JOURNAL OF BACTERIOLOGY, Dec. 1991, p. 7625–7635
0021-9193/91/237625-11$02.00/0

Vol. 173, No. 23

Upstream Gene of the *mgl* Operon Controls the Level of MglA Protein in *Myxococcus xanthus*

PATRICIA HARTZELL† AND DALE KAISER*

Department of Biochemistry and Department of Developmental Biology, Stanford University School of Medicine, Stanford, California 94305-5307

Received 24 April 1991/Accepted 25 September 1991

The *mgl* operon contains two open reading frames (ORFs) which are transcribed together. A collection of nonmotile mutants helped to define the downstream ORF as the *mglA* gene. Single mutations at the *mglA* locus completely abolish motility. A series of deletion mutations was constructed to determine the role of the upstream ORF (now called *mglB*). A strain carrying a deletion in *mglB* and with an intact *mglA* produces small colonies. The cells are motile, but their rate of swarm spreading is reduced. Measurements of cell movement showed that *mglB* mutant cells advanced, on average, less than 0.1 cell length in 5 min. The *mglB*$^+$ cells advanced an average of 1.3 cell lengths in the same time. Extracts of Δ*mglB* cells contain 15 to 20% as much of the 22-kDa MglA protein as do mglB$^+$ cells, as measured in Western immunoblots and enzyme-linked immunosorbent assays. However, the amount of *mgl* transcript is the same in the Δ*mglB* mutants as in the *mglB*$^+$ strain. Heterozygous partial diploids *mglB*/*mglA* with the wild-type alleles in *trans* have normal motility, demonstrating that the largest of the *mglB* deletions is not polar on *mglA*. Like other motility defects, a Δ*mglB* mutation alters fruiting body development and sporulation. The *mglB* mutants delayed aggregation, produced small immature fruiting bodies, and sporulated at 45 to 50% wild-type levels. All aspects of the *mglB* mutant phenotype are explained by the reduced levels of *mglA* protein and the assumption that it limits the amount of gliding.

Gliding motility is defined as translocation over a solid surface and involves movement in the direction of the long axis of the cell (8). In all known gliding organisms, movement is correlated with the production of slime. There is occasional reversal of direction, but motion is smooth, with no rotation apparent. No appendages, which would suggest a mechanism for gliding, have been identified. Gliding is employed by a diverse group of organisms, including *Cytophaga* and *Flexibacter* spp., the myxobacteria, and the filamentous cyanobacteria, all of which belong to evolutionarily different groups (33). These organisms range in length from 5 to 50 μm and glide at rates of 1 to 300 μm/min. While different explanations for gliding have been proposed for different organisms (directional extrusion of slime [24], polar release of surfactant [12], and movement by contraction [8, 26], for example), the molecular mechanism of gliding remains unsettled.

Genes that regulate the pattern of gliding motility have been found. Two genetically independent sets of genes, called the adventurous (A) and social (S) motility systems, have been identified in *Myxococcus xanthus* (18, 19). A single mutation at the *mglA* locus simultaneously blocks both A and S motility (17), and it is the only type of single mutation known which gives rise to completely nonmotile cells. Like other motility mutants, such as A$^-$, S$^-$, and *frz* (4, 28) strains, mutations at *mgl* also affect fruiting body development. Mutant *mglA* cells are completely defective in fruiting and sporulation (22). Gliding is essential for fruiting body development because, in part at least, it permits *M. xanthus* cells to achieve the critical cell density required for development (34) and to form the regular alignments that are necessary for transmission of the C-signal molecule (21, 22).

The *mglA* gene of *M. xanthus* encodes a 22-kDa cytoplasmic protein that is necessary for gliding during growth and development (22, 34). The *mglA* antigen was detected in extracts of *Myxococcus virescens* and *Stigmatella aurantiaca* as well as in extracts of *M. xanthus* (15). Sequence analysis of the *mgl* locus revealed two open reading frames (ORFs); the downstream ORF identified as *mglA* is the locus of mutations which abolish gliding (34). ORF1 is transcribed in the same mRNA with *mglA* and lies just upstream of it. Because of the location of ORF1, it seemed possible that its product might also play a role in gliding. To determine whether the ORF1 gene product is essential for gliding, a series of mutations in ORF1 was constructed and the motility of each was analyzed. Since ORF1 precedes *mglA* in their common transcription unit, we were mindful that a mutation in ORF1 could have a polar effect on *mglA* expression and thus indirectly alter gliding. Accordingly, deletions in ORF1 were designed to retain a functional *mglA*. The ORF1 mutants were analyzed for their effect on growth and on A- and S-system motility. All three deletions constructed were found to express *mglA* but at reduced levels, and all decreased gliding in proportion to their loss of *mglA*. The effects of ORF1 on the control of gliding and on *mglA* are the subjects of this report.

MATERIALS AND METHODS

Bacterial strains and cultivation. *M. xanthus* DK1622, which is fully motile (A$^+$ S$^+$) and kanamycin sensitive (Kan^s), was used as the wild-type reference strain (20). *M. xanthus* DK1218 (*cglB2*), DK1300 (*sglG1*), and DK1250 (*aglB1 tgl-1*) have been described elsewhere (19). DK476 (*asgA1 sglA1*), a strain of *M. xanthus* which is competent for electroporation, was used to construct multiple mutants.

* Corresponding author.
† Present address: Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA 90024-1489.

DK4050 (*mglA4*) and DK4151 (*mglA7*) are Kan^s nonmotile strains (35). Both mutants fail to produce detectable levels of *mglA* protein by immunoblot (15). All cultures were grown in CTT broth or on CTT agar plates (1% Casitone [Difco Laboratories, Detroit, Mich.], 10 mM Tris, 1 mM KH_2PO_4, 8-mM $MgSO_4$, final pH 7.5) at 33°C with vigorous aeration (17). Where applicable, kanamycin sulfate was added to CTT medium to a final concentration of 40 μg ml^{-1}. Growth of *M. xanthus* in liquid culture was monitored by measuring the light scattered from cells in a Klett colorimeter equipped with a red filter. *Escherichia coli* MC1061 was used during plasmid construction and for plasmid maintenance; it was grown on LB medium (27).

Plasmid constructions. Plasmids pPLH300 and pPLH325 were subcloned from pKNS123, which contains the original 12-kb *M. xanthus* DNA which was known to rescue the motility defect of all *mgl* mutants (35). pPLH300 contains a 1.75-kb *Bam*HI-*Sal*I fragment of *mgl* DNA from pKNS281 subcloned into a pUC18 vector digested with *Bam*HI and *Sal*I. The 1.75-kb region contains the *mgl* promoter region and both ORFs. pPLH325 contains a 2.2-kb *Pst*I fragment from pKNS123 cloned into a pBGS18 vector which contains the neomycin phosphotransferase (NPT) gene (32). pPLH325 differs from pPLH300 in that it includes an additional 500 bp of DNA 5′ to the *mgl* promoter. pPLH302 is identical to pPLH300 except that a 60-bp *Bgl*II region (bp 564 to 624 counted from the *Bam*HI site as bp 1 in the sequence described by Stephens et al. [34]) has been deleted from ORF1. A 1.8-kb *mgl* region from pPLH302 was isolated and subcloned into pBGS18 to produce pPLH322. A *Xho*I digest of pLS49 (a gift from L. Shimkets) was the source of the 5.5-kb Mx8 attachment fragment; this fragment was then introduced into the *Dra*I site of pPLH322 to create pPLH332. Likewise, pPLH335 was prepared by introducing the same 5.5-kb Mx8 attachment fragment into the *Dra*I site of pPLH325.

pPLH340 contains a 1.5-kb fragment of NPT gene inserted between the two *Bgl*II sites within ORF1. pPLH321 was derived from pPLH320 after digestion with *Bal*I to remove a 834-bp fragment from the *mgl* region beginning with bp 422 in ORF1 to bp 1256 in the *mglA* gene. Approximately 420 bp of flanking DNA remain at the 5′ end of ORF1, and 300 bp remain at the 3′ end. pPLH350 is a subclone of pPLH320 in which a 354-bp *Eco*RV-*Hin*cII (bp 315 to 669 with *Bam*HI as bp 1) region has been deleted within ORF1. In pPLH351, the same region from ORF1 has been deleted from pPLH330. pPLH330 differs from pPLH320 in that it contains the Mx8 attachment site.

ORF1 deletion strain construction. To ascertain the function of ORF1, three different types of ORF1 deletion mutations were constructed. pPLH322 and pPLH350 were introduced into DK6204 (described below) by electroporation to produce DK6216 and DK6250, respectively. Both plasmids contain sufficient *M. xanthus mgl* DNA to allow integration at the (partially) deleted *mgl* locus in strain DK6204. pPLH332 and pPLH351 were also introduced by electroporation into DK6204 to produce DK6213 and DK6251, respectively. DNA was electroporated into *M. xanthus* cells as described previously (25) except that the electroporation buffer did not contain glycerol. pPLH322 has suffered a 60-bp deletion within ORF1, and this 60-bp region is inside the region of DNA which has also been deleted, constructing DK6204. Since pPLH322 contains an intact copy of *mglA*$^+$, its integration will restore motility functions which require the presence of the *mglA* protein, but an intact copy of the ORF1 gene cannot be reconstructed by homologous recombination. Similarly, integration of pPLH350 at the *mgl* chromosomal site cannot produce an intact copy of the ORF1 gene. These four strains, DK6213, DK6216, DK6250, and DK6251, differ in that pPLH332 and pPLH351 integrate at the Mx8 attachment site while pPLH322 and pPLH350 incorporate into the *mgl* chromosomal locus by homologous recombination. DK6214 is a fully motile derivative of DK6204 containing pPLH335 integrated at the Mx8 attachment site. Colonies of DK6214 are indistinguishable from DK1622 (data not shown), and DK6214 served as a control for integration at the Mx8 attachment site.

DK6204 is a Kan^s *mgl* null mutant which is deleted for 57% of ORF1 and 84% of *mglA*. It was constructed by introducing pPLH321 into DK476 by electroporation. The resulting electroporant, designated DK6170, was motile and Kan^r as a result of integration at the *mgl* chromosomal location. An Mx4 stock (myxophage Mx4 *ts18 ts27 hrm* [14]) was prepared from DK6170 and was used to transfer its tandem duplication into DK1622 to create DK6202. Nonmotile, Kan^s segregants were obtained by culturing DK6202 in CTT liquid, without selection, for several generations. Aliquots were removed at various times and plated on CTT agar. After incubation at 33°C for 4 days without selection, 200 colonies were toothpicked to CTT plates with and without addition of kanamycin; 57 of these colonies were Kan^s, and 24 were nonmotile. DNA from three of these segregants was analyzed by Southern hybridization, which confirmed that the 834-bp *mgl* region was missing. Hence, nonmotile colonies examined were the result of excision of vector along with the original, chromosomal copy of *mgl*. Southern analysis of the original tandem duplication strain DK6202 indicated that the original *mgl* region was present in addition to the plasmid which was introduced containing the *mgl* deletion. The *mgl* null mutant is named DK6204. We were unable to detect any *mglA* protein in this strain by Western immunoblot analysis probed with anti-*mglA* immunoglobulin G.

An additional construction to confirm the ORF1 phenotype was prepared by obtaining segregants of a strain which harbored a tandem duplication of pPLH340. pPLH340 was initially introduced into DK476 and DK6204 by electroporation to produce DK6173 and DK6217, respectively. The resulting electroporants thus carried a copy of the wild-type *mgl* operon in tandem with pPLH340 integrated at the *mgl* chromosomal locus. Stable Kan^r electroporants were isolated, and Mx4 bacteriophage stock was prepared to transfer the tandem duplication into DK1622 to generate DK6205. A segregant of DK6205, DK6206, was obtained by culturing DK6205 in CTT liquid for several generations and subsequently plating dilutions on CTT agar. Colonies with visible motility defects were selected as candidates for the ORF1 mutation.

To evaluate the effect of the upstream *mglB* deletion on the expression of *mglA*, pPLH351 was electroporated in DK4050 and DK4155 to produce DK6264 and DK6267, respectively. The colonies of electroporants of each strain were examined on solid medium, and their motility was compared with the motility of DK1622 (wild type) and DK6251 on the same plates. As a control, pPLH330 was introduced into the same two recipients.

Congo red staining. Colonies were stained for slime components by flooding the colony with 400 μl of an aqueous solution of 0.02% Congo red and 100 mM sodium phosphate (pH 7.7) (2, 29, 30). DK6206 and DK6213, when stained with Congo red, turned red at the periphery of the colony, which

is a typical Congo red staining pattern for the wild-type colony.

Quantitation of cell movement. Time lapse photography was used to analyze the motility of individual cells of ORF1 mutants. DK6206, DK6213, DK6230, DK6231, DK1622 (wild type), and DK1250 (A^- S^-) were grown in CTT broth to a cell density of 4×10^8 to 5×10^8 cell per ml (100 Klett units [20]) and adjusted to 5 and 25 Klett units by dilution in a 1:1 (by volume) CTT deionized water solution. Activated charcoal was added to each sample to a final concentration of 50 μg/ml. Samples were prepared for examination in two-chamber Lab-Tek tissue culture slides (Miles Scientific, Naperville, Ill.) filled with 700 μl of 0.5 × CTT plus 1% agar. Aliquots (10 μl) of each mixture were spotted in separate chambers of the Lab-Tek slides and allowed to dry at room temperature (~25°C) for 20 min. The slides were covered and incubated at 33°C.

For DK1622 and the ORF1 mutants DK6206 and DK6213, photographs were taken at 5-min intervals, using Panatomic-X film. Cell movement was monitored by projecting negatives onto a grid and marking the change in position of 30 cells for each photograph. Both isolated cells and cells in contact were examined. The path of each cell was noted for 18 consecutive images taken at 5-min intervals. For the double mutants DK6230 and DK6231, photographs of the same microscopic field were taken with Kodak high-contrast Ektagraphic slide film (ASA 25) at 1-, 2-, and 4-h intervals after the initial spotting. The local distribution of charcoal particles was noted to ensure that the same field was photographed at each time point. Photographic negatives were examined under a dissecting microscope to determine whether the position of cells had changed during the 4-h incubation. For each strain, at least 200 individual cells were examined. During this time, growth was apparent for each strain, indicating that cells remained viable.

Spreading rate was determined as described by Kaiser and Crosby (20). For this assay, cells were grown to 4×10^8 cells per ml and centrifuged for 2 min at 13,500 × *g*. Cell pellets were then suspended in a volume of CTT broth calculated to yield either 100 or 500 Klett units. Charcoal particles were added to a final concentration of 0.2 mg/ml as position markers.

Quantitation of MglA protein by Western immunoblot. Extracts were prepared from DK6206 and from DK6213, electrophoretically separated on 12% sodium dodecyl sulfate (SDS)-polyacrylamide gels, and compared with extracts from DK1622 grown and harvested under identical conditions. To estimate the relative levels of MglA protein in each of the extracts, various amounts of protein (5, 10, 15, and 20 μg of total cell extract) were compared in Western immunoblots. The response was measured by using a 1:1,200 dilution of anti-*mglA* immunoglobulin G (15). Proteins were electrophoretically transferred to nitrocellulose, using a Transblot apparatus (Bio-Rad) equipped with a cooling coil, and run at 90 V for 2 h. The transfer buffer contained 20% methanol, 20 mM Trisma base, and 0.15 M glycine at a final pH of 8.6. The transferred proteins were visualized by staining with 0.2% Ponceau S in 3% trichloroacetic acid.

The nitrocellulose blot was incubated with a blocking buffer containing 3% gelatin in TBS (10 mM Tris, 150 mM NaCl, pH 7.5) for 1 to 2 h at room temperature (25°C) with gentle rotation. The blot was washed with three 5-min incubations of TBS containing 0.1% Tween (TTBS) and then reacted with primary antibody (anti-*mglA*) at a 1:900 dilution in 1% gelatin buffer in TTBS for 1 h at room temperature. The blot was then washed with three changes of TTBS buffer, secondary antibody (goat anti-rabbit conjugated to alkaline phosphatase diluted 1:2,000 in 0.4% gelatin in TTBS buffer) was added, and the mixture was incubated at room temperature for 1 h. The blot was washed three times with TTBS buffer and then three times with 0.15 M Tris-HCl (pH 9.6). Alkaline phosphatase activity was visualized upon incubation at 37°C with a solution containing 0.15 M Tris-HCl (pH 9.6), 4 mM $MgCl_2$, 0.1 mg of *p*-nitroblue tetrazolium chloride per ml, and 0.05 mg of 5-bromo-4-chloro-3-indolylphosphate (U.S. Biochemical) per ml. The bands were visible within 10 to 15 min.

Enzyme-linked immunosorbent assay (ELISA). Cultures of DK1622, DK6206, DK6213, and DK6204 were grown in CTT broth to a cell density of 5×10^8 cells per ml and were centrifuged. The pellet was suspended in 0.1 volume of TBS (20 mM Tris [pH 7.5], 500 mM NaCl) to 5×10^9 cells per ml. Lysis was achieved by addition of 10 μl of 10% SDS to 490 μl of each cell preparation. Each strain was assayed in triplicate at three different dilutions corresponding to 5×10^9, 2.5×10^9, and 1×10^9 cells per ml. Immulon microtiter plates (Dynatech, Alexandria, Va.) were first blocked with 300 μl of TBS and 2% albumin. Antigen preparations (cell extracts) were incubated for 4 h and then washed three times for 5 min each with TBS. The primary antibody, affinity-purified anti-*mglA*, was diluted 1:100 (90 μg/ml) and incubated at 4°C overnight. The wells were washed three times with TBS, secondary antibody, a 1:300 dilution of goat anti-rabbit conjugated to alkaline phosphatase, was added, and the mixture was incubated at room temperature for 2 h. The activity was determined after addition of a mixture of 0.1 M 3-[cyclohexylaminol]-2-hydroxy-1-propanesulfonic acid (pH 10.0), 0.5 mM $MgSO_4$, and 1 mg of *p*-nitrophenylphosphate per ml. After incubation at 37°C for 30 min, the reaction was terminated by addition of 2 volumes of 37.5 mM EDTA (pH 8.0). Activity per well was measured as A_{405} relative to control wells.

Protein determinations were performed using the Bradford reagent (7). For dilute samples, the BCA reagent (Pierce, Rockford, Ill.) was also used. In both cases, gamma globulin was used as the standard. SDS-polyacrylamide gels were prepared, and electrophoretic conditions were applied as described by Blackshear (5).

Northern (RNA) analysis. RNA was isolated as described by Stephens et al. (34) and then separated by electrophoresis on 1% agarose gels containing 2.2 M formaldehyde, 20 mM 3-[*N*-morpholino]propanesulfonic acid (MOPS; pH 7.0), 1 mM EDTA, and 5 mM sodium acetate. Buffer solutions and deionized water used to prepare the gel were pretreated with diethylpyrocarbonate at a final concentration of 0.2%. RNA and ^{32}P-labeled DNA molecular weight standards were lyophilized and hydrated in 20 μl of diethylpyrocarbonate-treated sample buffer (2.2 M formaldehyde, 50% deionized formamide, 20 mM MOPS [pH 7.0], 1 mM EDTA, 5 mM sodium acetate) and 1 μl of 1% bromophenol blue. RNA was heated at 65°C for 5 min and then electrophoresed at 100 V for approximately 4 h. The RNA and standards were transferred to Nytran (Schleicher & Schuell, Keene, N.H.) by capillary action for 24 h at room temperature against 10× SSC (1.5 M NaCl, 0.15 M sodium citrate) as described elsewhere (10). The RNA was then cross-linked to the Nytran by UV irradiation (Stratalinker 1800; Stratagene, La Jolla, Calif.; set at 0.12 mJ). The filter was prehybridized at 42°C with 10 ml of a solution containing 50% formamide, 0.75 M NaCl, 0.075 M sodium citrate, 1% SDS, and 5% dextran sulfate. After 4 h, the fluid was exchanged; fresh solution (10 ml) containing 1 μg of boiled sheared salmon

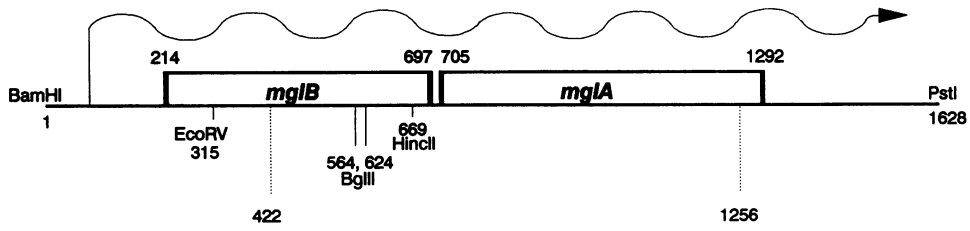

FIG. 1. The *mgl* transcription unit. A 1.6-kb DNA fragment contains *mglB* (bp 214 to 697) and *mglA* (bp 705 to 1292) and an upstream region sufficient for normal expression (34). Restriction sites, used to construct the null strain DK6204 (Δ*mglB mglA*; bp 422 to 1256) and *mglB* deletions are shown.

sperm DNA and 10^7 cpm of labeled probe was added, and hybridization was allowed to proceed at 42°C for 18 h. The filter was washed with 2× SSC–0.1% SDS at room temperature for 5 min and then three times for 40 min each at 65°C.

Northern blots were probed with *mgl* DNA and *dsg* DNA. *dsg* DNA (9) was used as a control to ensure that the same amount of RNA was loaded for wild-type and mutant strains. Probes were labeled with [^{32}P]CTP from DNA fragments excised from low-melting-point agarose gels (SeaPlaque). A 900-bp *dsg* fragment was prepared by restriction of pYLC74 (a gift from Y. Cheng), containing the cloned *dsg* gene, with restriction endonuclease *Sma*I. A 1,054-bp *mgl* fragment was isolated after restriction of pPLH300 with *Bam*HI and *Xho*I. To label each fragment, 15 μl of DNA (approximately 150 ng of DNA) in agarose was boiled with 15 μl of H_2O. The DNA was cooled, and the following additions were made: 10 μl of OLB [200 mM Tris (pH 7), 25 mM $MgCl_2$, 12 μM β-mercaptoethanol, 250 μM each dATP, dTTP, and dGTP, 1 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 6.6), 27 U of pd$(N)_6$ oligonucleotides (Pharmacia, Piscataway, N.J.) ml^{-1}, 50 μCi of [α-^{32}P]dCTP, 3 U of Klenow fragment (DNA polymerase I), and 20 μg of bovine serum albumin. The reaction was incubated at room temperature overnight and then terminated by addition of 50 μl of TES (10 mM Tris [pH 8], 1 mM EDTA, 0.1% SDS). Labeled probe was purified chromotographically, using a Bio-Gel P-10 (Bio-Rad) column equilibrated with TE (10 mM Tris [pH 8], 1 mM EDTA).

RESULTS

Deletion mutations. The *mgl* transcription unit (Fig. 1) consists of two adjacent ORFs, *mglA* and ORF1 (labeled *mglB* in Fig. 1), the function of ORF1 being the subject of this investigation. *mglA* encodes a 22-kDa protein that is essential for all gliding in *M. xanthus* (15). Point mutations, insertions, and deletions in *mglA* produce totally nonmotile cells (15, 34, 35). Without prejudice to the argument to be presented, yet hoping to simplify presentation of the experimental results, we will tentatively refer to ORF1 as *mglB*. To test whether *mglB* plays a role in the control of motility, null (deletion) mutants were sought. Construction of the deletions posed a special problem since mutations in *mglB*, even if the product of *mglB* had no direct role in controlling motility, might cause motility defects indirectly as a consequence of the joint transcription of *mglB* with *mglA* and the upstream position of *mglB* (Fig. 1). The strategy adopted to mitigate this concern was to compare three different internal deletions of *mglB* so that polar effects could be revealed by differences between the phenotypes of the three strains. To ensure that the three deletion strains were isogenic, apart from their *mglB* deletions, a gutted strain was first constructed, one deleted for both *mglB* and *mglA*, adopting the gutted terminology from reference 6. Motility of the gutted strain was determined to establish a baseline. Finally, to the gutted strain were added plasmids containing DNA fragments that had suffered three different internal *mglB* deletions. All three deletion fragments were designed to retain the beginning of *mglB*, in order to preserve transcription initiation, and a complete *mglA*$^+$ sequence, including its putative ribosome binding site. This design was intended to maximize the chance that *mglA*$^+$ would be normally expressed so that any change in motility could be interpreted as a direct action of *mglB*.

The gutted strain, DK6204, has a deletion extending from bp 422 in *mglB* to bp 1256 in *mglA* (Fig. 1). DK6204 produces no MglA protein antigen detected by ELISA (data below), forms very small compact colonies, and is nonmotile under all conditions tested. Details of its construction from pPLH321 (Fig. 2A) are given in Materials and Methods. Over 50,000 colonies of DK6204 have been examined, and no motile revertants have been observed even after exposure to UV mutagenesis.

Three different *mglB* deletion mutants designed to conserve an active *mglA*$^+$ were constructed. A 60-bp deletion internal to *mglB* was constructed by deleting the *Bgl*II fragment between bp 564 and 624 (Fig. 1). This 60-bp deletion mutation, incorporated into plasmids pPLH322 and −332 (Fig. 2 B and C), is internal to the coding region of *mglB* and therefore should preserve transcription initiation and reading frame.

However, because the 60-bp deletion should preserve the reading frame, it could not be excluded, a priori, that a truncated MglB protein lacking 20 amino acids would retain partial activity. To control for this possibility, the deleted *Bgl*II fragment was replaced by a 1.5-kb DNA fragment containing the complete NPT gene (3). This second fragment type was realized in pPLH340 (Fig. 2D).

Although replacement of the *Bgl*II fragment by NPT gene in the DK6206 construction should completely inactivate *mglB* and yield an *mglB* null mutation, one might still object that transcription from the NPT gene promoter might prevent *mglA* transcription, and by this indirect means motility might be reduced. Accordingly, a large *mglB* deletion was constructed as a second control. This third deletion removed bp 315 to 669, or 73%, of *mglB* and also conserves the reading frame (Fig. 1). The third deletion is carried by pPHL350 and pPHL351 (Fig. 2E and F).

Duplication strains. The 60-bp (short) in-frame deletion, the NPT gene replacement, and the long in-frame deletion were separately placed as duplications in either an *mgl*$^+$ genetic background or the gutted background of DK6204 for comparison of phenotype. Construction and genotypes of the resulting tandem duplication and segregant strains are

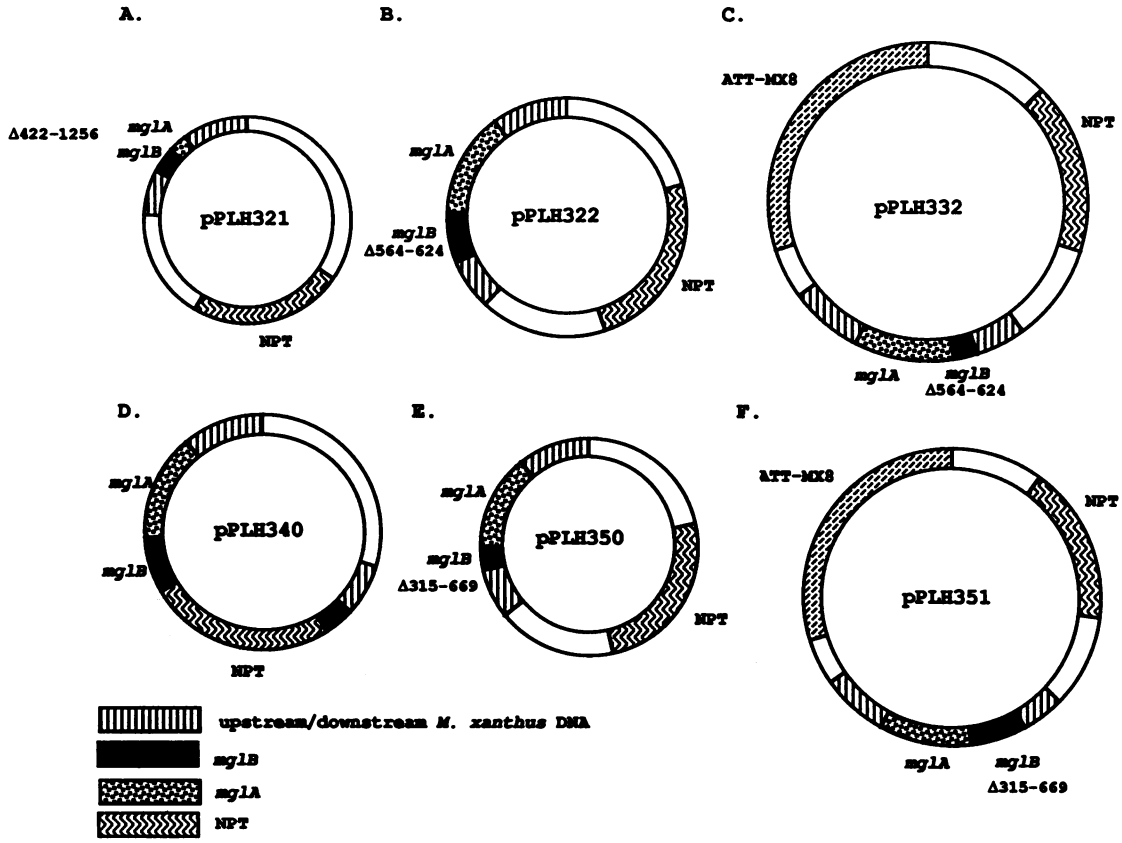

FIG. 2. Plasmids used in the construction of *mgl* mutant strains.

summarized in Table 1. Line 1 of Table 1 summarizes the origin of the gutted strain, DK6204, which is detailed in Materials and Methods. The NPT gene replacement plasmid pPLH340 (Fig. 2) was integrated at the *mgl* locus, resulting in a tandem duplication at the *mgl* chromosomal location with plasmid sequences at the novel joint (Table 1, line 2). Segregants which had lost, by homologous recombination, the original chromosomal DNA along with adjacent vector sequences were obtained after culturing the cells for several generations in liquid medium without antibiotic selection. Approximately 7,000 segregants were examined from three independent cultures. Though initially all colonies had the sharp edges of nonmotile strains, after 5 days two of the cultures yielded a total of 27 colonies showing some motility. All 27 had an identical colony morphology. All of these strains were found to have lost the vector DNA, as determined by DNA hybridization with a probe prepared from vector sequences. These segregants, all Kan^r as a consequence of the NPT gene, are represented by strain DK6206 (Table 1, line 3). DK6250 and DK6251 (Table 1, lines 4 and 5) both lack the long, 354-bp fragment internal to *mglB* (Fig. 1 and 2). Integration within the *mgl* operon in the construc-

TABLE 1. Construction of *mglB* deletions[a]

Recipient	DNA donor	Integration site	Duplication	Segregant	Genotype[b]
1. DK476 (Mot$^+$)	pPLH321[c]	*mgl* operon	DK6170	DK6204	Δ*mglBA*
2. DK476 (Mot$^+$)	pPLH340[c]	*mgl* operon	DK6171	No	Deletion of *Bgl*II; insertion of NPT gene
3. DK1622 (Mot$^+$)	DK6171[d]	*mgl* operon	DK6205	DK6206	Deletion of *Bgl*II
4. DK6204 (Mot$^-$)	pPLH350[c]	*mgl* operon	DK6250	No	*mglA*$^+$/Δ*mglBA*
5. DK6204 (Mot$^-$)	pPLH351[c]	Att	DK6251	No	*mglA*$^+$/Δ*mglBA*
6. DK6204 (Mot$^-$)	pPLH322[c]	*mgl* operon	DK6216	No	*mglA*$^+$/Δ*mglBA*
7. DK6204 (Mot$^-$)	pPLH332[c]	Att	DK6213	No	*mglA*$^+$/Δ*mglBA*
8. DK4050 (*mglA4*)	pPLK351[c]	Att	DK6264	No	Δ*mglB mglA*$^+$/*mglB*$^+$ *mglA4*
9. DK4155 (*mglA7*)	pPLH351[c]	Att	DK6267	No	Δ*mglB mglA*$^+$/*mglB*$^+$ *mglA7*
10. DK4050 (*mglA4*)	pPLH330[c]	Att	DK6274	No	*mglB*$^+$ *mglA*$^+$/*mglA4*
11. DK4155 (*mglA7*)	pPLH330[c]	Att	DK6277	No	*mglB*$^+$ *mglA*$^+$/*mglA7*

[a] Additional steps of strain construction are detailed in Materials and Methods. Mot, motility; Att, attachment.
[b] Genotype of segregant or final duplication.
[c] Donor DNA introduced by electroporation.
[d] Donor DNA introduced by transduction with myxophage Mx4.

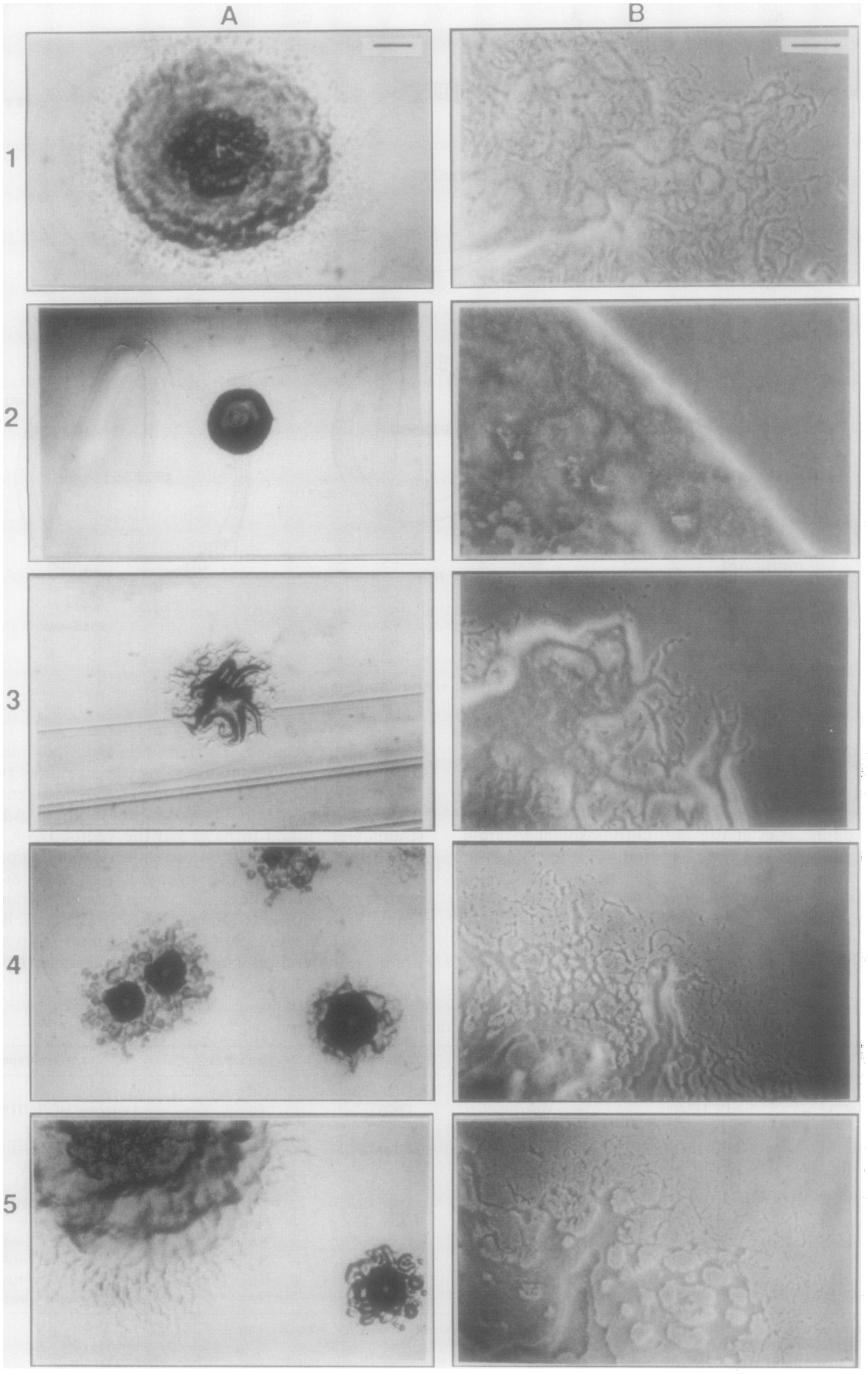
A
B
1
2
3
4
5

FIG. 3. Colony morphology of *mglB* mutants. (A) Colony morphology of DK1622 (wild type) (row 1), DK4141 (*mglA9*) (row 2), DK6206 (*mglB*) (row 3), DK6216 (*mglB*) (row 4), and DK6250 (*mglB*) (colony in lower right corner of photograph in row 5) on CTT agar. (Scale bar for panel A shown at the top is 0.5 mm long.) (B) Colony edge morphology of DK1622 (wild type) (row 1), DK4141 (*mglA9*) (row 2), DK6206 (*mglB*) (row 3), DK6216 (*mglB*) (row 4), and DK6250 (*mglB*) (row 5) on CTT agar. (Scale bar for panel B is 0.1 mm long.) Panel A, row 5, shows the colony morphology of DK1622 (left) and DK6250 (right) grown together on the same CTT agar plate. Only a portion (approximately one-half) of the DK1622 colony is visible in the field.

tion of DK6250 ensured that there would be a normal sequence upstream of *mglB* in the resulting tandem duplication. Comparison of DK6250 with DK6251 would show whether the phenotype would depend on insertion of the plasmid-borne *mgl* operon into the (ectopic) Mx8 prophage attachment site or the normal *mgl* chromosomal site.

To control directly against the possibility that deletions in *mglB* might reduce motility by a polar effect of an upstream *mglB* mutation on the expression of the downstream *mglA* gene, doubly heterozygous partial diploids were constructed with $mglB^+$ and $mglA^+$ in *trans* to each other. A plasmid bearing the 354-bp *mglB* deletion and an Mx8 prophage attachment site (pPLH351) was introduced by electroporation into each of two well-characterized *mglA* point mutants, *mglA4* and *mglA7* (35), as described in Table 1. These double heterozygotes have the genotype ΔmglB $mglA^+/mglB^+$ *mglA* (*mglA4* or *mglA7*). Independent Kan^r electroporants of each recipient were examined and are named DK6264 and DK6267 in Table 1. Positive controls were constructed with pPLH330 ($mglB^+$ $mglA^+$) DNA introduced into the same two recipients. From negative controls with no DNA added but subjected to a mock electroporation, no motile colonies were observed. Seventy-six percent of the DK6264-type Kan^r electroporants (36 of 47 colonies) and 83% of the DK6267-type (46 of 55 colonies) spread as rapidly on agar as did the DK6274-type and DK6277-type positive controls. DK1622 was included on these test plates as a second positive control, and the motile electroporants were found to have the same colony spreading morphology as did DK1622. DK6251 was a negative control on each plate. The high frequency of motile Kan^r strains shows that *mglB* and *mglA* can function in *trans*, even when $mglA^+$ is expressed from the Mx8 attachment site and $mglB^+$ is expressed from the normal *mgl* chromosomal site.

The *mglB* null phenotype. Because of active cell movement in the broad swarming zone at the periphery of a *Myxococcus* colony, the rate of colony spreading is a sensitive indicator of cell motility (18–20). As it turned out, the short-*mglB*-deletion mutants, the NPT gene replacement mutants and the long-deletion mutants all produced the same abnormal colony phenotype (Fig. 3A, rows 4 and 5). The diameter of the colony was 10 to 12 mm after 5 days, about one-quarter the size of an mgl^+ colony of the same age (Fig. 3A, row 5, mgl^+ to the left and Δ*mglB* to the right). All three types of *mglB* mutants had the same doubling time as did DK1622 cells; their small colony size is therefore not due to reduction in growth rate (Table 2). Instead, the small colony size of Δ*mglB* mutants DK6206 and DK6231 correlates with a spreading rate about one-quarter that of DK1622, the standard fully motile strain (Table 2). Though less than the rate of DK1622, the spreading rate of the *mglB* deletion mutants was still 10-fold greater than that of the gutted strain, DK6204. The spreading rate of DK6204, a totally nonmotile strain, can be taken as a measure of the effect of growth on colony spreading, and the data of Table 2 show it to be negligible in the comparison of *mglB* mutants with $mglB^+$ cells.

Reduction of spreading from the wild-type rate has been reported with both A^+ S^- and A^- S^+ strains (20). However, as shown in Fig. 3B, rows 3 to 5, the cell arrangement in the *mglB* deletion mutants resembles that of neither an A^+ S^- nor an A^- S^+ strain. There are both many isolated cells (a characteristic of A motility) and large rafts of cells (a characteristic of S motility) (19). A test of whether the *mglB* deletion inactivates either motility system A or motility system S can be based on the fact that A^- S^- mutants are nonmotile, no matter which A^- or S^- mutations are combined (19). An *mglB* lesion was transduced separately into an A^- recipient and an S^- recipient so that nonmotile (A^- S^-) recombinants could be detected if they were produced. A transducing phage stock prepared from DK6206 (Table 1) carried the Δ*mglB* lesion into DK1218 (*cglB2*) and DK1300 (*sglG1*). The colony edges of Kan^r transductants from both DK1218 and DK1300 were examined microscopically for motility defects. Initially the double mutants DK6230 (*mglB cglB2*) and DK6231 (*mglB sglG1*) produced small, nonspreading colonies, but after several transfers on CTT growth medium, a few motile flares were evident at the colony edges and some spreading was evident.

Individual cells of A^+ S^-, A^- S^+, and *frz* strains move at the same rate as do A^+ S^+ frz^+ cells (4, 20). However, A^- S^+ mutant cells do not move at all when dispersed (19), and individual *frzA*, *-B*, *-C*, *-E*, or *-F* mutants cells reverse their direction of movement less frequently than do frz^+ cells (4), and thus should progress farther in a given direction. To examine the movement of individual Δ*mglB* cells, time lapse studies were carried out. A comparison of *mglB* mutants DK6213 and DK6206 with DK1622 is shown in Table 3. Both *mglB* mutants moved at approximately 0.1 cell length in 5 min, whereas the $mglB^+$ cells moved 1.26 cell lengths, 10-fold more rapidly. Over a 2-h period, 54% of the mutant cells in the field moved, whereas 68% of the $mglB^+$ cells moved during this time. In both cases, movement was detected for isolated cells as well as for cells which were in contact with one another. Overall, the properties of Δ*mglB* mutants resemble those of neither A^-, S^-, nor *frz* mutants, but their behavior resembles that of hypomorphic *mglA* mutants that have decreased but not eliminated both A and S motility.

Levels of MglA protein in *mglB* mutants. Extracts prepared

TABLE 2. Growth and spreading rates of *mglB* deletion mutants

Strain	Doubling time (h)	Spreading rate[a] (μm/min) at density of:	
		100 Klett units	500 Klett units
DK1622	4.6	0.34	0.48
DK6204	4.4	0.005	0.01
DK6206	4.8	0.09	0.11
DK6213	4.5	0.07	0.10

[a] Measured at 33°C.

TABLE 3. Movement of individual *mglB* mutant cells detected by time lapse photography

Strain	Mutation[a]	No. of cells examined	% of cells moving	Cell length(s)/ 5 min[b]
DK1622	*mglB*$^+$	52	68	1.26
DK6204	Δ*mglBA*	27	<3	—[c]
DK6206	Δ*mglB*	36	54	0.12
DK6213	Δ*mglB*	49	47	0.085

[a] Origin of the deletion mutants is described in Table 1.
[b] Measurements of cell movement were made on photographs containing about 20 cells per field that were taken at 5-min intervals over a 1-h period. Only cells which moved were used to calculate speed averages.
[c] No DK6204 cells were observed to move.

from DK6206 and DK6213 were examined by Western immunoblot to determine whether these deletion *mglB* strains produced MglA protein (antigen). Both did. To quantitate the levels of MglA protein, ELISA assays were performed; in addition, each of the cell extracts was diluted to an endpoint just sufficient to detect a signal by Western blotting. Various amounts of the extracts were separated on 12% SDS-polyacrylamide gels, and the protein material was transferred to nitrocellulose and subsequently probed with purified anti-*mglA* antibody. Bands of MglA were evident in the upper part of Fig. 4 when 7 μg of DK1622 protein were added, but 21 or 28 μg of DK6206 or DK6213 extracts was required for even faint bands to appear. The *mglB* deletion mutants produced 11, 15, and 17% the amount of MglA protein as *mgl*$^+$ cells by ELISA (Fig. 4).

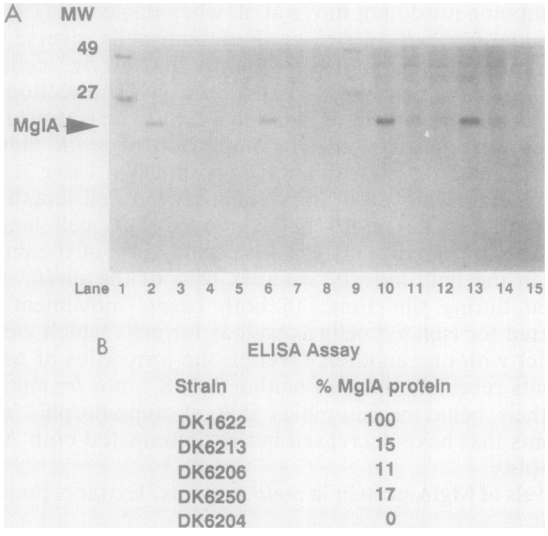

FIG. 4. Immunological detection of MglA protein in *mglB* mutants. (A) Western immunoblot. Extracts of DK1622, DK6206, and DK6213 were separated by electrophoresis on 12% denaturing gels and transferred to nitrocellulose. Nitrocellulose was probed with purified anti-*mglA* immunoglobulin G and visualized with secondary antibody conjugated to alkaline phosphatase. The arrow indicates the position of *mglA*. Lanes: 1, prestained molecular weight (MW) markers (positions indicated in thousands); 2, DK1622 (7 μg of protein); 3, DK6206 (7 μg); 4, DK6213 (7 μg); 5, DK6204 (7 μg); 6, DK1622 (14 μg); 7, DK6206 (14 μg); 8, DK6213 (14 μg); 9, prestained markers; 10, DK1622 (21 μg); 11, DK6206 (21 μg); 12, DK6213 (21 μg); 13, DK1622 (28 μg); 14, DK6206 (28 μg); 15, DK6213 (28 μg). (B) ELISA quantitation of the level of MglA protein in DK1622 (wild type) and *mglB* motility mutants.

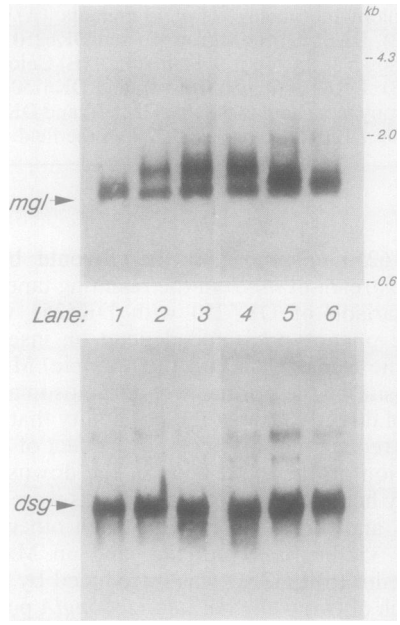

FIG. 5. Northern analysis of *mglB* mutants. Lanes: 1, fully motile control (DK6204 with *mgl*$^+$ DNA integrated at the Mx8 attachment site); 2, DK6206; 3, DK6216; 4, DK1622; 5, DK6250; 6, DK6213. Five micrograms of total RNA was loaded per lane. RNA in the top panel was probed with labeled *mgl* DNA; RNA in the bottom panel was probed with a labeled *dsg* DNA fragment.

***mgl* RNA.** Despite a 5- to 10-fold reduction in MglA protein levels, no corresponding decrease in *mgl* RNA levels was apparent in the *mglB* mutants (Fig. 5). RNA from growing DK1622, DK6206, DK6216, DK6250, and DK6213 cells was probed with DNA specific for the *mgl* region. DNA from the *dsg* gene, a vital gene that is expressed during growth (9), was used to ensure that similar amounts of RNA from each strain had been loaded into the gel. Although there is some change in the distribution of RNA lengths between strains, overall amounts of *mgl* RNA were similar. No interpretation of the length distribution is offered at this time.

Congo red dye binding. Gliding *Myxococcus* cells deposit a slime trail, and the dye Congo red is adsorbed by *Myxococcus* colonies (29, 30). Arnold and Shimkets (2) have correlated Congo red binding with a loose network of thick fibers that surrounds cells and adheres to agar. This network is likely to be at least one of the constituents of slime. They showed that *dsp* mutants lacked thick fibers and failed to react with Congo red dye (2). Colonies of the *mglB* mutants gave a positive reaction with Congo red. Suspensions of *mglB* mutant cells bound Congo red at the same apparent rate and affinity as did *mglB*$^+$ cells. The rate of Congo red binding to DK1622 was 0.26 μg/5 × 10^8 cells per min; to DK6204, it was 0.31 μg; to DK6206, it was 0.28 μg; and to DK6213, it was 0.23 μg. These data suggest that *mglB* mutants retain the material of which the fibers are made.

DK6213 and DK6206 were grown on CTT agar plates supplemented with L-cysteate (1). L-Cysteate is a precursor in the synthesis of sulfonolipids and has been shown to play a role in motility of some gliding organisms (1). No rescue of the *mglB* mutants was observed.

Role of *mglB* in development. When starved of nutrients, *M. xanthus* aggregates, builds fruiting bodies, and sporulates. Motility is required for fruiting body development and

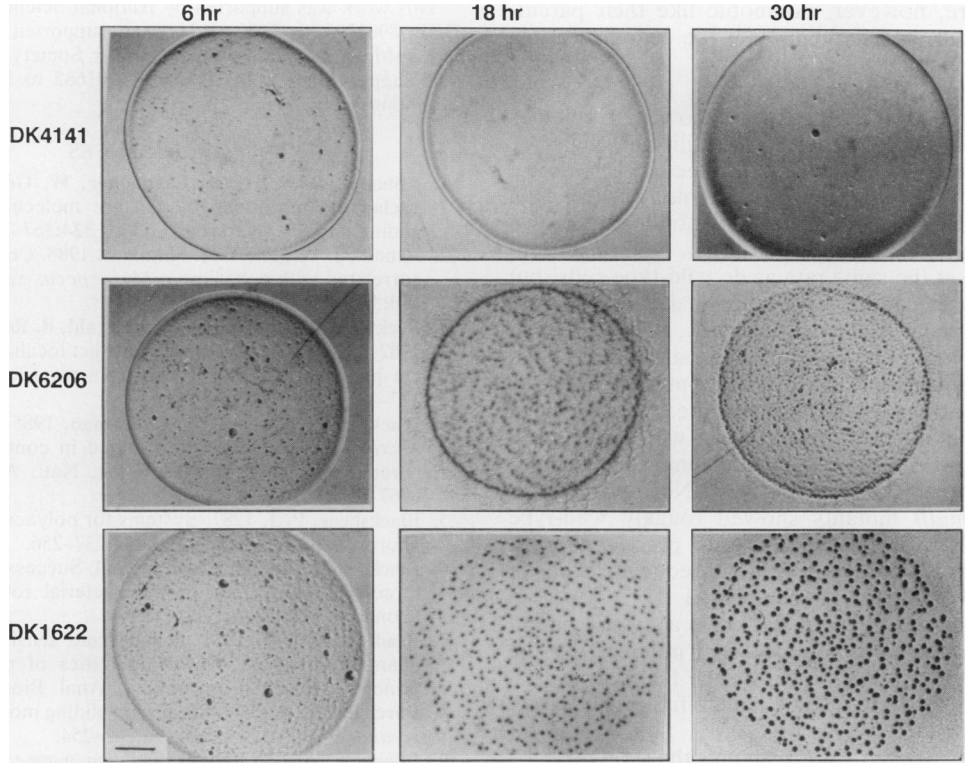

FIG. 6. Development of *mgl* mutants. The developmental phenotype was assayed by spotting 20 μl of a suspension of cells (5×10^9 cells per ml in TPM) on starvation medium as described previously (23). Plates were incubated at 33°C and photographed in bright field at 6, 18, and 30 h after the onset of starvation. Shown is fruiting body development of DK1622 (wild type), DK6206 (*mglB*), and DK4141 (*mglA9*). Scale bar (lower left) is 0.5 mm long.

sporulation (21, 22). Altered patterns of cell movement are reflected in the shape and number of multicellular aggregates (4, 13, 19). When starved, the *mglB* mutants delayed aggregation for 10 to 12 h relative to $mglB^+$ cells (data not shown). Aggregates that did form were smaller than $mglB^+$ cells, which is evident in Fig. 6. Microscopic examination revealed that roughly twice the number of cells remained outside the *mglB* aggregates compared with DK1622 and produced 40 to 45% of the number of spores produced by $mglB^+$ cells.

DISCUSSION

Two ORFs are transcribed into the same mRNA from the *mgl* operon (34). Deletion mutants were constructed to investigate the possible function of the upstream open frame (*mglB*) in gliding motility. Their construction posed a special problem because *mglB* is upstream of and cotranscribed with *mglA*, and *mglA* is known to be essential for gliding (34). This problem was approached by constructing three different deletion mutants: a 60-bp in-frame deletion within *mglB*, a 354-bp deletion within *mglB*, and an insertion of the NPT gene within the 60-bp deletion of *mglB*. Although a deletion of 20 amino acids from the central part of a gene would be expected to render the gene inactive, the insertion or the 354-bp internal deletion of a 484-bp gene would certainly destroy all activity. As it turned out, all three deletion strains have the same phenotype, which thus corresponds to the null genetic state. Since that phenotype results from reduced gliding, affecting both A and S motility, *mglB* is an appropriate designation for this ORF. (*mgl* indicates a mutual gliding function, mutual with respect to A and S motility.)

The *mglB* mutant phenotype is immediately evident in the size and form of mutant colonies. Δ*mglB* colonies are one-quarter or less the diameter of wild-type colonies of the same age. These small colonies are, however, larger than Δ*mglA* colonies. Δ*mglB* colonies are surrounded by a zone of swarming cells, which is absent from Δ*mglA* colonies. In time lapse studies, single cells of Δ*mglB* strains were observed to move, but their movement was less than 10% the rate of mgl^+ cells. Similar time lapse experiments with *mglA* mutants showed no movement at all (19). Consistent with the low rate of movement of individual cells, the *mglB* mutant swarms spread fourfold more slowly than did wild-type swarms.

The size of Δ*mglB* mutant colonies resembles those of A^- S^+ and A^+ S^- strains, and all three types of mutants show reduced rates of swarm spreading (18, 19, 21; this work). A general test of whether *mglB* is an A or an S gene is based on the fact that any A^- S^- strain is nonmotile, no matter which A or S genes are involved (19). Δ*mglB* mutants failed this test, because colonies of both A^- Δ*mglB* and S^- Δ*mglB* strains initially had the sharp edges characteristic of nonmotile strains. The fact that both A^- Δ*mglB* and S^- Δ*mglB* strains are initially nonmotile implies that Δ*mglB* is not an A^- or an S^- mutation. If it were, one or the other double mutant, but not both, should have been motile (19). Isolated cells of these double mutants, when followed in time lapse photography, were nonmotile. In all of these qualities, the Δ*mglB* mutants resembled *mglA* mutants.

Upon transfer to fresh plates, both A^- Δ*mglB* and S^- Δ*mglB* strains began to produce small flares at their colony edges, indicative of cell movement. Bacteria picked from the

Δ*mglB* flares were, however, nonmotile like their parent, ruling reversion out, and implying that the cells had a low probability of moving.

Null *mglA* strains or the gutted strain, DK6204, never produced such flares. Detection of some motility in colonies does not conflict with the absence of motility in single A^- Δ*mglB* and S^- Δ*mglB* cells studied by time lapse, because the density of cells is much higher in a colony than in the single-cell experiments, and both A and S motility are known to increase markedly with cell density (20). Null (insertion) *frz* mutants move at the same rate as do wild-type cells, but they reverse less frequently and individual cells progress farther (4). Therefore, *mglB* is neither an A, S, nor *frz* gene.

Extracts of the *mglB* mutants contained substantially less MglA protein, as measured with an affinity-purified antibody to *mglA* (15). It is reasonable to associate the greatly reduced but nonvanishing motility of *mglB* mutants with the reduced levels of MglA protein because cells are completely nonmotile in the absence of MglA protein (15). Northern hybridization of four *mglB* mutants showed roughly wild-type levels of *mgl* RNA. If the $mglB^+$ gene product were a positive regulator of *mgl* transcription, reduced levels of *mglA* transcript would have been expected.

The reduced amounts of MglA protein in a Δ*mglB* mutant is not due to a polar effect of the *mglB* mutation on the expression of *mglA*. This is shown by the fact that Δ*mglB* $mglA^+/mglB^+$ *mglA* heterozygotes have a full mgl^+ phenotype.

By whatever mechanism *mglB* regulates the level of MglA protein, less MglA protein in Δ*mglB* mutant cells also explains the observed changes in their fruiting body development and sporulation. The MglA protein is required for aggregation (22, 35). Δ*mglB* mutants are delayed approximately 10 to 12 h in aggregation, they fail to form mature, dark fruiting bodies even after 5 to 7 days, and they produce half the wild-type level of heat-resistant spores. All three defects could arise from less MglA protein and a consequent decrease in motility. Since aggregation depends on cell movement, reduced motility would be expected to delay aggregate formation. Even though aggregates may eventually form from cells with less MglA protein, they may never achieve the high density of cell packing of $mglB^+$ strains, and hence not turn as dark, as is observed. Moreover, with less motility, fewer cells may enter the aggregates even after many days. Since C-factor signalling is required for sporulation, and a high cell density is needed for C-factor signalling (21, 31), fewer spores would be expected.

Sequence of the ORF1 gene product. The *mglB* gene product has a predicted molecular weight of 17,000 (34). One region of its amino acid sequence bears a resemblance to the calcium binding site of yeast calmodulin (11). Residues 35 to 46 of MglB (with matching residues underlined) are Asp Lys Asn Gly Gln Leu Ile Ser Ser Ala Gly (34). Corresponding residues in yeast calmodulin are Asp Asn Asn Gly—Ser Ile Ser Ser Ser Glu. Calcium may be required for gliding, since addition of the calcium ionophore A23187 or the calcium chelator EGTA to *M. xanthus* cells growing on plates inhibits their gliding (16). Calcium induces the myxobacterium *S. aurantiaca* to glide and to produce two new protein products (36). Isolation of the MglB protein may provide a way to investigate potential molecular function(s) of calcium in gliding as well as the role of *mglB*.

ACKNOWLEDGMENTS

P.H. thanks Yvonne Cheng for excellent help and training.

This work was supported by National Science Foundation grant DCB 8903705 to D.K. P.H. was supported by a postdoctoral fellowship from the American Cancer Society. Sequence analysis was supported by grant 1 U41 RR-01685 to Intelligenetics, Inc., Mountain View, Calif.

REFERENCES

1. **Abbanat, D. R., E. R. Leadbetter, W. Godchaux III, and A. Escher.** 1986. Sulphonolipids are molecular determinants of gliding motility. Nature (London) **324**:367–369.
2. **Arnold, J. W., and L. J. Shimkets.** 1988. Cell surface properties correlated with cohesion in *Myxococcus xanthus*. J. Bacteriol. **170**:5771–5777.
3. **Beck, E., G. Ludwig, E. A. Auerswald, B. Reiss, and H. Schaller.** 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. Gene **19**: 327–336.
4. **Blackhart, B. D., and D. R. Zusman.** 1985. "Frizzy" genes of *Myxococcus xanthus* are involved in control of frequency of reversal of gliding motility. Proc. Natl. Acad. Sci. USA **82**: 8767–8770.
5. **Blackshear, P. J.** 1987. Systems for polyacrylamide gel electrophoresis. Methods Enzymol **104**:237–256.
6. **Block, S. M., and H. C. Berg.** 1984. Successive incorporation of force-generating units in the bacterial rotary motor. Nature (London) **309**:470–472.
7. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
8. **Burchard, R. P.** 1974. Studies on gliding motility in *Myxococcus xanthus*. Arch. Microbiol. **96**:247–254.
9. **Cheng, Y., and D. Kaiser.** 1989. *dsg*, a gene required for cell-cell interaction early in *Myxococcus* development. J. Bacteriol. **171**:3719–3726.
10. **Davis, L. G., M. D. Dibner, and J. F. Battey.** 1986. Basic methods in molecular biology. Elsevier Publishing Co., New York.
11. **Davis, T. N., M. S. Urdea, F. R. Masiarz, and J. Thorner.** 1986. Isolation of the yeast calmodulin gene: calmodulin is an essential protein. Cell **47**:423–431.
12. **Dworkin, M., K. H. Keller, and D. Weisberg.** 1983. Experimental observations consistent with a surface tension model of gliding motility of *Myxococcus xanthus*. J. Bacteriol. **155**:1367–1371.
13. **Fink, J. M., and J. F. Zissler.** 1989. Defects in motility and development of *Myxococcus xanthus* lipopolysaccharide mutants. J. Bacteriol. **171**:2024–2028.
14. **Geisselsoder, J., J. M. Campos, and D. R. Zusman.** 1978. Physical characterization of bacteriophage MX4, a generalized transducing phage for *Myxococcus xanthus*. J. Mol. Biol. **119**: 179–189.
15. **Hartzell, P., and D. Kaiser.** 1991. Function of *mglA*, a 22-kilodalton protein essential for gliding in *Myxococcus xanthus*. J. Bacteriol. **173**:7615–7624.
16. **Heumann, H. G., and H. Kühlwein.** 1979. Effects of drugs that influence eucaryotic motile processes on motility of *Cystobacter fuscus* (Myxobacterales). Z. Allg. Mikrobiol. **19**:547–552.
17. **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.
18. **Hodgkin, J., and D. Kaiser.** 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): genes controlling movement of single cells. Mol. Gen. Genet. **171**:167–176.
19. **Hodgkin, J., and D. Kaiser.** 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): two gene systems control movement. Mol. Gen. Genet. **171**:177–191.
20. **Kaiser, D., and C. Crosby.** 1983. Cell movement and its coordination in swarms of *Myxococcus xanthus*. Cell Motility **3**:227–245.
21. **Kim, S., and D. Kaiser.** 1990. Cell motility is required for the transmission of C-factor, an intercellular signal that coordinates fruiting body morphogenesis of *Myxococcus xanthus*. Genes Dev. **4**:896–905.

22. **Kroos, L., P. Hartzell, K. Stephens, and D. Kaiser.** 1988. A link between cell movement and gene expression argues that motility is required for cell-cell signalling during fruiting body development. Genes Dev. **2**:1677–1685.
23. **Kroos, L., A. Kuspa, and D. Kaiser.** 1986. A global analysis of developmentally regulated genes in *Myxococcus xanthus*. Dev. Biol. **117**:252–266.
24. **Kühlwein, H.** 1953. Weitere Untersuchungen an Myxobakterien. Arch. Microbiol. **19**:365–371.
25. **Kuspa, A., and D. Kaiser.** 1989. Genes required for developmental signalling in *Myxococcus xanthus*: three *asg* loci. J. Bacteriol. **171**:2762–2772.
26. **Lünsdorf, H., and H. Reichenbach.** 1989. Ultrastructural details of the apparatus of gliding motility of *Myxococcus fulvus* (Myxobacterales). J. Gen. Microbiol. **135**:1633–1641.
27. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. **McBride, M. J., R. A. Weinberg, and D. R. Zusman.** 1989. "Frizzy" aggregation genes of the gliding bacterium *Myxococcus xanthus* show sequence similarities to the chemotaxis genes of enteric bacteria. Proc. Natl. Acad. Sci. USA **86**:424–428.
29. **McCurdy, H. D.** 1969. Studies on the taxonomy of the Myxobacterales. I. Record of Canadian isolates and survey of methods. Can. J. Microbiol. **15**:1453–1461.
30. **McCurdy, H. D., J. Ho, and W. J. Dobson.** 1978. Cyclic nucleotides, cyclic nucleotide phosphodiesterase, and development in *Myxococcus xanthus*. Can. J. Microbiol. **24**:1475–1481.
31. **O'Connor, C., and D. Zusman.** 1989. Patterns of cellular interactions during fruiting body formation in *Myxococcus xanthus*. J. Bacteriol. **171**:6013–6024.
32. **Spratt, B. G., P. J. Hedge, S. teHeesen, A. Edelman, and J. K. Broome-Smith.** 1986. Kanamycin-resistent vectors that are analogues of plasmids pUC8, pUC9, pEMBL8 and pEMBL9. Gene **41**:337–342.
33. **Stackebrandt, E., and C. R. Woese.** 1981. The evolution of prokaryotes. Symp. Soc. Gen. Microbiol. **32**:1–31.
34. **Stephens, K., P. Hartzell, and D. Kaiser.** 1989. Gliding motility in *Myxococcus xanthus*: the *mgl* locus, its RNA and predicted protein products. J. Bacteriol. **171**:819–830.
35. **Stephens, K., and D. Kaiser.** 1987. Genetics of gliding motility in *Myxococcus xanthus* molecular cloning of the mgl locus. Mol. Gen. Genet. **207**:256–266.
36. **Womack, B. J., D. F. Gilmore, and D. White.** 1989. Calcium requirement for gliding motility in myxobacteria. J. Bacteriol. **171**:6093–6096.