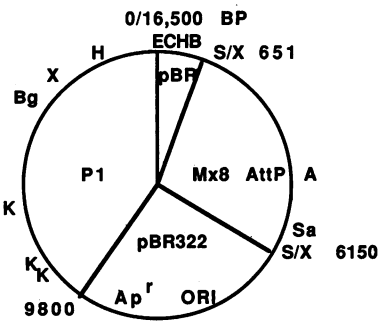

FIG. 1. Restriction map of the 16.5-kbp site-specific integration vector pLJS60. The unique cloning sites in this vector include *Apa*I (A), *Bam*HI (B), *Bgl*II (Bg), *Cla*I (C), *Eco*RI (E), *Sac*I (Sa), and *Xho*I (X). Even though *Hin*dIII (H) and *Kpn*I (K) cut two and three times, respectively, they are also useful cloning sites in that the intervening regions between the sites are not essential. S/X denotes that an *Xho*I fragment was ligated with *Sal*I fragment to produce joints that were not hydrolyzed by either enzyme. Another derivative, pLJS59, containing the *Xho*I fragment in the opposite orientation is also available. BP, Base pairs.

ligated with *Sal*I-digested pREG422. The orientation of the *Xho*I fragment was determined by double digestion with *Eco*RI and *Sac*I, and a restriction map is shown in Fig. 1. Plasmids pLJS61, pLJS62, and pLJS64 contain portions of Tn*5 lac* ΩDK4435 isolated from *M. xanthus* DK5204 and inserted into pLJS60. Plasmid pLJS61 was constructed by ligating *Hin*dIII-digested chromosomal DNA from DK5204 into *Hin*dIII-digested pLJS60. *E. coli* MC1061 cells were transformed and selected by ampicillin resistance (50 μg/ml), which is encoded by the vector, and kanamycin resistance (Km^r) (25 μg/ml), which is encoded by the insert. Plasmid pLJS62 was constructed by ligating the 9.2-kbp *Sal*I fragment from pLJS61 with *Xho*I-digested pLJS60. Plasmid pLJS64 was constructed by digesting pLJS62 with *Bam*HI and ligating it, which deleted the *M. xanthus* promoter sequences but left the *lacZ* gene intact. These plasmids were transferred from *E. coli* to *M. xanthus* by specialized P1 transduction with selection for Km^r (40 μg/ml) as described previously (14). Individual transductants were streak purified before further use. The segregation analysis was performed as described by Avery and Kaiser (1). Plasmid pLJS79 was constructed by ligating the 1.6-kbp *Hin*dIII-*Bam*HI fragment from the ΩDK4435 promoter region of pLJS62 to *Hin*dIII-*Bam*HI-digested pUC19. pLJS79 will hybridize with the native promoter fragment, with the cloned and integrated promoter fragment, or with any plasmid containing a gene or origin of replication that is homologous with those found on pUC19.

Submerged-culture development. The submerged-culture fruiting technique used was similar to that of Kuner and Kaiser (8). Cells were grown in CTT broth to a density of 60 to 80 Klett units and then diluted into fresh CTT to a density of 3 Klett units. An 8-ml amount was added to a standard petri dish (100 by 15 mm) (or 24 ml to a larger petri dish [150 by 15 mm]), and the plates were incubated at 32°C for 20 h. During this time, the cells adhered to the plastic surface and grew in a thin layer. To induce development, plates were washed twice with 10 ml of sterile distilled water at room temperature, and then 8 ml of inducing buffer (10 mM MOPS [3-*N*-morpholinopropanesulfonic acid, pH 6.8], 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% sodium pyruvate) was added, and plates were incubated at 32°C.

β-Galactosidase assay. Cells were scraped off the plastic surface of the submerged-culture plates with a razor blade and transferred to a 1.5-ml conical centrifuge tube, and the cell pellets were stored at −20°C until all the samples were collected. Samples were thawed at room temperature, suspended in buffer containing 0.1 M $NaPO_4$, 0.01 M KCl, 0.001 M $MgSO_4$, and 0.5 mM dithiothreitol, and sonicated twice on ice at 60 μW for 45 s each with a microtip (Heat Systems Ultrasonics). Samples were centrifuged in a microfuge for 1 min to remove the debris, and the supernatant was assayed for β-galactosidase activity with the substrate *o*-nitrophenyl-β-D-galactoside as described by Kroos et al. (7). Protein was assayed with the BCA reagents (Pierce Chemical Co.) with bovine serum albumin standards. β-Galactosidase specific activity units are given as nanomoles of *o*-nitrophenol produced per minute per milligram of protein and were calculated as described by Kroos et al. (7).

RNA isolation. Developing cells in submerged culture were harvested by pouring off the inducing buffer, adding 10 ml of urea buffer (8.0 M urea, 350 mM NaCl, 50 mM Tris hydrochloride [pH 7.5], 20 mM disodium EDTA, 2% [wt/vol] Sarkosyl, 5% [vol/vol] phenol) to the petri plate (150 by 15 mm), scraping the cells off the plate with a razor blade, transferring the cell extract to a 50-ml centrifuge tube, and vortexing continuously for approximately 1 min to lyse the cells. To this solution, 1 volume of phenol-chloroform (3:1) containing 5% isoamyl alcohol was added, and the mixture was shaken for 60 min at room temperature. The phenol extraction was repeated after removing the phenol-chloroform phase by centrifugation at 16,000 × *g* for 20 min. The nucleic acid in the aqueous phase was ethanol precipitated, and the pellets were washed in 70% ethanol, dried, and suspended in 8 ml of RNase-free TE buffer. NaCl and LiCl were added to this solution to 200 mM and 2 M, respectively. The RNA was precipitated by placing this mixture at 0°C for 45 min and then sedimented by centrifugation at 12,000 × *g* and 4°C for 25 min. The RNA pellets were washed with ice-cold 2 M LiCl and resuspended in 2 ml of TE buffer (9). DNase I (RNase free; Boehringer Mannheim) was used to remove contaminating DNA.

RNA hybridization. Analysis of RNA by a dot hybridization procedure has been described by Kafatos et al. (4) and Thomas (18). Briefly, 20 μg of each RNA sample was diluted serially in a twofold manner, with a final dosage of 10, 5, 2.5 1.25, and 0.625 μg. Each RNA sample was diluted 10 times with a denaturing solution containing 50% formamide, 6% formaldehyde, and 10 mM PO_4 buffer (13.8 mg of monosodium plus 241 mg of disodium phosphate in 100 ml), heated for 10 min at 70°C, chilled on ice, and mixed with an equal volume of 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). A total volume of 200 μl was applied to nitrocellulose with a Minifold apparatus (Schleicher & Schuell, Inc., Keene, N.H.), and the filter was baked at 80°C for 2 h under vacuum. The filter was prehybridized for 6 h or overnight at 42°C in 10 ml of a solution containing 50% (vol/vol) deionized formamide, 1 mg of Ficoll per ml, 1 mg of polyvinylpyrrolidone per ml, 1 mg of bovine serum albumin per ml, 0.5% sodium dodecyl sulfate (SDS), 100 μg of sonicated, denatured salmon sperm DNA per ml, 0.75 M NaCl, 50 mM NaH_2PO_4, and 5 mM EDTA. This buffer was then replaced with another 2 ml of the same buffer containing the denatured radiolabeled probe DNA, and incubation was continued for at least 12 h at 42°C. The filter was washed with two changes of buffer containing 0.02 M NaH_2PO_4, 2 mM EDTA, and 0.1% SDS and then washed with another two changes of buffer consisting of 2 mM NaH_2PO_4, 0.2 mM

EDTA, and 0.1% SDS at 55°C for a total of about 80 min to 2 h.

[α-^{32}P]CTP (3,000 Ci/mmol; Amersham)-labeled DNA probes were prepared by the primer extension method (2) from either template plasmids or internal DNA fragments with specific activities of 1×10^8 to 2×10^8 cpm/μg. In control experiments it was found that the amount of 16S and 23S RNA remained a constant percentage of the total RNA throughout development. Therefore, the RNA samples were normalized against rRNA with a probe prepared from pNO1301 (courtesy of R. L. Gourse) containing 16S and 23S rRNAs by hybridization with approximately 10^7 cpm of labeled pNO1301, followed by autoradiography with Kodak XAR-5 film and scanning densitometry of the autoradiogram. A 2.2-kbp internal fragment of the *lacZ* gene of *E. coli* was excised with *Cla*I and *Eco*RI, electroeluted (9), and radiolabeled for detection of *csgA*$^+$-dependent gene expression in strains with Tn*5 lac* ΩDK4435.

DNA hybridization. Myxobacterial chromosomal DNA was purified by the method of Shimkets and Asher (12). Southern blots were performed as described by Maniatis et al. (9). Probe DNA (pLJS79) was radiolabeled to a high specific activity by primer extension (2). Restriction digests of chromosomal DNA were separated by field inversion electrophoresis in a horizontal gel apparatus as described by Shimkets and Asher (12). Pulses were applied with a Hoefer PC750 pulse controller. Initial pulse times were 150 ms in the forward direction and 50 ms in the reverse direction, with a linear ramp of 0.6 h^{-1}.

RESULTS

Developmentally regulated promoter. Tn*5 lac* is a transposable element that contains a promoterless *lacZ* gene (5). When it is inserted in the proper orientation next to an active promoter, transcription of the *lacZ* gene occurs and β-galactosidase is produced. Tn*5 lac* ΩDK4435 is inserted next to an *M. xanthus* promoter whose developmental expression is dependent on the function of the *csgA* gene (6). Expression of this promoter was assayed by hybridization of the *lacZ* mRNA with a radiolabeled probe which is internal to the *lacZ* gene and by measuring β-galactosidase specific activity. In the *csgA*$^+$ background, *lacZ* mRNA appeared after 12 to 18 h of development, about 12 h prior to the increase in β-galactosidase activity (Fig. 2). In *csgA* mutant LS248, neither β-galactosidase nor *lacZ* mRNA was expressed during development. The basal level of β-galactosidase detected in *csgA* cells and early developing *csgA*$^+$ cells appeared to be due to nonspecific hydrolysis of the substrate. There was a rather large difference in the sensitivity of the two assays. The *csgA*$^+$ β-galactosidase peak at 48 h had a specific activity of 220 nmol of *o*-nitrophenol formed per min per mg of protein, or 220 U. Assuming that 1 μg of purified enzyme has 500 U of activity (19), this assay detected 440 ng of β-galactosidase at the 48-h time point and can make reliable measurements in these cell extracts down to about 40 ng of β-galactosidase per mg of protein. The sensitivity of the RNA dot blot hybridizations was determined by diluting RNA samples on nitrocellulose and hybridizing these with a radiolabeled probe containing an *E. coli* ribosomal operon. The limit of detection with this technique was about 20 pg of rRNA (data not shown).

Cloning and insertion into the chromosome. The cloning vector used in these experiments, pLJS60, is shown in Fig. 1. It contains the AttP site from bacteriophage Mx8 (15), the β-lactamase gene and origin of replication from pBR322, and

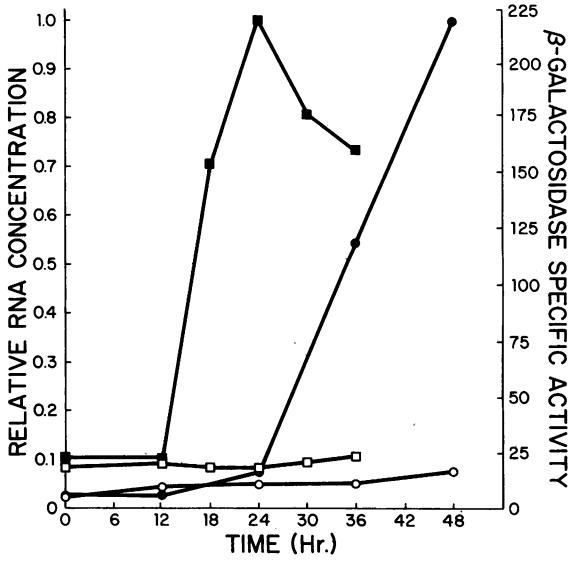

FIG. 2. Developmental expression of *lacZ* under the control of an *M. xanthus* promoter. *csgA*$^+$ strain DK5204 and *csgA* mutant LS248 containing Tn*5 lac* ΩDK4435 were assayed for β-galactosidase activity (units as defined in text) and relative *lacZ* mRNA levels. Symbols: ●, β-galactosidase activity of DK5204; ○, β-galactosidase activity of LS248; ■, relative amount of DK5204 *lacZ* mRNA; □, relative amount of LS248 *lacZ* mRNA.

the incompatibility and partitioning functions from coliphage P1. The P1-derived portion of the vector was used to transfer the plasmid from *E. coli* to *M. xanthus* by specialized transduction with coliphage P1 (14). The AttP portion of the vector was used to integrate the plasmid into the *M. xanthus* chromosome by site-specific recombination as described by Stellwag et al. (15). Portions of Tn*5 lac* ΩDK4435 were cloned into pLJS60 to generate three plasmids with different lengths of *M. xanthus* sequences upstream from the beginning of *lacZ* (Fig. 3). Plasmid pLJS61 contains 11 kbp of *M. xanthus* DNA, pLJS62 contains 1.3 kbp, and pLJS64 contains no *M. xanthus* DNA. These plasmids were transferred to *M. xanthus* by specialized P1 transduction, with selection for Km^r. They are incapable of replicating in *M. xanthus*, and cells become Km^r only when the plasmids integrate into the chromosome.

Since two of the plasmids contain a region of homology with the chromosome, they could integrate by either homologous recombination or site-specific recombination. Plasmid pLJS64, which has no homology with the chromosome, produced just as many transductants as the other two

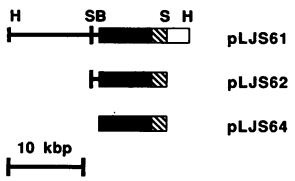

FIG. 3. Plasmids containing the *csgA*$^+$-dependent promoter associated with Tn*5 lac* ΩDK4435 were constructed as described in Materials and Methods. Thin bar represents sequences derived from *M. xanthus*. Thick bar represents sequences derived from Tn*5 lac* and includes the *lac* operon (black boxes), the kanamycin phosphotransferase gene (striped boxes), and other Tn*5* sequences (white boxes). Relevant restriction sites include *Hin*dIII (H), *Sal*I (S), and *Bam*HI (B).

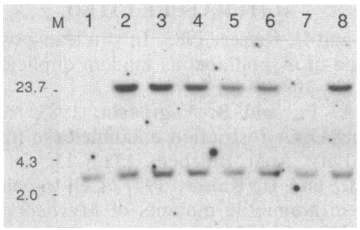

FIG. 4. Southern hybridization of *Apa*I-digested chromosomal DNA from wild-type cells and transductants containing pLJS62 or pLJS64. Lanes: 1, DK1622; lanes 2–6, independent Km^r strains generated by transducing pLJS62 into DK1622; lane 7, LS463, a Km^s segregant of the transductant in lane 4; lane 8, LS462, a Km^r transductant generated by transducing pLJS64 into DK1622. Sizes are shown to the left (kilobase pairs).

plasmids, providing the first evidence that the plasmids preferentially integrated by site-specific recombination (data not shown). A second line of evidence was given by the unusual stability of the integrated plasmids. Several transductants were grown nonselectively for several hundred generations and plated for single colonies, and the colonies were tested for kanamycin sensitivity. The cells segregated Km^s progeny at an average frequency of 0.0007% per generation. For comparison, merodiploids formed by homologous recombination segregate at a frequency of 0.1% per generation (1, 14). For a third line of evidence, the chromosomal location of integrated pLJS62 molecules was determined by Southern blotting. Chromosomal DNA was prepared from five independent transductants and digested with *Apa*I, which cuts the plasmid DNA once in the AttP region. The restriction fragments were separated by electrophoresis in agarose, transferred to nitrocellulose, and hybridized with radiolabeled pLJS79, which contains the 1.6-kbp *M. xanthus* fragment from pLJS62 as well as the β-lactamase gene and origin of replication from pBR322. DNA from the wild-type strain, DK1622, had one 2.9-kbp homologous fragment, the native *Apa*I fragment (Fig. 4, lane 1). DNA extracted from five independent transductants formed by the integration of pLJS62 into the chromosome was also run. In all five transductants, the native fragment was intact, which would not be the case if the plasmid integrated by homologous recombination. A second band of about 25 kbp was formed by integration of the plasmid into a different chromosomal location. The uniform size of the band for all transductants suggests a common site of integration, which is likely to be the chromosomal Mx8 attachment site. A Km^s segregant from one of these transductants retained only the native fragment (Fig. 4, lane 7). A transductant formed by the integration of pLJS64, which has no homology with the chromosome and can only integrate by site-specific recombination, had a pattern of bands similar to that of the transductants shown in lanes 2 to 6 (lane 8). The fact that no transductants were formed when the Mx8 portion of the vector was deleted from pLJS64 (data not shown) argues that the integration process is mediated by the Mx8-derived sequences. In each case the integrated plasmid appeared to be present at a copy number of 1 per chromosome, since a third band, the length of the plasmid, would have been detected if a multimeric form of the plasmid had integrated. Together, these results demonstrate that plasmids containing the Mx8-derived sequences preferentially integrate at a unique location by a process different from homologous recombination.

Expression of cloned DNA. To determine whether the cloned sequences were developmentally expressed in their new chromosomal location, pLJS61 and pLJS62 were transduced into a wild-type strain, DK1622, and *csgA* mutant, LS202. In the *csgA*$^+$ background, pLJS61 and pLJS62 showed developmental regulation of *lacZ* expression (Fig. 5B and C). For pLJS62, the results shown are the average of determinations for three different transductants, and the variation between strains is given by the error bars (Fig. 5B). There seemed to be little variability between individual transductants. The maximum expression of the merodiploids was only about half that of the parental strain DK5204 (Fig. 5A). A Km^s segregant from a pLJS62 transductant in the wild-type lost the ability to produce β-galactosidase (Fig. 5D). Furthermore, a transductant formed from pLJS64 in the wild type did not express β-galactosidase activity, suggesting that there was not a substantial amount of readthrough transcription at the site of integration. In the *csgA* background, expression of the cloned promoter in pLJS61 or pLJS62 was negligible (Fig. 5B and C). Together, these results suggest that genes moved to distant locations maintain the ability to be induced at the appropriate time during development.

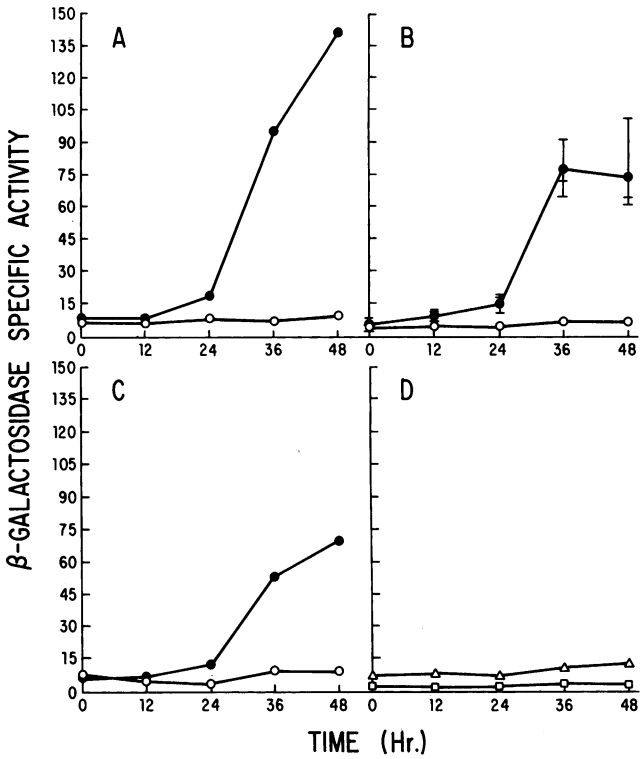

FIG. 5. Expression of *lacZ* under the control of a cloned *M. xanthus* promoter. (A) DK5204, Tn*5 lac* ΩDK4435, in *csgA*$^+$ background (●) and LS248, Tn*5 lac* ΩDK4435, in *csgA* background (○). (B) Average activity of three independent DK1622 transductants containing pLJS62 (●) and LS202(pLJS61) (○). The values for the transductants are shown with horizontal slashes, and the range is given with the vertical line. (C) DK1622 containing pLJS61 (●) and LS202 containing pLJS61 (○). (D) DK1622 containing pLJS64 (△) and Km^s segregant of DK1622 containing pLJS62 (□).

DISCUSSION

Cell-cell communication has been implicated in the control of *M. xanthus* development (6, 13). Mutations in genes involved in cell communication, such as *csgA*, prevent

expression of certain developmental markers, such as Tn*5* *lac* ΩDK4435 (6). One level of control is at the level of transcription, since neither β-galactosidase nor *lacZ* mRNA was detected in *csgA* cells containing this fusion. A 12-h lag was observed between initiation of transcription and appearance of β-galactosidase activity in *csgA*$^+$ cells. The precise reason for this lag is unknown, but one possibility is the large difference in the sensitivity of the two techniques, although posttranscriptional or translational regulatory mechanisms cannot be ruled out.

Plasmids that replicate in *M. xanthus* have not yet been discovered, and alternative strategies have been adopted for creating merodiploids for genetic analysis. DNA has been integrated into the chromosome by homologous recombination between the cloned allele and the chromosomal copy (10, 14) and by site-specific recombination mediated by the bacteriophage Mx8 system (15). The former method has been used by many workers, and two disadvantages have been noted; gene conversion (10, 12, 14, 16) and marker rescue (12, 16), which arise out of the recombination event that brings the two alleles together (12, 14). In a careful comparison of these two methods for integrating DNA into the chromosome, it was found that gene conversion (12, 16) and marker rescue (12) do not occur after site-specific integration. Several additional advantages to the site-specific integration technique were discovered during the course of this work. First, there was a single integration site, and quantitative comparisons may be made between expression of different DNAs integrated at this site. Second, within the resolution of this technique, there is little if any background expression. Third, the integrated DNA was present at a single copy per chromosome, permitting precise copy number control. Fourth, the integrated DNA was extraordinarily stable, segregating at a frequency of 0.0007% per generation in the absence of selection. Fifth, genes integrated at this location were expressed well and maintained their developmental regulation. The developmentally regulated protein S gene was expressed in a similar vector, although the time of expression and level of expression were not compared with those in the wild type (17). Finally, there was little variation in expression between independent transductants. Therefore, integration of cloned genes by site-specific recombination provides perhaps the most reliable system for dominance and complementation tests, as well as for measuring the activity of cloned promoters in vivo. With this system it should be possible to elucidate the mechanisms by which this promoter is developmentally regulated by CsgA-dependent cell communication.

ACKNOWLEDGMENTS

We thank Sheilah Asher and Scott Smith for their able technical assistance.

This work was supported by grants PCM-8351306 and DCB-8710705 from the National Science Foundation.

LITERATURE CITED

1. **Avery, L., and D. Kaiser.** 1983. In situ transposon replacement and isolation of a spontaneous tandem duplication. Mol. Gen. Genet. **191**:99–109.
2. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
3. **Hodgkin, J., and D. Kaiser.** 1977. Cell-to-cell stimulation of movement in nonmotile mutants of *Myxococcus*. Proc. Natl. Acad. Sci. USA **74**:2938–2942.
4. **Kafatos, F. C., C. Weldon, and A. Efstratiadis.** 1979. Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucleic Acids Res. **7**: 1541–1552.
5. **Kroos, L., and D. Kaiser.** 1984. Construction of Tn*5lac*, a transposon that fuses *lacZ* expression to endogenous promoters, and its introduction into *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **81**:5816–5820.
6. **Kroos, L., and D. Kaiser.** 1987. Expression of many developmentally regulated genes in *Myxococcus xanthus* depends on a sequence of cell interactions. Genes Dev. **1**:840–854.
7. **Kroos, L., A. Kuspa, and D. Kaiser.** 1986. A global analysis of developmentally regulated genes in *Myxococcus xanthus*. Dev. Biol. **117**:252–266.
8. **Kuner, J., and D. Kaiser.** 1982. Fruiting body morphogenesis in submerged cultures of *Myxococcus xanthus*. J. Bacteriol. **151**: 458–461.
9. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **O'Conner, K. A., and D. R. Zusman.** 1983. Coliphage P1-mediated transduction of cloned DNA from *Escherichia coli* to *Myxococcus xanthus*: use for complementation and recombinational analyses. J. Bacteriol. **155**:317–329.
11. **Shimkets, L. J.** 1987. Control of morphogenesis in myxobacteria. Crit. Rev. Microbiol. **14**:195–227.
12. **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.
13. **Shimkets, L. J., and M. Dworkin.** 1981. Excreted adenosine is a cell density signal for the initiation of fruiting body formation in *Myxococcus xanthus*. Dev. Biol. **84**:51–60.
14. **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.
15. **Stellwag, E., J. M. Fink, and J. Zissler.** 1985. Physical characterization of the genome of the *Myxococcus xanthus* bacteriophage Mx8. Mol. Gen. Genet. **199**:123–132.
16. **Stephens, K., and D. Kaiser.** 1987. Genetics of gliding motility in *Myxococcus xanthus*: molecular cloning of *mgl* locus. Mol. Gen. Genet. **207**:256–266.
17. **Teintze, M., M. Inouye, and S. Inouye.** 1988. Characterization of calcium-binding sites in developmental-specific protein S of *Myxococcus xanthus* using site-specific mutagenesis. J. Biol. Chem. **263**:1199–1203.
18. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
19. **Ullman, A.** 1984. One-step purification of hybrid proteins which have β-galactosidase activity. Gene **29**:227–231.