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Regulation of Cohesion-Dependent Cell Interactions in *Myxococcus xanthus*

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Myxococcus xanthus **has two nearly independent genetic systems, A and S, which appear to mediate adventurous (single-cell) movement and social (group) movement, respectively. In addition to a notable reduction in group movement, social motility mutants exhibit decreased biofilm formation, cell cohesion, dye binding, fibril production, and fruiting body formation. The *stk-1907* allele, containing transposon Tn*5* insertion ΩDK1907, was introduced into wild-type cells and many social motility mutants. This allele, which was epistatic to most social motility mutations, caused wild-type and most mutant cells to exhibit increased group movement, cell cohesion, dye binding, and production of cell surface fibrils. The presence of the *stk-1907* allele in *dsp* mutants, which almost completely lack cell surface fibrils, did not result in these phenotypic changes; therefore, *stk-1907* is hypostatic to *dsp* mutations. Those mutants which exhibited increased group movement and cell cohesion with the *stk-1907* allele also had increased fruiting body formation, but no significant changes in spore production were observed. These results suggest that fibrils may mediate cell cohesion, dye binding, and group movement. Additionally, the results suggest that the *dsp* locus contains genes involved in subunit synthesis, transport, and/or assembly of fibrils. The wild-type and mutant alleles of *stk* were cloned and studied in merodiploids. The mutant allele is recessive, suggesting that Tn*5* ΩDK1907 caused a null mutation in a gene which acts as a negative regulator of fibril synthesis. The *stk-1907* allele appears to cause utilization of the A motility system for group movement, possibly because of increased fibril production.**

Gliding bacteria require a solid surface on which to move. No flagella have been observed on the surfaces of gliding bacteria, and the mechanism of motility is not known, although many possibilities have been suggested (12, 27, 33). There are several taxonomically unrelated groups of gliding bacteria, including the myxobacteria, *Cytophaga* and *Flexibacter* spp., and because of the very distant relationship between these groups, there may be more than one mechanism for gliding motility. In *Myxococcus xanthus*, the most well-studied myxobacterium, gliding motility appears to be controlled by two nearly independent multigene systems, the adventurous (A) motility system, which controls the movement of individual cells, and the social (S) motility system, which controls the movement of groups of cells (17–21). Also involved in gliding in *M. xanthus* are the *frz* genes, which show striking homology to the chemotaxis genes of *Salmonella typhimurium* and control the frequency of direction reversal (5, 6, 31). Because *M. xanthus* preys on other bacteria and undergoes a complex developmental process during which tens of thousands of cells move toward an aggregation center to form a spore-filled fruiting body, motility is essential for both the growth and developmental phases of the life cycle.

Mutations in the S motility system affect many other phenotypic characteristics. *M. xanthus* cells have been shown to agglutinate in an energy-dependent manner when suspended in a nonnutritive buffered solution containing Mg^{2+} and Ca^{2+} (37). However, when cells carry a mutation in one of the S motility system genes, including *sgl*, *tgl*, or *dsp*, they have a reduced ability to agglutinate (1, 37). Additionally, mutations in the *dsp* locus have been shown to decrease the cells' ability to bind the diazo dye Congo red and to cause reduced expression of extracellular fibrils (1, 2), one of the two types of extracellular appendages of *M. xanthus*. In general, fibrils, which are approximately 50 nm in diameter and distributed over the entire surface of the cell (2), are observed on cells exhibiting group movement but are not observed on cells that are moving adventurously (3). Pili are the other type of extracellular appendage observed on *M. xanthus* and are located exclusively at the cell poles. Cells containing a *sgl* or *tgl* mutation do not express pili (22), although pili are observed on *dsp* mutants (2). Mutations in *sgl*, *tgl*, and *dsp* also affect fruiting body formation. Hodgkin and Kaiser (21) observed that approximately 75% of the S motility system mutants failed to form proper fruiting bodies, and Shimkets (38) observed that *dsp* mutants completely failed to aggregate during development. A *dsp* mutation disrupts the program of developmental gene expression about 9 h after initiation (29).

In this work, we characterize a mutation which increases group movement, cell cohesion, dye binding, and fruiting body formation in wild-type cells and in most social motility mutants. The results suggest that cell cohesion-dependent processes of *M. xanthus* are under the control of a negative regulator.

MATERIALS AND METHODS

Bacteria, growth media, and phages. *M. xanthus* strains used in this study are listed in Table 1. *M. xanthus* strains were grown vegetatively in CTT broth (19), with vigorous shaking, or on CTT agar (CTT broth with 1.5% Bacto-Agar [Difco Laboratories]) at 32°C. Kanamycin monosulfate was used at a concentration of 40 μg ml^{-1}, and oxytetracycline was used at a concentration of 20 μg ml^{-1}. When transductants were selected, the concentration of oxytetracycline was initially 8 μg ml^{-1} and was increased to 20 μg ml^{-1} 24 h later. *Escherichia coli* strains were grown in L broth (30) or on L agar (L broth with 1.5% agar) at 37°C. The following

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TABLE 1. Strain list and transduction of *M. xanthus*[a]

Strain	Relevant genotype	Reference or source	No. of transductants exhibiting the *stk* phenotype/total no. of transductants examined (% cotransduction)[b]	Representative strain with *stk-1907*
DK101	*sglA1*	19	24/24 (100)	LS1101
DK321	*cglB2 sglA1*	20	24/24 (100)	LS1123
DK323	*cglC1 sglA1*	20	24/24 (100)	LS1124
DK360	*cglE1 sglA1*	20	19/19 (100)	LS1103
DK370	*cglF1 sglA1*	20	24/24 (100)	LS1125
DK1250	*aglB1 tgl-1*	21	24/24 (100)	LS1126
DK1251	*aglJ1 tgl-2*	21	32/32 (100)	LS1127
DK1255	*cglE1 tgl-1*	21a	18/18 (100)	LS1104
DK1622	Wild type	22	24/24 (100)	LS1102
DK1693	*aglB1 dsp-1693*	31a	0/31 (0)	LS1128
DK1973	*cglD1 sglA1*	43	21/21 (100)	LS1129
DK1985	*stk-1907 sglA1*	43a	NA[c]	NA
DK2160	*aglB1 dsp-2160*	31a	0/24 (0)	LS1130
DK2227	*agl sgl-2227*	31a	14/14 (100)	LS1105
DK2608	*cglC1*	41	23/23 (100)	LS1131
DK2616	*cglB2*	41	18/18 (100)	LS1132
DK3088[d]	*stk-1907 sglA1*	15a	NA	NA
DK3112	*agl sgl-3112*	31a	11/11 (100)	LS1106
DK3119	*agl sgl-3119*	31a	0/23 (0)	LS1107
DK3260	*dsgA429*	9	16/16 (100)	LS1108
DK3379	*tgl-1050*	41a	15/15 (100)	LS1109
DK3460	*mglA1*	37	11/11 (100)	LS1110
DK3468	*dsp-1680*	37	0/13 (0)	LS1111
DK3469	*dsp-2105*	38	0/13 (0)	LS1112
DK3470	*dsp-1693*	38	0/15 (0)	LS1113
DK3471	*dsp-1694*	38	0/11 (0)	LS1114
DK3472	*dsp-2160*	38	0/18 (0)	LS1115
DK3473	*sgl-3163*	37	18/18 (100)	LS1116
DK3475	*sgl-3112*	37	20/20 (100)	LS1117
DK3481	*sgl-2234*	37	26/26 (100)	LS1118
DK3482	*tgl-3114*	37	17/17 (100)	LS1119
DK4398	*asgB480*	25	22/22 (100)	LS1120
DK5057	*asgA476*	24	18/19 (95)	LS1121
DK5061	*asgC767*	24	12/12 (100)	LS1122

[a] Myxophage Mx4 grown on DK3088 (*stk-1907*) was used to infect the *M. xanthus* strains with selection for oxytetracycline resistance.
[b] Colonies grown on CTT agar were examined for morphology typical of *stk* mutants, including lighter coloration, compact growth, and rough edges corresponding to thick flares of cells gliding away from the center of the colony.
[c] NA, strain provided the original *stk* mutation and was not transduced.
[d] This strain contains a Tn*5-132* replacement of the Tn5ΩDK1907 of DK1985.

antibiotics and chemicals were added when necessary: ampicillin, 50 or 100 μg ml^{-1}; kanamycin, 40 μg ml^{-1}; chloramphenicol, 35 μg ml^{-1}, isopropylthio-β-D-galactoside (IPTG), 1 mM; and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal), 10 μg ml^{-1}. Coliphage P1 *cam clr100* (36) was used to transfer plasmids from *E. coli* to *M. xanthus* (40). Myxophage Mx4 (7) transductions were performed by the method of Rhie and Shimkets (35) with *M. xanthus* DK3088 as the donor strain.

Agglutination assay. Agglutination of *M. xanthus* was measured by a modification of the method previously described by Shimkets (37). The cells were grown to 5×10^8 cells ml^{-1}, sedimented at 8,000 × *g* at 4°C for 5 min, washed in 10 mM MOPS (morpholinepropanesulfonic acid, pH 6.8), and suspended in 10 mM MOPS (pH 6.8)–10 mM $MgCl_2$–1 mM $CaCl_2$ (agglutination buffer) to a calculated density of 5×10^8 cells ml^{-1}. The cell suspensions were incubated at room temperature for 2 h without shaking. The A_{625} was measured in a Beckman DU-40 spectrophotometer. Relative agglutination values were determined by dividing the A_{625} of the cell suspension after 2 h of incubation by its initial A_{625}. The quotient thus obtained for the wild-type strain, DK1622, was then divided by the quotient for each strain, yielding the relative agglutination value for that strain.

Dye binding assay. *M. xanthus* cells were grown to 5×10^8 cells ml^{-1}, sedimented by centrifugation at 8,000 × *g* at 4°C for 5 min, washed in 10 mM MOPS, and suspended in CTT broth to a calculated cell density of 5×10^9 cells ml^{-1}. Twenty-five microliters of the cell suspension was spotted onto CTT agar plates containing Congo red (Sigma Chemical Co., St. Louis, Mo.) (20 μg ml^{-1}), trypan blue (Sigma Chemical Co.) (20 μg ml^{-1}), or Calcofluor white M2R (fluorescent brightener 28; Sigma Chemical Co.) (50 μg ml^{-1}). The plates were incubated at 32°C for 96 h, and dye binding was determined qualitatively by observing the color of the colonies. Calcofluor white binding was determined by observing the colonies under long-wavelength UV light (366 nm) (Mineralight Lamp, UVGL-25; UVP, Inc., San Gabriel, Calif.).

Development assay. *M. xanthus* strains were grown to a density of 5×10^8 cells ml^{-1}, centrifuged at 8,000 × *g* for 5 min at 4°C, washed in TPM buffer (10 mM Tris-hydrochloride [pH 7.5], 1 mM KH_2PO_4, 8 mM $MgSO_4$), sedimented as described above, and resuspended in TPM buffer to a

calculated cell density of 5×10^9 cells ml^{-1}. Twenty-five microliters of the cell suspension was spotted onto TPM agar (TPM buffer containing 1.5% Bacto-Agar) plates. The plates were incubated at 32°C for 96 h and were observed for aggregation and fruiting body formation. Aggregation was scored by comparing the size and number of aggregates produced by each strain with the results for the wild-type strain, DK1622, which forms well-defined fruiting bodies. Sporulation was measured by scraping the cells off the TPM agar surface with a razor blade and suspending them in 200 μl of TPM buffer. The cell suspensions were sonicated for 15 s at 60 μW cm^{-2} (Heat Systems Ultrasonics) to disrupt vegetative cells and clumps of spores. The spores were counted in a Petroff-Hauser counting chamber by phase-contrast microscopy.

Scanning electron microscopy. *M. xanthus* cells were grown to 5×10^8 cells ml^{-1} in CTT, sedimented for 5 min at 8,000 × *g* at 4°C, washed in 10 mM MOPS, suspended in agglutination buffer (10 mM MOPS [pH 6.8], 10 mM $MgCl_2$, 1 mM $CaCl_2$) to 5×10^7 cells ml^{-1}, and incubated at room temperature for 30 min. Two hundred microliters of the cell suspensions was collected on a 0.2-μm-pore-size Nuclepore filter, fixed in Parducz solution (32), washed in 0.1 M cacodylate (pH 7.2) containing 5% sucrose, and dehydrated sequentially in 50, 70, 90, 95, and 100% ethyl alcohol. The samples were dried and examined as described by Arnold and Shimkets (1) in a Phillips 505 scanning electron microscope.

DNA isolation and manipulations. *M. xanthus* genomic DNA was prepared by the method of Shimkets and Asher (39). *E. coli* plasmid DNA was isolated and manipulated by the conventional techniques (30). Restriction enzymes and T4 DNA ligase were used as recommended by the suppliers of the enzymes. Agarose gel electrophoresis was performed with 0.7% agarose gels with either Tris-borate or Tris-acetate buffer (30). DNA fragments were eluted from agarose gels by use of Geneclean (Bio 101, La Jolla, Calif.). *E. coli* transformations were performed by the methods of either Hanahan (16) or Dower et al. (11). For colony hybridizations, the method of Shimkets and Asher was used (39). For Southern hybridizations, restriction enzyme-digested DNA was electrophoresed, transferred to a Hybond nylon filter (Amersham), and hybridized by the methods of Maniatis et al. (30), with the following exceptions. The prehybridization and hybridization fluids contained 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5% sodium dodecyl sulfate (SDS), 5× Denhardt's solution, and 100 μg of salmon sperm DNA per ml; the posthybridization washing solutions all contained 0.1× SSC and 0.5% SDS; and all washes were performed at 68°C. DNA probes were radiolabeled by the incorporation of [α-^{32}P]dCTP (Amersham), using the random primer extension method of Feinberg and Vogelstein (13).

Molecular cloning. (i) Construction of pJRD1. *M. xanthus* DK1985 genomic DNA, which contains the transposon Tn*5* in the *stk* locus (*stk-1907*), and plasmid pUC19 DNA (44) were digested with *Kpn*I, ligated, and used to transform *E. coli* JM109. Selection was for ampicillin resistance (Ap^r), conferred by pUC19, and for kanamycin resistance (Km^r), conferred by Tn*5*.

(ii) Construction of pJRD2. *M. xanthus* DK1622 genomic DNA was digested with *Kpn*I and *Eco*RI, and the restriction products were separated by agarose gel electrophoresis. Fragments in the size range of 3.0 to 5.0 kbp were eluted from the gel, ligated to *Kpn*I-*Eco*RI-digested pREG429 (15), and used to transform *E. coli* MC1061 with selection for Km^r and Ap^r. The transformants were screened by colony hybridization, using the 1.9-kbp *Bam*HI-*Eco*RI DNA fragment from pJRD1 as the radiolabeled probe.

(iii) Construction of pJRD101/-201, pJRD102/-202, pJRD 103/-203, and pJRD104/-204. The Tn*5*-containing, 9.3-kbp *M. xanthus Eco*RI fragment of pJRD1 was ligated with either *Eco*RI-digested pUC19 or *Eco*RI-digested pLJS49 (39) and used to transform *E. coli* MC1061 with selection for Ap^r and Km^r, creating pJRD101 and pJRD201, respectively. The 3.6-kbp *Kpn*I-*Eco*RI *M. xanthus* fragment of pJRD2 was ligated with *Kpn*I-*Eco*RI-digested pUC19 and used to transform *E. coli* JM109 with selection for Ap^r, creating pJRD102. The 1.7-kbp *Kpn*I-*Bam*HI *M. xanthus* fragment of pJRD2 was ligated with *Kpn*I-*Bam*HI-digested pUC19 and used to transform *E. coli* JM109 with selection for Ap^r, creating pJRD103. The 1.9-kbp *Eco*RI-*Bam*HI *M. xanthus* fragment of pJRD2 was ligated with *Bam*HI-*Eco*RI-digested pUC19 or pLJS49 and used to transform *E. coli* JM109 with selection for Ap^r, creating pJRD104. The plasmids pJRD102, pJRD103, and pJRD104 were digested with *Eco*RI and *Hin*dIII. The resulting 3.6-kbp *M. xanthus* fragment of pJRD102, 1.7-kbp *M. xanthus* fragment of pJRD103, and 1.9-kbp *M. xanthus* fragment of pJRD104 were ligated with *Eco*RI-*Hin*dIII-digested pLJS49 and used to transform *E. coli* MC1061 with selection for AP^r and Km^r, creating pJRD202, pJRD203, and pJRD204, respectively.

Construction of *stk* locus merodiploids. Plasmids pJRD201, pJRD202, pJRD203, and pJRD204 were transferred from *E. coli* to *M. xanthus* strains by specialized transduction with the bacteriophage P1 (40). Plasmid pLJS49, the vector of pJRD201, pJRD202, pJRD203, and pJRD204, does not replicate in *M. xanthus* and preferentially integrates at the temperate myxophage Mx8 *attB* attachment site, thereby leaving the recipient's native chromosomal allele intact (28).

RESULTS

Effect of the *stk* mutation on colony morphology. Transposon insertion ΩDK1907 in DK1985 caused the strain DK101 to become more cohesive or "stickier"; hence the locus containing this transposon insertion was designated *stk*, and the allele was designated *stk-1907* (Table 1). Colonies exhibiting the cohesive phenotype appeared rougher, with a dry, wrinkled surface, and had lighter coloration, more compact morphology, increased group movement or decreased single cell movement along the colony edges, and rough edges. Additionally, the cells clumped inordinately when grown in CTT broth, even with vigorous shaking. To determine what effect the *stk-1907* allele might have on strains carrying mutations that reduce cohesion, these strains were infected with the generalized transducing myxophage Mx4 grown on DK3088, which contains transposon Tn*5-132* in the *stk* locus, and selected for resistance to oxytetracycline, encoded by Tn*5-132*. In many strains, including the wild type (DK1622), the Stk phenotype cotransduced with resistance to oxytetracycline 100% of the time, suggesting that *stk-1907* may be a null mutation in a gene that normally represses cohesion (Table 1). The effect of *stk-1907* on the colony morphology of DK1622 is illustrated in the phase-contrast micrographs taken of the edges of colonies of DK1622 and its *stk-1907*-containing counterpart, LS1102 (Fig. 1). DK1622 exhibited both social (group) and adventurous (single-cell) motility (Fig. 1). With LS1102, single-cell movement decreased and group movement increased, as evidenced by fewer individual cells and larger groups of cells along the edge of the colony (Fig. 1).

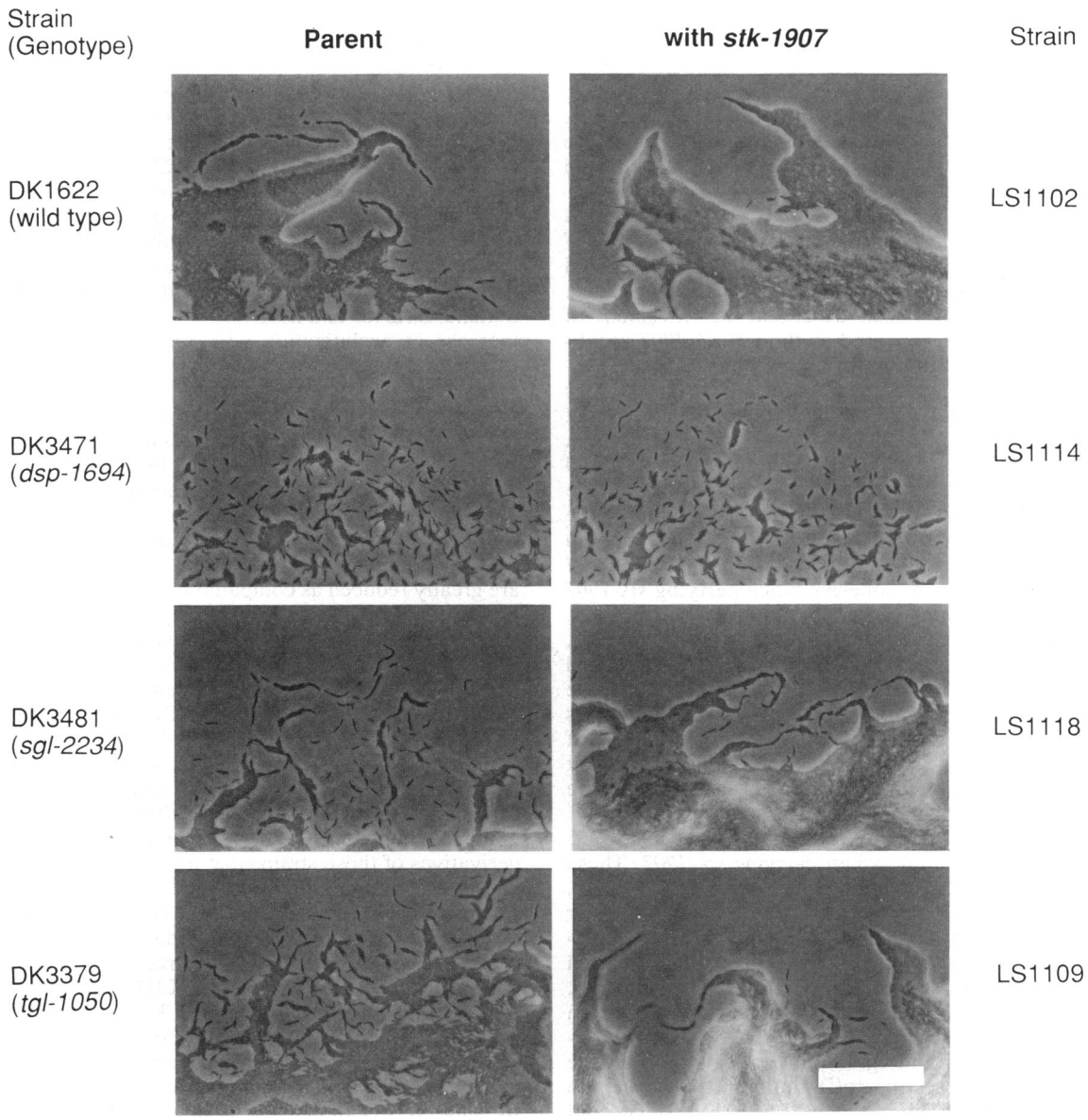

FIG. 1. Cell behavior of wild-type, *dsp*, *sgl*, and *tgl* cells before and after receiving the *stk-1907* allele. Colonies were grown on CTT agar at 32°C for 24 h, and the edges were photographed by phase-contrast microscopy. Bar, 100 μm.

Strains that contain mutations to which *stk-1907* was epistatic included the *asg* mutants DK4398, DK5057, and DK5061, which are defective in the production of extracellular proteases that release amino acids and peptides responsible for development-specific cell-cell signaling (25, 26, 34); the *dsg* mutant DK3260; the *sgl* (DK101, DK3473, DK3475, and DK3481) and *tgl* (DK3379 and DK3482) mutants, all of which are defective in social motility; and the *mgl* mutant, which is defective in both motility systems. A representative of the *sgl* group of mutants, DK3481, and its *stk-1907* derivative, LS1118, were photographed by phase-contrast microscopy to observe any changes in the colony edges caused by *stk-1907* (Fig. 1). DK3481 had fewer groups of cells and more individual cells at the colony edge than did the wild type, characteristics typical of social motility mutants. In comparison with DK3481, LS1118 had more groups of cells and fewer individual cells at the colony edge, behavior much more typical of wild-type cells. When viewed with a phase-contrast microscope, a representative *tgl* mutant, DK3379, appeared to have fewer groups of cells and more individual cells along the edge of the colony than did the wild type. LS1109, which is DK3379 carrying *stk-1907*, had fewer individual cells and more groups of cells at the colony edge than did both DK3379 and DK1622. The phenotypic change caused by *stk-1907*, which is observed as more group movement and less movement as single cells, is typical of cells that have intact social motility systems, suggesting that *stk* may play a role in regulating social behaviors.

In some strains, such as the *dsp* mutants and DK3119, none of the transductants acquiring *stk-1907* exhibited the Stk phenotype. The lack of a phenotypic change is evident in the phase-contrast micrographs of a representative *dsp* strain, DK3471, and its *stk-1907* counterpart, LS1114 (Fig.

1). Both DK3471 and LS1114 had very few groups of cells but many individual cells along their colony edges. These results suggest that DK3119 and the *dsp* mutants contain mutations which are epistatic to *stk-1907* and are therefore not affected by *stk-1907*, unlike the other S motility mutants. Interestingly, *sgl-3119* and the *dsp* locus map relatively close to each other on the *M. xanthus* genome, well removed from other *sgl* genes and the *tgl* locus (8).

The introduction of the *stk-1907* allele into many of the S motility mutants, i.e., those carrying mutations in most *sgl* or *tgl* genes, resulted in an increase in group movement and a decrease in single-cell movement along the colony edge. Because S motility has been described as movement of groups of cells (21), it appeared as though *stk-1907* genetically restored the S motility system by alleviating deficiencies caused by the S motility mutations. If *stk-1907* does genetically restore social motility, then one would expect *mgl* cells and nonmotile cells with mutations in both the A motility system, which includes the *agl* and *cgl* genes, and the S motility system to exhibit social motility upon the introduction of *stk-1907*. This result was generally not the case, as most mutants which carried a mutation in both motility systems remained nonmotile with *stk-1907*. The *mgl* mutant DK3460 was more cohesive when carrying *stk-1907* but remained nonmotile (data not shown). In addition, strains DK323 (*cglB2 sglA1*), DK3112 (*agl sgl-3112*), DK1250 (*aglB1 tgl-1*), and DK1251 (*aglJ1 tgl-2*) exhibited the Stk phenotype with *stk-1907* but remained nonmotile (data not shown). Group movement was restored to strains with mutations in the *sgl* and *tgl* loci upon the introduction of *stk-1907* (Fig. 1). But the social motility system itself was not restored, suggesting that group movement does not require an intact S motility system.

Interestingly, strains DK360 (*cglE1 sglA1*) and DK1255 (*cglE1 tgl-1*) became motile when carrying *stk-1907*. These results appear to be due to suppression of the *cglE* defect, rather than suppression of *sglA1* or *tgl-1*, because other mutants containing mutations in *sglA1* and *tgl-1* in combination with an A system mutation do not become motile when carrying the *stk-1907* allele (e.g., LS1124, LS1125, LS1126, and LS1127; Table 1). The *stk-1907* allele also caused an increase in motility in strain DK1973 (*cglD1 sglA1*), confirming the observation of Sodergren and Kaiser (43a) that the *stk-1907* allele suppresses the *cglD* mutation. However, the type of motility observed in strains LS1103, LS1104, and LS1129 was primarily group movement, even though the A motility system was responsible for the motility.

Agglutination assay. An agglutination assay was used to determine if cells containing the *stk-1907* allele had a quantifiable change in cohesiveness. The assay measures the changes in the optical density of a cell suspension over time as the cells fall out of suspension because of clumping. Relative agglutination, determined as described in Table 2, footnote *b*, allows comparison of the cohesiveness of each strain with that of the wild type, with 1.0 being the wild-type level of agglutination. LS1102, the wild-type strain containing *stk-1907*, had a relative agglutination value of 1.6, up from 1.0 for DK1622. The results showed that all strains which exhibited the Stk colony phenotype when carrying *stk-1907* also exhibited an increase in agglutination as compared with their corresponding parental strains (Table 2). Agglutination of the *asg* mutants, DK4398, DK5057, and DK5061, which were shown previously to agglutinate less efficiently than the wild type (24), increased to levels near that of the wild type when the mutants were carrying the *stk-1907* allele. Agglutination increased to wild-type levels with the introduction of *stk-1907* in strain DK3260, a *dsg* mutant, as it did in strain DK3460, an *mgl* mutant. Previously, Shimkets (37) observed that the *mgl* mutant DK3460 agglutinates as well as the wild-type strain DK1622, and we are uncertain of the reason why DK3460 showed reduced agglutination in this study (Table 2). The social motility mutants DK101, DK360, DK2227, DK3481, DK1255, DK3379, and DK3482, which exhibited changes in colony morphology after the introduction of *stk-1907*, also showed an increase in agglutination (Table 2). The other social motility mutants, which did not change phenotypically upon the introduction of *stk-1907*, including DK3119 and the *dsp* mutants DK3468 and DK3471, did not show any appreciable increase in agglutination with the *stk* mutant allele. The results of this assay suggest that the *stk* locus is involved in regulating both cell cohesiveness and group movement. The results also suggest that *sgl-3119* and *dsp* mutants are different from other S system mutants in that they cannot be suppressed to participate in social behavior.

Dye binding assay. Another phenotypic characteristic that has been correlated with group movement and agglutination in *M. xanthus* is the ability to bind the dye Congo red (1). The dye binding capabilities of the noncohesive *dsp* mutants are greatly reduced as compared with the wild type, suggesting that the Congo red receptor plays an essential role in cell cohesion (1). Other dyes which have been shown to bind to *M. xanthus* cells include trypan blue and Calcofluor white (42a). To determine if *stk-1907* causes an increase in dye binding, cells were placed onto CTT agar containing appropriate amounts of either Congo red, trypan blue, or Calcofluor white, and the colonies were observed for dye binding after 96 h (Table 2). The wild-type strain DK1622 bound all three dyes well, but with the *stk-1907* allele (LS1102), the binding of two of the three dyes increased. In general, the *stk* derivatives of those strains that became more cohesive after the introduction of *stk-1907* bound the dyes at least as well as, and often better than, DK1622. These results suggest that the *stk* mutation increases the production of the Congo red, trypan blue, and Calcofluor white receptors in most strains, but not in the *dsp* strains or in DK3119.

Extracellular fibril production. To determine if the increased cell cohesion and dye binding caused by *stk-1907* were caused by an increase in extracellular fibril production, the cell surfaces of strains both with and without the mutant *stk* allele were examined by scanning electron microscopy. Scanning electron microscopy has been utilized to observe fibrils on the surface of *M. xanthus* cells previously (2, 3), and Arnold and Shimkets (2) found that fibrils observed by scanning electron microscopy were comparable in size and number to those observed by transmission electron microscopy of negatively stained samples. Structures similar to fibrils, "slime threads," were observed by Fluegel with a light microscope (14).

Although the wild-type strain DK1622 produced many fibrils, the presence of the mutant *stk* allele resulted in a dramatic increase in the number of fibrils (Fig. 2). In contrast, the *dsp* mutant strain DK3471 produced few fibrils, and there was no observable difference between the amount of fibrils produced by DK3471 and the amount produced by its *stk* counterpart, LS1114. The surface of *mgl* strain DK3460 has relatively few fibrils as compared with the wild type, but the number of fibrils increased dramatically in strain LS1110, which carries the *stk-1907* allele. As with the *mgl* mutant, the *sgl* mutant DK3481 and the *tgl* mutant DK3379 both had fewer fibrils than the wild type, and their isogenic *stk-1907* mutants, LS1118 and LS1109, respec-

TABLE 2. Effect of *stk* mutation on agglutination, dye binding, and development[a]

Parent/ transductant	Relative agglutination[b]	Dye binding[c]			Development	
		Congo red	Trypan blue	Calcofluor white	Aggregation[d]	% of wild-type sporulation[e] (mean ± range)
Wild type						
DK1622/	1.00	++	++	++	+++	100 ± 0
LS1102	1.66	++	+++	+++	++	69 ± 2
asg						
DK4398/	0.87	++	++	++	++	40 ± 0
LS1120	1.09	++	+++	+++	++	10 ± 0
DK5057/	0.11	+	++	++	−	1 ± 0
LS1121	1.07	++	+++	+++	++	3 ± 2
DK5061/	0.36	+	++	++	++	53 ± 6
LS1122	0.69	++	+++	+++	++	41 ± 3
dsg						
DK3260/	0.22	++	++	+++	+	6 ± 1
LS1108	1.24	++	+++	+++	++	35 ± 7
dsp						
DK3468/	0.03	−	−	−	−	7 ± 7
LS1111	0.04	−	−	−	−	15 ± 15
DK3471/	0.04	ND[f]	ND	ND	−	<1 ± 0
LS1114	0.04	ND	ND	ND	−	<1 ± 0
mgl						
DK3460/	0.04	−	−	−	−	4 ± 3
LS1110	1.49	++	+++	+++	−	5 ± 4
sgl						
DK101/	0.04	+	++	−	+	83 ± 8
LS1101	0.99	++	+++	+++	++	67 ± 4
DK360/	0.03	−	−	−	−	<1 ± 0
LS1103	0.25	++	++	+++	−	<1 ± 0
DK2227/	0.04	−	−	−	−	<1 ± 0
LS1105	0.20	++	+++	+++	−	<1 ± 0
DK3119/	0.03	−	+	−	−	<1 ± 0
LS1107	0.04	−	+	−	−	<1 ± 0
DK3481/	0.06	−	−	−	−	6 ± 1
LS1118	0.42	++	++	+++	++	19 ± 1
tgl						
DK1255/	0.05	−	−	−	−	<1 ± 0
LS1104	0.25	++	+	+++	−	<1 ± 0
DK3379/	0.04	−	−	−	+	25 ± 1
LS1109	1.14	++	+	+++	++	8 ± 1
DK3482/	0.08	ND	ND	ND	±	<1 ± 0
LS1119	0.87	ND	ND	ND	++	<1 ± 0

[a] Parental strains and otherwise isogenic transductants containing *stk-1907* were assayed for agglutination, dye binding, aggregation, and sporulation as described in Materials and Methods.

[b] Relative agglutination was determined by dividing the absorbance of the cell suspension after 2 h of incubation at room temperature by its initial absorbance. The quotient obtained for the wild-type strain, DK1622, was then divided by the quotient for each strain, yielding the relative agglutination value for that strain.

[c] The relative level of dye binding was qualitatively assessed, using as references strains DK3468 (binding designated −) and DK1622 (binding designated ++).

[d] Aggregation was scored by comparing with the wild type, DK1622 (size, shape, and number of fruiting bodies). −, no aggregation; +++, DK1622-like aggregation.

[e] Spores were counted in a Petroff-Hauser counting chamber following sonication to disrupt vegetative cells after 96 h of development.

[f] ND, not determined.

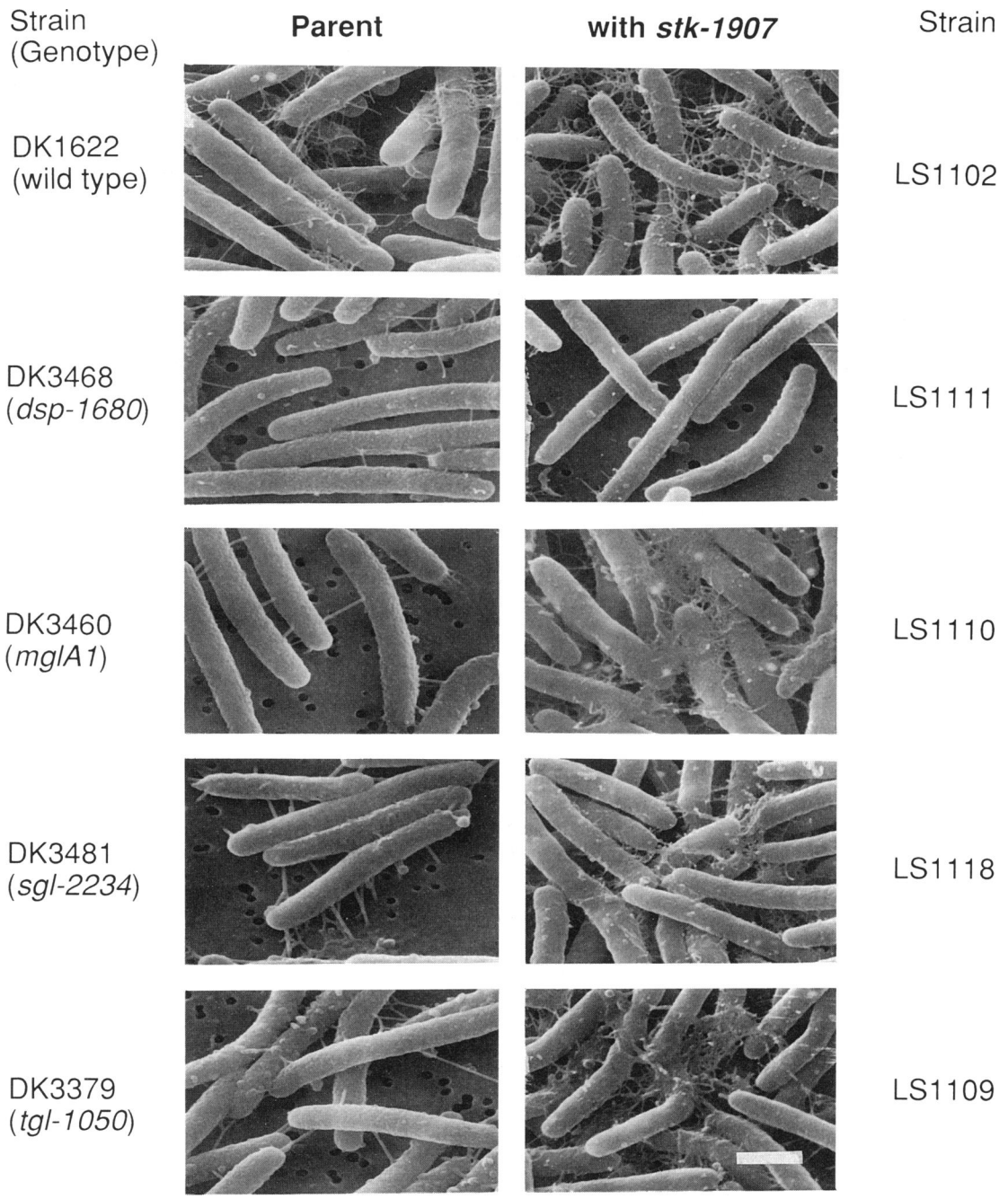

FIG. 2. Fibril production by wild-type, *dsp*, *mgl*, *sgl*, and *tgl* strains before and after receiving the *stk-1907* allele, observed by scanning electron microscopy. Cells were incubated in agglutination buffer for 30 min at room temperature and prepared for scanning electron microscopy as described in Materials and Methods. Bar, 1 μm.

tively, both produced more extracellular fibrils than their parental counterparts. The increase in the production of extracellular fibrils caused by *stk-1907* correlates with the observed increases in agglutination and dye binding, suggesting that the fibrils were at least partially responsible for cohesion and dye binding.

Development of *stk* mutants. Because the *stk-1907* allele restored group movement, a behavior thought to be important to development, to the social motility mutants DK101, DK3481, DK3379, and DK3482, *stk* mutants were observed for fruiting body formation (Fig. 3). DK1622 formed small, closely spaced, spore-filled fruiting bodies when starved for nutrients. Fruiting bodies of the *stk* mutant LS1102 were similar in size, less closely spaced, and fewer in number, suggesting that the *stk* mutation had a deleterious effect on the development of the wild-type strain. However, this reduction in the number of fruiting bodies produced by LS1102 as compared with DK1622 may have been because fewer LS1102 cells were present. LS1102 was extremely clumpy when grown in liquid culture, and because of this

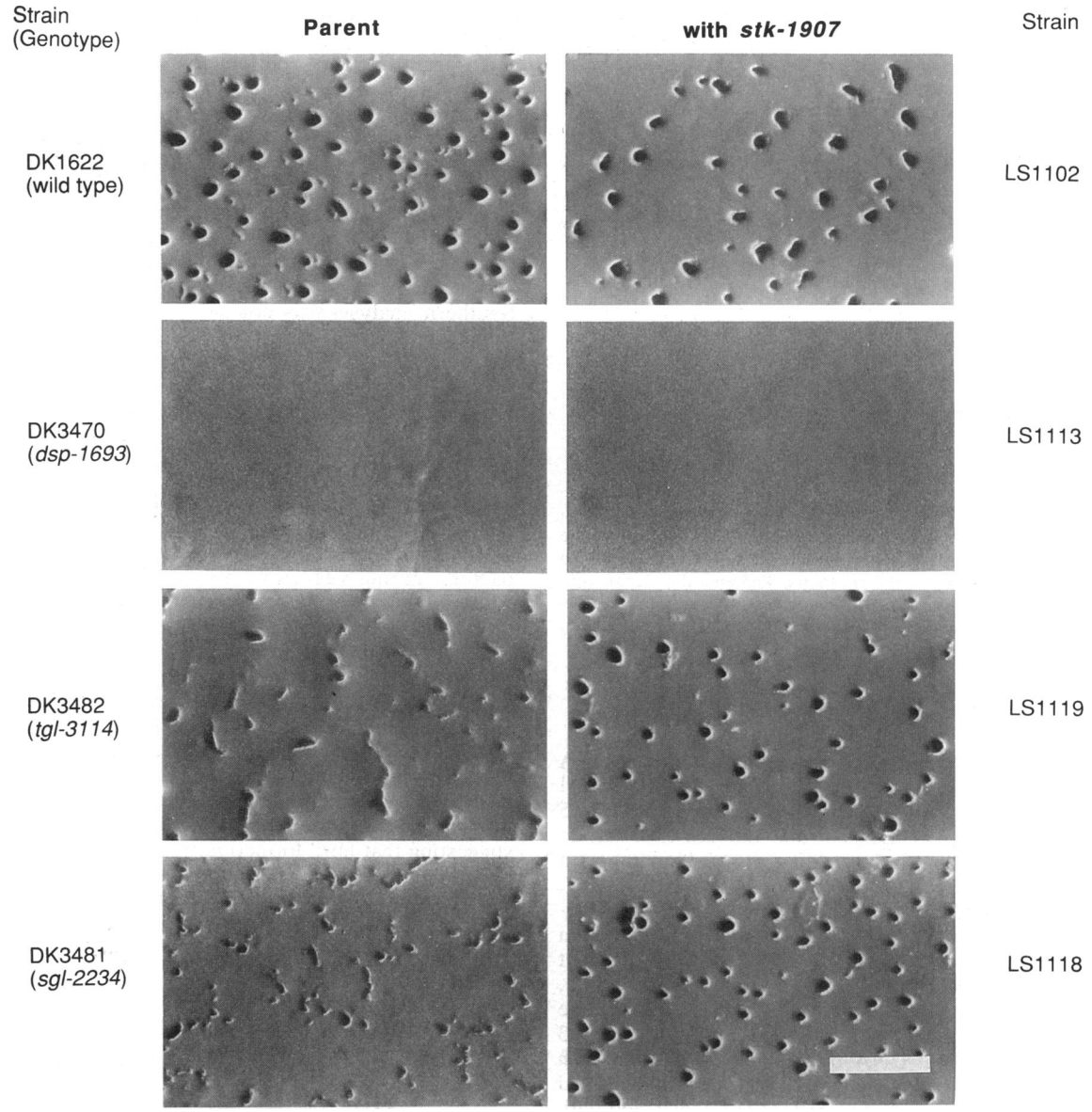

FIG. 3. Fruiting body morphology of wild-type, *dsp*, *sgl*, and *tgl* strains before and after receiving the *stk-1907* allele. The cells were placed on TPM agar at a density of 1.7×10^7 cells cm^{-2} and incubated at 32°C for 96 h. Bar, 1 mm.

clumpiness, it was very difficult to accurately estimate the number of cells. DK3470, a *dsp* mutant, did not form fruiting bodies, and neither did the otherwise isogenic *stk* mutant, LS1113. DK3481 and DK3482 aggregated very poorly, but after the introduction of *stk-1907* both made fruiting bodies closely resembling those of the wild type. A summary of the effects of the *stk* mutation on development of other strains is given in Table 2. Excluding the *dsp* mutants and the nonmotile strains DK3460, DK360, DK2227, DK3119, and DK1255, all strains showed some improvement in their ability to form fruiting bodies after receiving the *stk-1907* allele.

Spores were harvested after development for 96 h on TPM plates. The wild type produced fewer spores with the *stk-1907* allele. The *stk-1907* allele had little effect on spore production in those strains which aggregated better with the *stk-1907* allele, such as DK101, DK3481, DK3379, and DK3482, and in those strains in which *stk-1907* had no effect on aggregation, DK3468 and DK3471. Some strains showed a modest increase in spore production, such as DK3260, but others showed a modest decrease, such as DK4398. These results suggest that the *stk-1907* allele can restore proper aggregation but cannot compensate for the lack of proper developmental signals necessary for sporulation.

Genetic analysis of the *stk* locus. The *stk-1907* allele was cloned from the *stk* mutant DK1985, taking advantage of the kanamycin resistance conferred by Tn*5* and the absence of a *Kpn*I site in the transposon. A 14.3-kbp *Kpn*I fragment, which contains the 5.7-kbp Tn*5* insertion and *M. xanthus* sequences flanking both sides, was cloned into pUC19 (pJRD1) (Fig. 4). The 1.9-kbp *Bam*HI-*Eco*RI fragment located to the right of the transposon was used as a probe to locate the wild-type clone from a pool of clones yielding a 3.6-kbp *Kpn*I-*Eco*RI fragment (pJRD2) (Fig. 4). The restric-

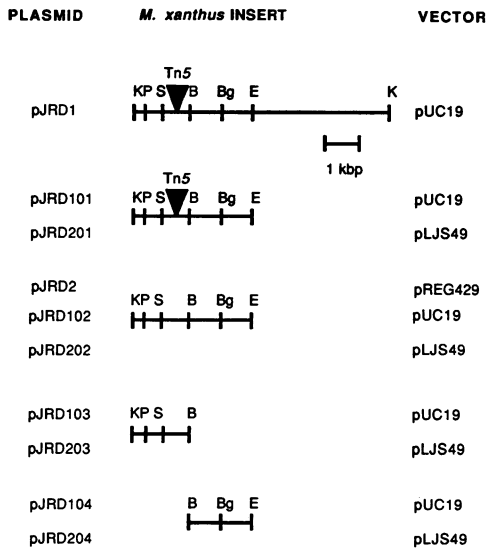

FIG. 4. Restriction maps of the *stk*$^+$ and *stk-1907* alleles and the plasmids used in this study. The triangles indicate the location of the Tn*5* (or Tn*5-132*) insertion in the *stk* locus. Tn*5* encodes kanamycin resistance, and Tn*5-132* encodes oxytetracycline resistance. Restriction enzyme abbreviations: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; K, *Kpn*I; P, *Pst*I; and S, *Sal*I.

tion maps of the clones were compared with genomic DNA in a Southern blot, utilizing the 1.9-kbp *Bam*HI-*Eco*RI fragment of pJRD2 as the probe (Fig. 5). Lane 1 of Fig. 5 contained chromosomal DNA from the *stk-1907* strain DK1985 digested with *Kpn*I, yielding a 14.3-kbp fragment identified by the probe. Lane 2 contained pJRD1 DNA (Fig. 4) digested with *Kpn*I, which also yielded a 14.3-kbp fragment, indicating that the cloned DNA originated from DK1985. The faint band present in lane 2 with an apparent molecular size of 3.4 kbp probably represents supercoiled, undigested plasmid DNA which was not visible in the ethidium bromide-stained agarose gel (not shown). Lane 3 contained chromosomal DNA from DK1622 digested with *Kpn*I and *Eco*RI, which yielded a 3.6-kbp fragment identified by the probe. Lane 4 contained pJRD2 DNA digested with *Kpn*I and *Eco*RI, which also yielded a 3.6-kbp fragment, verifying that the clone originated from DK1622.

Transposon insertions are capable of causing two types of mutations. One type is a null mutation, which occurs when the transposon inserts into the reading frame of a gene, disrupting expression. The other type of mutation results when outward-reading promoters near the end of the transposon stimulate transcription of distal genes (4). To determine which type of mutation is caused by ΩDK1907, restriction fragments containing the wild-type *stk* locus and the mutant *stk* locus were subcloned into a plasmid capable of site-specific integration at the Mx8 *attB* site of the chromosome (28). The 9.3-kbp *Eco*RI fragment containing ΩDK1907 did not restore cohesion to DK101, suggesting that the wild-type locus at the native site is dominant to the mutant allele at the *attB* site (Fig. 6). In the converse experiment, when the 3.6-kbp *Kpn*I-*Eco*RI wild-type fragment was introduced at the *attB* site, the phenotype of DK3088, which carried *stk-1907* at the native chromosomal location, reverted to *stk*$^+$ in all 48 transductants examined (Fig. 6), suggesting that the wild-type allele is dominant. Together these results suggest that ΩDK1907 causes a null mutation.

To begin to define the location of the *stk* transcriptional unit, two smaller subclones containing either the 1.7-kbp *Kpn*I-*Bam*HI fragment or the 1.9-kbp *Bam*HI-*Eco*RI fragment of the wild-type *stk* locus were constructed and were introduced at the *attB* site of DK3088. Only 1 of the 37 transductants examined containing the *Kpn*I-*Bam*HI wild-type fragment had the *stk*$^+$ phenotype (Fig. 6). The lone *stk*$^+$ transductant was probably due to a homologous recombination event in which the plasmid insert and the native allele recombined to restore a wild-type allele (28), although Southern analysis was not performed. The *Bam*HI-*Eco*RI fragment also failed to restore the *stk*$^+$ phenotype to DK3088 when at the *attB* site. These results suggest that the transcription unit for *stk* spans the internal *Bam*HI site of the 3.6-kbp *Kpn*I-*Eco*RI fragment.

DISCUSSION

Adhesion to surfaces and/or cohesion to other cells is necessary for gliding motility, which is essential in all phases of the life cycle of *M. xanthus*. *M. xanthus* hunts prey bacteria during vegetative growth and forms multicellular fruiting bodies during development; both processes require coordinated cell movement. The identification of *stk*, a locus that regulates cell cohesion and adhesion and is therefore involved in motility, represents a significant step toward understanding the genetics of *M. xanthus* social processes requiring cell-cell contact. The *stk-1907* allele was produced by a Tn*5* insertion and is likely to be a null mutation because the wild-type allele is dominant in merodiploids.

The *stk-1907* allele increased the ability of *mgl*, *tgl*, and most *sgl* mutants to cohere, bind dyes, and produce fibrils, suggesting that fibril formation, rather than pilus formation, plays a major role in mediating cell cohesion and dye binding. The *dsp* and *sgl-3119* mutants containing *stk-1907* showed no increase in fibril synthesis and were unable to agglutinate despite the presence of pili (2). Cells which

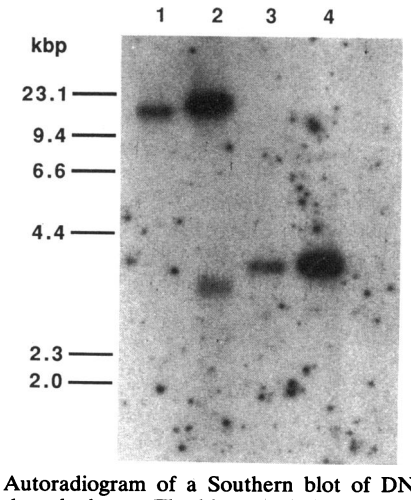

FIG. 5. Autoradiogram of a Southern blot of DNA of strains containing the *stk* clones. The blot was hybridized to the 1.9-kbp *Bam*HI-*Eco*RI fragment from pJRD104 (Fig. 4) as described in Materials and Methods. Lanes 1 and 2, respectively, genomic DNA from *stk* mutant DK1985 and its derivative plasmid pJRD1, both digested with *Kpn*I; lanes 3 and 4, respectively, genomic DNA from wild-type strain DK1622 and its derivative plasmid pJRD2, both digested with *Kpn*I and *Eco*RI.

produced more fibrils because of the *stk-1907* mutation also exhibited more group movement and less single-cell movement, suggesting that fibrils may be important for group movement. The restoration of group movement to the *sgl* and *tgl* mutants raised the question of whether these S system mutations were being suppressed by the *stk-1907* allele. Because the *stk-1907* allele did not induce movement of most strains which carry a mutation in both the A system and the S system or of *mgl* strains, *stk-1907* does not restore S motility. The *stk-1907* allele did, however, restore motility to *cglD* S^- and *cglE* S^- mutants, suggesting that the *stk-1907* suppression works through the A motility system. To overcome the ambiguities that arise from the adventurous motility system directing group movement, the terms group movement and single-cell movement will be used to refer to the behavior of the cells, while S motility and A motility will be used to refer to the two genetically separable motility systems.

The A motility and S motility systems of *M. xanthus* coordinate the movement of cells on solid surfaces. *M. xanthus* cells typically move in groups and as single cells when both of the motility systems are functional. The factors involved in determining whether a cell will move as part of a group or as a single cell are not all known; however, cell density is known to be involved (23). In order for cells to move via the S motility system, and thus in groups, they must be no more than one cell length from one another (23), and therefore cell density is likely to play a determining role in whether a cell may use the A or the S motility system. While A motile cells exhibit some stimulation with increasing cell density, particularly at low cell densities, the rate of S motile cell movement continues to increase with cell density until very high cell densities are achieved. The presence or absence of fibrils is another factor that we believe to be involved in determining whether cells move as groups or as individuals. Cells moving as individuals possess few fibrils compared with cells moving as groups (3), and

stk allele at native location (Strain)	*stk* allele at *attB* site (Plasmid)	Number of transductants exhibiting the *stk* + phenotype/total number of transductants examined (% cotransduction)
***stk*+** (DK101) K B E	***stk-1907*** (pJRD201) Tn*5* K B E	48/48 (100%)
stk-1907 (DK3088) Tn*5-132* K B E	***stk*+** (pJRD202) K B E	48/48 (100%)
Tn*5-132* K B E	(pJRD203) K B	1/37 (3%)
Tn*5-132* K B E	(pJRD204) B E	0/48 (0%)

FIG. 6. Examination of *stk* merodiploids. Wild-type derivative DK101 was transduced with plasmid pJRD201 (Fig. 4), which introduced the *stk-1907* allele at the *attB* site. In the converse experiment, the *stk* mutant DK3088 was transduced with either plasmid pJRD202, pJRD203, or pJRD204 (Fig. 4), which introduced subclones of the wild-type *stk* locus into the *attB* site. The results of the merodiploid construction are given in the right-hand column.

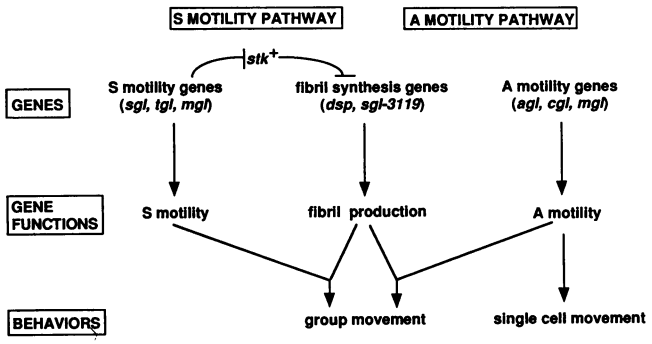

FIG. 7. Model outlining the regulation pathways of group and single-cell movement in *M. xanthus*. The social (S) motility pathway leads to group movement, whereas the adventurous (A) motility pathway can lead to either single-cell or group movement. In the S motility pathway, the social motility genes influence the activity of fibril synthesis genes, most probably by repressing *stk*. The function of *stk*, when not repressed, appears to be to repress fibril synthesis genes. The fibril synthesis genes encode products necessary for fibril production. These fibrils are necessary for group movement, which can be exhibited only when fibrils are present and either the S motility apparatus or the A motility apparatus is present. In addition, the A motility pathway can lead to single-cell movement regardless of the presence of fibrils.

cells which normally move predominantly as individuals (those with a mutation in the S motility system) will move predominantly in groups when fibril synthesis is stimulated by *stk-1907* (this work). While cell density and fibrils are important in determining the type of movement expressed by a cell, many other factors are likely to be involved.

The regulation of fibril synthesis in *M. xanthus* is likely to play a central role in determining whether cells exhibit group movement or single-cell movement. A model is proposed to explain the regulation of fibril synthesis and its effect on cell behavior in *M. xanthus* (Fig. 7). Movement of cells in groups is often the result of a functional S motility pathway; products of the S motility genes (*sgl* and *tgl*) and the fibril synthesis genes interact, resulting in group movement. A mutation of any S motility system gene results in decreased numbers of cell surface fibrils, suggesting that the S motility genes usually stimulate the expression of fibril synthesis genes. When the *stk* locus contains transposon insertion ΩDK1907 (*stk-1907*), which creates a null mutation, the number of fibrils observed on the surface of the cell increases, suggesting that a functional *stk* locus usually represses fibril synthesis. The *dsp* and *sgl-3119* loci are likely to contain the genes for fibril synthesis, because mutations in these loci were epistatic to *stk-1907* and their mutants did not synthesize many fibrils. Taken together, these results suggest the simple possibility that S motility genes repress the expression of *stk*, which usually represses the expression of *dsp* and *sgl-3119*, resulting in increased fibril synthesis.

A mutation in any one of the A motility genes (*agl* and *cgl*) results in cells moving exclusively in groups, indicating that the A motility system is solely responsible for single-cell movement (23). However, it appears as though the A motility pathway can also be used in group movement (Fig. 7). While a mutation in any one of the S motility genes (*sgl* and *tgl*) results in a decrease in the number of cells moving in groups, group movement does not appear to be abolished (Fig. 1). Furthermore, introduction of *stk-1907* into cells containing a mutation in an S motility gene usually results in a dramatic increase in group movement (Fig. 1), which must

be directed by the A motility system since the S motility system is mutated. It appears as though an increase in fibrils, caused by *stk-1907*, results in an increase in group movement. A direct relationship between the *stk* locus and A motility is suggested by the observation that the motility defects in *cglD* and *cglE* mutants are suppressed by *stk-1907*. S^- *cglD* and S^- *cglE* cells become motile and exhibit group movement when *stk-1907* is introduced, but motility is not restored to other A^- S^- cells or *mgl* mutants. Exactly how the suppression is accomplished is unknown, but the mechanism is likely to be elucidated as more is learned about the functions and cellular locations of CglD and CglE and the structures and functions of the fibrils.

The observation that dyes bound to cells which produced many fibrils but did not bind to cells reduced in surface fibrils suggests that the fibril material acts as the receptor for Calcofluor white, trypan blue, and, as previously reported (1), Congo red. This raises the possibility that the fibrils might act as receptors for physiologically or developmentally important molecules. For example, the attachment of cohesins to the fibrils could result in agglutination. Indeed, certain development-specific protein epitopes are found exclusively on the fibrils of *M. xanthus* (10), and CsgA, an important development-specific signaling molecule, was localized to the extracellular matrix and cell surface by immunogold labeling (42). Fibrils may also play a role in the exchange of intercellular signals necessary for movement directed by the A motility system. The *cglD* and *cglE* mutations were suppressed when the synthesis of fibrils was increased, suggesting that the signals necessary for stimulation of the A motility system, which may be less abundant in *cglD* and *cglE* mutants (20), are more efficiently transferred in the presence of fibrils. Alternatively, the increase in fibril synthesis and cohesion could increase the local cell density or change the way cell density is perceived within a colony, which could result in cell movement if the *cglD* and *cglE* genes are involved in sensing cell density. In addition, the *stk-1907* allele restored to most mutants the ability to form fruiting bodies but not spores, suggesting that fibril production may be necessary for the cell-cell signaling and cell alignments which are essential during fruiting body formation. Taken together, these results suggest that fibrils are involved with cell-cell recognition and may provide a means of presenting proteins to other cells.

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