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Defects in Contact-Stimulated Gliding during Aggregation by *Myxococcus xanthus*

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During development, *Myxococcus xanthus* cells glide toward foci of aggregation and produce compact multicellular mounds. We studied development in strains with defects in contact-stimulated gliding. Contact stimulation involves a mechanism influenced by contacts between neighboring cells which stimulates the gliding motility of single cells (Hodgkin and Kaiser, Proc. Natl. Acad. Sci. USA 74:2938–2942, 1977; Hodgkin and Kaiser, Mol. Gen. Genet. 171:167–176, 1979). Most mutants containing a mutation in a single gene affecting contact stimulation (*cgl* gene) were able to form foci of aggregation during development. However, the aggregates were diffuse, suggesting that contact stimulation is important for morphogenetic movements during aggregation. A mutant containing a mutation in the *cglF3* gene showed a striking delay in aggregation, suggesting that the *cglF3* gene affects a mechanism stimulating cells moving to foci or affects a mechanism for coordinating early cell behavior. Mutants containing the *cglF3* mutation in combination with a *cglB*, *cglC*, *cglE*, or *cglF1* mutation had severe defects in aggregation and failed to recover from the early delay. The severity of the defects in mutants containing two *cgl* mutations suggests that *cgl* genes are critical for development. We propose that *cgl* genes stimulate cell movement or control specific contacts between cells during aggregation.

Myxococcus xanthus is a gram-negative bacterium that provides a model for multicellular behavior during development (34, 35). The organism lives in the soil and feeds on other microorganisms and organic material. Vegetative cells move by gliding and display cooperative interactions as they travel in swarms (30, 32, 33). Upon starvation, cells begin a developmental cycle and move to centers of aggregation, where they form multicellular mounds that mature into fruiting bodies containing spores.

Myxobacteria move by gliding upon a surface (2). Gliding involves the slow smooth movement of cells in the direction of their long axis without the use of flagella (2, 12, 14). Although models for gliding motility have been proposed (3, 4, 6, 23, 24, 28, 31), the mechanism of gliding is not understood, and the components of the motility apparatus have not been identified. Highly ordered strands have been observed by electron microscopy in *Myxococcus fulvus* (24). These lie underneath the outer membrane and may be ultrastructural elements of an apparatus that undergoes conformational change to promote motility (24).

Individual *M. xanthus* cells frequently move in groups and behave as if they are coordinated. During the early phase of development, cells glide in circles or spirals where foci of aggregation initiate (30, 32). As aggregating cells form raised mounds, they move in spirals within terraces that become stacked (30). The movement of cells within patterns suggests that cells interact through a complex process which coordinates cell behavior (30).

A system involving cell-cell contacts or cell-cell signaling may control the gliding apparatus of *M. xanthus* and enable individual cells to move coordinately in groups (17). Gliding motility by vegetative cells is controlled by at least two systems of genes. The social (S) system is required for the rafts of peninsulas of cells to move out from the edge of a vegetative swarm (17). At least 10 genes affecting S motility have been identified (15, 36, 37). S motility may be mediated in part by pili, which may touch the surface of adjacent cells and stimulate motility through contact (16). Cell interactions appear to be required for S motility, because cells guided by S motility are nonmotile when they are separated from each other (17).

The adventurous (A) system is required for single cells to move out from the edge of a vegetative swarm (17). At least 25 genes for A motility have been detected by screening strains for defects in motility (14; M. Kalos and J. F. Zissler, Proc. Natl. Acad. Sci. USA, in press). Genes for the biosynthesis of cell surface lipopolysaccharide (LPS) also may be involved in A motility (9). One class of mutants defective in A motility is called the contact-stimulated gliding (*cgl*) class. Mutants in this class become transiently A motile after contact with wild-type cells. There are five groups of mutants stimulatable for A motility: CglB, -C, -D, -E, and -F (13). Mixing cells from different groups restores A motility. Motility stimulation results from extracellular complementation and requires that cells be in contact or in proximity (13). Mutations of a single stimulation class generally map at the same genetic locus locus (14, 40). The CglF group of mutants is an exception, because the *cglF1*, *cglF2*, and *cglF3* mutations map at distinct genetic loci (Kalos and Zissler, in press). The fact that complementation of mutants is efficient argues that stimulation for A motility may occur normally between wild-type cells (14). This suggests that contacts or signals between neighboring cells may be important in regulating A motility during swarming and possibly during fruiting (14, 15).

Motility defects caused by frizzy (*frz*) mutations affect the frequency of reversal in the direction of gliding (1). *frz* mutants produce frizzy filaments of aggregating cells during development, which would be characteristic of cells unable to migrate to centers of aggregation (43, 44). The genes identified by *frz* mutations have been cloned and sequenced and show striking homology to genes in a pathway for enterobacterial chemotaxis (27). Therefore, *frz* mutations

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could block a signal transduction pathway which may move cells to centers of aggregation by chemotaxis (30). Other motility defects, either the one-step *mgl* defect or the two-step A^- S^- defect, totally block developmental aggregation (15). Motility may be required not only to bring cells into aggregates, but also to bring cells into contact for cell-to-cell signaling (19). Cell-to-cell signaling appears to be critical for the expression of specific developmental genes (19).

We discovered that strains containing a mutation in a single *cgl* gene were aberrant in forming compact multicellular mounds during development. A strain containing a *cglF3* mutation displayed a striking delay in aggregation. Double mutants containing a *cglF3* mutation in combination with another *cgl* mutation were severely defective in aggregation. The severe defects in strains containing two *cgl* mutations suggest that genes for contact stimulation are critical for development.

MATERIALS AND METHODS

Bacterial strains. JZ007 is our reference strain, identical to strain DK1622 isolated in the laboratory of D. Kaiser (16). JZ007 (Table 1) is wild type for the adventurous (A) and social (S) motility systems (A^+ S^+). DK1218 (*cglB2*), DK1219 (*cglC1*), DK1230 (*cglE1*), DK1234 (*cglF1*), DK1212 (*aglJ1*), and DK1215 (*aglA1*) are A^- S^+ mutants isolated in the laboratory of D. Kaiser (15). *cgl* mutants are defective in the contact-stimulated gliding mechanism for A motility. DK306 (*mgl-1 sglA1*) is an A^- S^- mutant isolated in the laboratory of D. Kaiser (14). JZ302 (*cglF2*::Tn*phoA*), JZ306 (*cglB*::Tn*phoA*), and JZ334 (*cglF3*::Tn*phoA*) are A^- S^+ mutants containing transposon Tn*phoA* insertions Ω302, Ω306, and Ω334, respectively, at the *cgl* locus (Kalos and Zissler, in press). The symbol Ω refers to the insertion site of the transposon.

Strains JZ369 through JZ383 contain two A motility mutations. These were constructed by generalized transduction of a recipient strain containing a point mutation affecting A motility. Transducing lysates of myxophage Mx4 were grown on donor strains containing the Tn*phoA* insertion at a *cgl* locus, and transductants were selected by using the kanamycin resistance marker encoded by Tn*phoA*. Strains JZ384 through JZ401 (*cgl lac*$^+$) contain a Tn*phoA* insertion at a *cgl* locus and a Tn*5 lac* insertion in a gene expressed during development. These strains were constructed by generalized transduction of a *cgl* mutant containing Tn*phoA* in which the kanamycin resistance gene was replaced by an oxytetracycline resistance gene (Kalos and Zissler, in press). A transducing lysate of Mx4 was grown on a strain containing a Tn*5 lac* transposon insertion isolated previously in the laboratory of D. Kaiser (21). The Tn*5 lac* transposon encoded kanamycin resistance. Transductants which were resistant to both kanamycin and oxytetracycline were selected. These contained both transposons and were *cgl lac*$^+$. The Tn*5 lac* insertion itself does not cause a detectable defect in developmental aggregation and fruiting (20, 21). Strains JZ402, JZ403, and JZ404 are fully motile (A^+) derivatives of A^- Tn*5 lac* strains. These were constructed by transducing JZ389, JZ396, and JZ392 with Mx4 grown on JZ007, selecting A^+ S^+ strains that were kanamycin resistant, and checking for loss of oxytetracycline resistance.

Media. *M. xanthus* strains were grown in liquid CT medium (5) at 32°C in a gyratory shaker (shaken at 250 to 320 rpm). All antibiotic-resistant strains were grown in the presence of the appropriate antibiotic(s). Kanamycin monosulfate was used at a final concentration of 50 μg/ml. Oxytetracycline hydrochloride was used at a concentration of 12.5 μg/ml. Growth on solid medium involved CTT plates (26) with 1.5% (wt/vol) Bacto-agar (Difco).

Assay for development. *M. xanthus* development was studied on an agar surface at 32°C with TPM plates, which contained 1.5% (wt/vol) Bacto-agar in TPM buffer. TPM buffer contained 10 mM Tris hydrochloride (Tris-HCl, pH 7.6), 1 mM KH_2PO_4-K_2HPO_4 (pH 7.6), and 8.3 mM $MgSO_4$. Before plating, cells were grown to log phase in liquid CT medium, pelleted at 4,320 × *g* for 5 min, and resuspended at 3.2×10^9 cells per ml in cold TPM buffer. For low- and high-cell-density fruiting assays, 3×10^8 and 1×10^9 cells, respectively, from this suspension were diluted to 0.5 ml in cold TPM buffer and spread onto freshly poured 100-cm^2 TPM plates. The plates were dried under a hood and incubated at 32°C. Progression of cells through the developmental stages of aggregation and mound formation was monitored by observing the plates under a dissecting microscope.

Lysate generation. Bacteriophage lysates were made on *M. xanthus* by mixing 0.1 ml of bacteriophage Mx4 and 0.2 ml of cells at 10^8/ml (multiplicity of infection of 0.01). Adsorption occurred at room temperature for 5 min; 2.5 ml of CTT top agar (CTT broth containing 0.7% [wt/vol] Bacto-agar) was then added, and the mixture was plated onto freshly poured CTT plates. Plates were incubated at 28°C until confluent lysis was observed (20 to 30 h). Lysates were harvested by scraping the top agar into 30-ml glass tubes; 2.5 ml of phage buffer (10 mM Tris-HCl [pH 7.2], 86 mM NaCl, 0.1 mM $CaCl_2$, 1 mM $MgSO_4$) per plate was then added to the tubes, and the suspension was gently dispersed. After a few drops of chloroform were added, the suspension was incubated at 4°C for 2 h or overnight. Top agar and cell debris were removed by centrifugation at 9,750 × *g* for 15 min. The resulting supernatant fluid containing the bacteriophage was poured into a fresh tube, and the lysates were stored at 4°C over a few drops of chloroform.

Generalized transduction. Recipient strains were grown in liquid CT medium to approximately 5×10^8 cells per ml. A 0.5-ml sample of cells was mixed with 0.1 ml of phage (multiplicity of infection of 0.1 to 1), and the mixture was incubated for 5 min at 32°C for adsorption. The mixture was plated directly onto CTT plates containing kanamycin at 50 μg/ml. Plates were dried under a hood and incubated for 5 to 6 days at 32°C. Kanamycin-resistant transductants were selected and streaked out three times on fresh CTT plates containing kanamycin at 50 μg/ml. The absence of Mx4 phage was confirmed by spotting 5 μl of liquid cultures onto a lawn of the sensitive indicator strain JZ007 and incubating at 28°C.

Slide motility assays. Slide motility assays were performed as described previously (Kalos and Zissler, in press).

Phenotypic complementation assays. Phenotypic complementation assays were performed as described previously (Kalos and Zissler, in press).

PhoA enzyme assays. Changes in PhoA (alkaline phosphatase) enzyme levels throughout development were examined under the condition of submerged fruiting (10, 11, 22). Strains were grown in CT medium to log phase and diluted in fresh CT medium to 10^7 cells per ml; 100 μl of this suspension was then inoculated into individual wells of 96-well tissue culture plates (Plastek), and the plates were incubated for 22 h at 32°C (in order to allow cells to adhere to the bottom of the wells). The CT medium was removed, and the wells were washed once in 200 μl of fresh CT medium which had been warmed to 32°C; 100 μl of fresh warm CT medium

TABLE 1. Bacterial strains

Strain	Relevant genotype	Origin or reference
JZ007	Reference	Derived from DK1622
JZ302	JZ007 Ω302 (*cglF2*::Tn*phoA*)	Kalos and Zissler, in press
JZ306	JZ007 Ω306 (*cglB*::Tn*phoA*)	Kalos and Zissler, in press
JZ334	JZ007 Ω334 (*cglF3*::Tn*phoA*)	Kalos and Zissler, in press
JZ311	JZ007 Ω311	Kalos and Zissler, in press
JZ335	JZ007 Ω334 (*cglF3*::Tn*phoA*-tet)	Kalos and Zissler, in press
JZ337	JZ007 Ω302 (*cglF2*::Tn*phoA*-tet)	Kalos and Zissler, in press
JZ338	JZ007 Ω306 (*cglB*::Tn*phoA*-tet)	Kalos and Zissler, in press
DK306	*mgl-1 sglA1*	14
DK1218	*cglB2*	15
DK1219	*cglC1*	15
DK1230	*cglE1*	15
DK1234	*cglF1*	15
DK1212	*aglJ1*	15
DK1215	*aglA1*	15
DK4499	DK1622 Ω4499 (Tn*5 lac*)	20
DK4290	DK1622 Ω4273 (Tn*5 lac*)	20
DK4514	DK1622 Ω4514 (Tn*5 lac*)	20
DK4521	DK1622 Ω4521 (Tn*5 lac*)	20
DK5204	DK1622 Ω4435 (Tn*5 lac*)	20
DK5206	DK1622 Ω4455 (Tn*5 lac*)	20
JZ369	JZ007 Ω302 (*cglF2*::Tn*phoA*) *cglB2*	Mx4 (JZ302) × DK1218
JZ370	JZ007 Ω302 (*cglF2*::Tn*phoA*) *cglE1*	Mx4 (JZ302) × DK1230
JZ372	JZ007 Ω302 (*cglF2*::Tn*phoA*) *cglF1*	Mx4 (JZ302) × DK1234
JZ373	JZ007 Ω306 (*cglB*::Tn*phoA*) *cglC1*	Mx4 (JZ306) × DK1219
JZ374	JZ007 Ω306 (*cglB*::Tn*phoA*) *cglE1*	Mx4 (JZ306) × DK1230
JZ376	JZ007 Ω306 (*cglB*::Tn*phoA*) *cglF1*	Mx4 (JZ306) × DK1234
JZ377	JZ007 Ω334 (*cglF3*::Tn*phoA*) *cglB2*	Mx4 (JZ334) × DK1218
JZ378	JZ007 Ω334 (*cglF3*::Tn*phoA*) *cglC1*	Mx4 (JZ334) × DK1219
JZ379	JZ007 Ω334 (*cglF3*::Tn*phoA*) *cglE1*	Mx4 (JZ334) × DK1230
JZ381	JZ007 Ω334 (*cglF3*::Tn*phoA*) *cglF1*	Mx4 (JZ334) × DK1234
JZ382	JZ007 Ω334 (*cglF3*::Tn*phoA*) *aglA1*	Mx4 (JZ334) × DK1215
JZ383	JZ007 Ω302 (*cglF2*::Tn*phoA*) *aglA1*	Mx4 (JZ302) × DK1215
JZ384	JZ007 Ω334 (*cglF3*::Tn*phoA*) Ω4499 (Tn*5 lac*)	Mx4 (DK4499) × JZ335
JZ385	JZ007 Ω302 (*cglF2*::Tn*phoA*) Ω4499 (Tn*5 lac*)	Mx4 (DK4499) × JZ337
JZ386	JZ007 Ω306 (*cglB*::Tn*phoA*) Ω4499 (Tn*5 lac*)	Mx4 (DK4499) × JZ338
JZ387	JZ007 Ω334 (*cglF3*::Tn*phoA*) Ω4273 (Tn*5 lac*)	Mx4 (DK4290) × JZ335
JZ388	JZ007 Ω302 (*cglF2*::Tn*phoA*) Ω4273 (Tn*5 lac*)	Mx4 (DK4290) × JZ337
JZ389	JZ007 Ω306 (*cglB*::Tn*phoA*) Ω4273 (Tn*5 lac*)	Mx4 (DK4290) × JZ338
JZ390	JZ007 Ω334 (*cglF3*::Tn*phoA*) Ω4514 (Tn*5 lac*)	Mx4 (DK4514) × JZ335
JZ391	JZ007 Ω302 (*cglF2*::Tn*phoA*) Ω4514 (Tn*5 lac*)	Mx4 (DK4514) × JZ337
JZ392	JZ007 Ω306 (*cglB*::Tn*phoA*) Ω4514 (Tn*5 lac*)	Mx4 (DK4514) × JZ338
JZ393	JZ007 Ω334 (*cglF3*::Tn*phoA*) Ω4521 (Tn*5 lac*)	Mx4 (DK4521) × JZ335
JZ394	JZ007 Ω302 (*cglF2*::Tn*phoA*) Ω4521 (Tn*5 lac*)	Mx4 (DK4521) × JZ337
JZ395	JZ007 Ω306 (*cglB*::Tn*phoA*) Ω4521 (Tn*5 lac*)	Mx4 (DK4521) × JZ338
JZ396	JZ007 Ω334 (*cglF3*::Tn*phoA*) Ω4435 (Tn*5 lac*)	Mx4 (DK5204) × JZ335
JZ397	JZ007 Ω302 (*cglF2*::Tn*phoA*) Ω4435 (Tn*5 lac*)	Mx4 (DK5204) × JZ337
JZ398	JZ007 Ω306 (*cglB*::Tn*phoA*) Ω4435 (Tn*5 lac*)	Mx4 (DK5204) × JZ338
JZ399	JZ007 Ω334 (*cglF3*::Tn*phoA*) Ω4455 (Tn*5 lac*)	Mx4 (DK5206) × JZ335
JZ400	JZ007 Ω302 (*cglF2*::Tn*phoA*) Ω4455 (Tn*5 lac*)	Mx4 (DK5206) × JZ337
JZ401	JZ007 Ω306 (*cglB*::Tn*phoA*) Ω4455 (Tn*5 lac*)	Mx4 (DK5206) × JZ338
JZ402	JZ007 Ω4273 (Tn*5 lac*)	Mx4 (JZ007) × JZ389
JZ403	JZ007 Ω4435 (Tn*5 lac*)	Mx4 (JZ007) × JZ396
JZ404	JZ007 Ω4514 (Tn*5 lac*)	Mx4 (JZ007) × JZ392

was then added to each well, and the plates were incubated for 2 h at 32°C. The supernatant (the 2-h supernatant) was removed from each well, and this was saved to assay PhoA enzyme activity present at this time point (T_0). Wells were washed once for 5 min with 200 μl of dH_2O at 32°C, and then 100 μl of MOPS-salts buffer (10 mM MOPS [morpholinepropanesulfonic acid, pH 7.2], 2 mM $CaCl_2$, 4 mM $MgSO_4$) at 32°C was added to each well. The point at which the MOPS buffer was added was defined as T_0 in development. Plates were incubated at 32°C to allow development to occur. At 2-h intervals, 100 μl of 2× PhoA buffer (2 M Tris [pH 9.6], 2 M NaCl, 4 mM $MgSO_4$) and 25 μl of a 1:1 mixture of $CHCl_3$ and methanol were added to each of four wells per strain. The contents of each well were mixed and then transferred to a fresh 96-well plate; 25 μl of a 5-mg/ml solution of *para*-nitrophenylphosphate (1 mM final concentration) was then added to each well, and this plate was incubated for 180 min at 37°C. Samples (50 μl) from each of the four wells per strain were pooled, and the PhoA enzyme activity in each pool (representing intracellular plus extracellular alkaline phosphatase activity) was determined by recording the A_{405}. Hydrolysis of 1 mM *para*-nitrophenylphosphate increased linearly in this assay with an increased concentration of PhoA enzyme.

β-Galactosidase assays. β-Galactosidase was assayed by spotting 3×10^6 or 1×10^7 cells per cm^2 onto TPM agar plates containing the chromogenic β-galactosidase substrate X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) at a final concentration of 40 μg/ml. The plates were incubated at 32°C. The expression of β-galactosidase was determined by visually monitoring the color of the colonies for 10 days. Quantitative measurements of β-galactosidase expression were based on the method described by Kroos et al. (21). Cells were grown to log phase in CT medium, harvested, suspended in cold TPM buffer, and spotted on TPM plates at a density of 10^7 cells per cm^2. The spots were allowed to dry under a hood, and then development was allowed to proceed at 32°C.

At various times throughout development, cells from duplicate spots were scraped from the agar into TPM buffer containing 0.02% sodium dodecyl sulfate. Cells on ice were disrupted by sonication at 80 W for 30 to 60 s. Spores were disrupted by adding glass beads (170-μm diameter) and sonicating on ice at 100 W for 2 to 3 min with intermittent cooling. Cell and spore disruption was verified by microscopic observation. Cell debris was removed by centrifugation at 9,600 rpm for 90 s in an Eppendorf centrifuge, and the supernatant fraction was assayed for protein content and β-galactosidase activity. Supernatant fractions were stored at −70°C until all samples were collected. Protein concentration in samples was determined by the Bio-Rad protein microassay procedure, reading the A_{580} of the assay samples, and with bovine gamma globulin as the protein standard.

Hydrolysis of the β-galactosidase substrate ONPG (*o*-nitrophenyl-β-D-galactopyranoside) was detected by adding 25 to 50 μl of samples to 100 μl of Z buffer (0.1 M Na_2HPO_4 [pH 7.0], 0.01 M KCl, 0.001 M $MgSO_4$, 0.005 M β-mercaptoethanol) containing 1 mg of ONPG per ml in individual wells of a 96-well plate, incubating for a maximum of 2 h at 37°C until sufficient yellow color had developed, and reading the A_{405} in wells spectrophotometrically with a 96-well plate reader. To account for variable amounts of yellow pigments in different samples (21), the absorbance of wells at the beginning of the assay was determined, and these values were subtracted from the final absorbance values. β-Galactosidase specific activity (in nanomoles of ONPG per minute per milligram of protein) was calculated as described by Kroos et al. (21).

RESULTS

***cgl* mutants have defects in development.** The aggregation of cells during the fruiting process was examined for different *cgl* mutants. Figure 1 compares the fruiting behavior of the reference strain JZ007 with the behavior of an *agl* mutant, different *cgl* point mutants isolated previously (15), and the *cgl* mutants containing Tn*phoA* at locus Ω306 (*cglB*) and at locus Ω302 (*cglF2*). The reference strain is normal for A and S motility, while the *agl* and *cgl* mutants are normal for S motility but defective for A motility.

Figure 1 shows the fruiting behavior of strains tested at low cell density (3×10^6 cells per cm^2, Fig. 1A) and high cell density (1×10^7 cells per cm^2, Fig. 1B). During development in the reference strain, cells moved into aggregation centers and formed compact mounds which darkened as the fruits matured and produced spores. Fruiting by the reference strain was complete by 24 h and was not defective when tested at either low or high cell density (Fig. 1A and B, a). Fruiting by the *aglJ1* mutant (DK1212) appeared to be similar at both low and high cell densities to fruiting by the reference strain, producing compact mounds of cells which darkened into mature fruits (Fig. 1A and B, b). Fruiting by another *agl* mutant (DK1215), which contained the *aglA1* mutation, also appeared to be normal at both low and high cell densities (data not shown).

cgl mutants, like *agl* mutants, are defective in A motility. However, *cgl* mutants differ from *agl* mutants in that they can be rescued by phenotypic complementation (13). We observed that each of the *cgl* mutants was defective in the fruiting process (Fig. 1A and B, c, d, e, and f). The degree of the defect depended on the density of the cells in the assay and varied for different strains. At low cell density, each of the strains with *cgl* point mutations (*cglB2*, *cglC1*, *cglE1*, and *cglF1*) formed irregular, diffuse, or multilobed mounds of cells. At high cell density, the *cgl* mutants formed more distinct mounds. High cell density did not completely rescue the fruiting defect of the *cglF1* mutant, however, since this mutant formed irregularly shaped mounds at both low and high cell densities (Fig. 1A and B, f). High cell density also did not completely rescue the fruiting defect of the *cglB2* mutant (Fig. 1B, c), which produced somewhat smaller and more numerous mounds than the reference strain when tested under this condition. High cell density dramatically rescued the fruiting defect of the *cglC1* mutant and the *cglE1* mutant (Fig. 1B, d and e).

Fruiting by the *cgl* mutants containing Tn*phoA* at Ω306 (*cglB*::Tn*phoA*) and at Ω302 (*cglF2*::Tn*phoA*) was similar to fruiting by the *cgl* point mutants, since these Tn*phoA* mutants produced irregular and diffuse mounds of cells when tested at low cell density (Fig. 1A and B, g and h). At high cell density, these strains produced more compact mounds, but the mounds still were not completely normal because they were slightly diffuse.

Figure 2 shows the fruiting phenotype of strain JZ334, which contains a Tn*phoA* insertion at Ω334 (*cglF3*::Tn*phoA*). This mutant was severely delayed in aggregation. Completion of fruiting required approximately three to five times as long as in the reference strain. While JZ007 cells formed mature fruiting bodies at both low and high cell densities by 24 h, JZ334 cells at 24 h had failed to move to focal centers of aggregation and remained in a smooth lawn. By 70 h, JZ334 cells formed immature fruiting bodies. By 144 h, development of JZ334 reached a terminal point, and no further change in fruiting behavior could be observed. At this point, distinct mounds of cells had formed, but they were smaller than those produced by the reference strain. In contrast to the defect observed for other *cgl* mutants, the fruiting defect of JZ334 was not rescued by high cell density, at least through 70 h (Fig. 2A and B). However, by 144 h, mounds produced at high cell density appeared to be darker than mounds produced at low cell density.

With respect to the number of fruits observed in these assays, all of the A motility mutants depicted in Fig. 1 and 2 produced a larger number of fruits than the reference strain, particularly when tested at high cell density.

Particular *cgl* double mutants have severe developmental defects. Although *cgl* mutations affected development, the mutations appeared to be weak, since they did not completely block aggregation, even when cells were tested at lower cell density. The weak effects of individual *cgl* mutations could be explained if the products of *cgl* genes are not critical for development. Alternatively, different *cgl* gene products might be critical but function in redundant or parallel pathways. In this case, a defect in one particular *cgl* gene could be complemented by another *cgl* gene whose

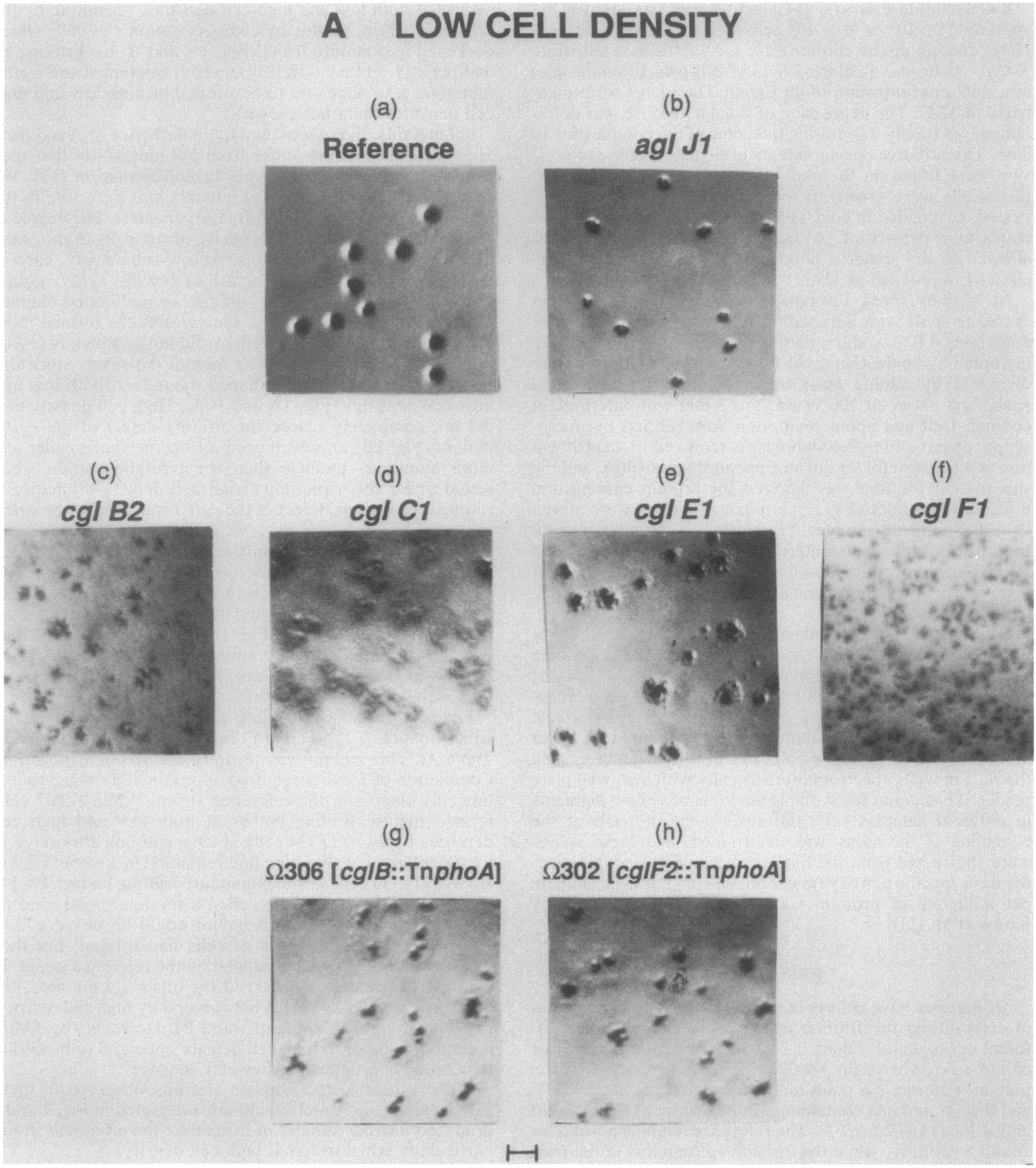

FIG. 1. Fruiting phenotypes of the reference strain JZ007 (a), the *aglJ1* mutant DK1212 (b), the *cgl* point mutants DK1218 (*cglB2*) (c), DK1219 (*cglC1*) (d), DK1230 (*cglE1*) (e), and DK1234 (*cglF1*) (f), and the *cgl*::Tn*phoA* mutants JZ306 (*cglB*::Tn*phoA*) (g) and JZ302 (*cglF2*::Tn*phoA*) (h). Panels A and B show development in the 24-h period at low and high cell densities, respectively. Bar, 50 μm.

product provides a similar function or at least a function that allows cells to bypass the mutational block. This hypothesis leads to the prediction that mutations in particular pairs of *cgl* genes might block separate pathways and severely block development.

To test this hypothesis, we used generalized transduction and made *cgl* double mutants containing a point mutation in one *cgl* gene and a Tn*phoA* insertion in a different *cgl* gene. Double mutants were generated by transducing a recipient strain which contained a *cgl* point mutation from a particular

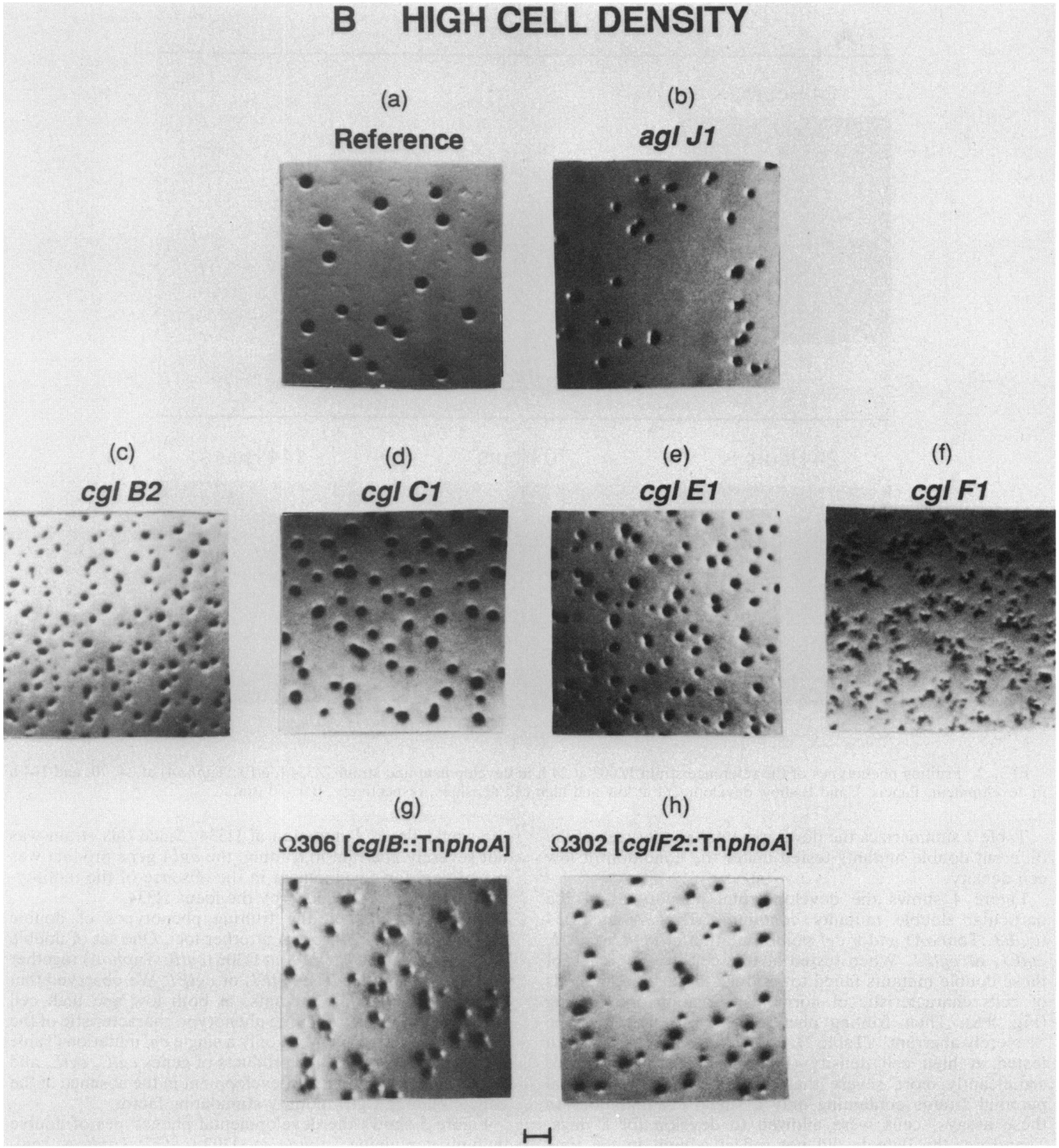

FIG. 1—*Continued*

phenotypic complementation class (i.e., *cglB2*, *cglC1*, *cglE1*, or *cglF1*). The transducing lysate contained myxophage Mx4 grown on a donor strain having a Tn*phoA* insertion in a different *cgl* gene. The antibiotic resistance gene in Tn*phoA* provided a selectable marker for isolating transductants containing the Tn*phoA* insertion mutation.

Slide motility assays showed that each of the transductants remained defective in A motility. Phenotypic complementation assays showed that particular mutants constructed in this way were double *cgl* mutants, because they were defective in both the factor affected by the *cgl* point mutation and the factor affected by the Tn*phoA* insertion (data not shown). Although each mutant had two mutations blocking A motility, slide motility assays showed that each double mutant possessed social (S) motility (Fig. 3, and data not shown).

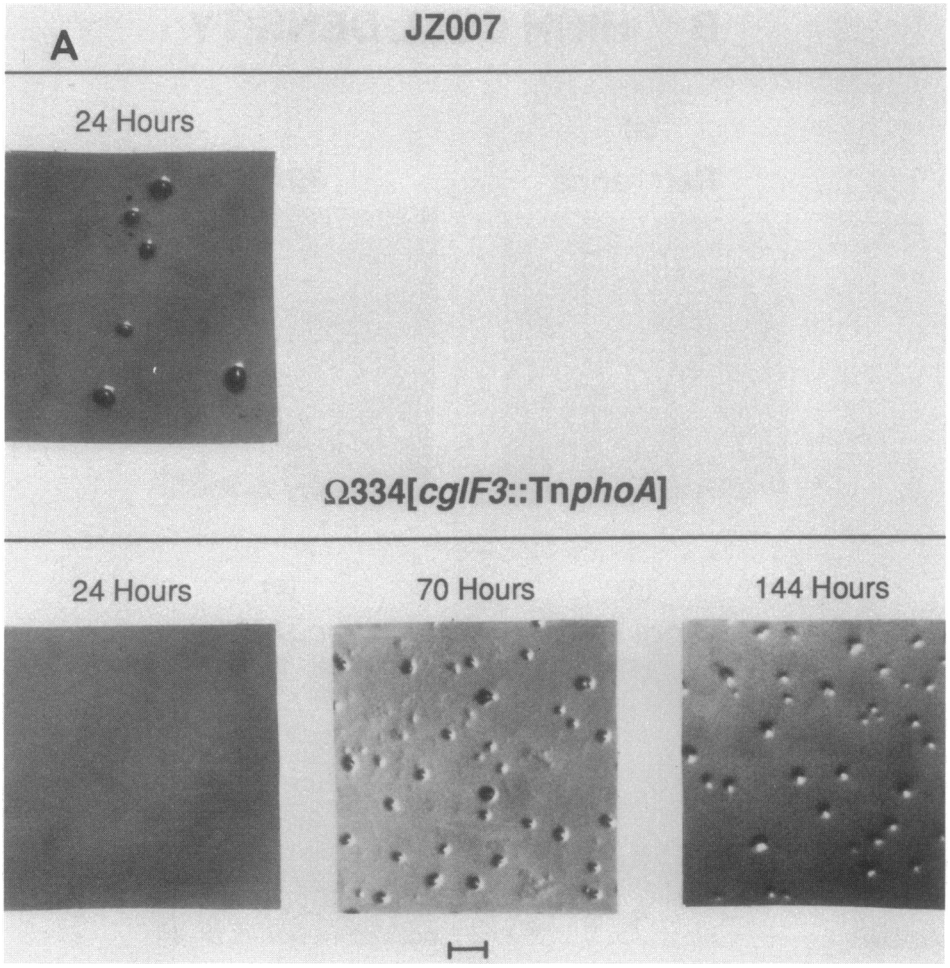

FIG. 2. Fruiting phenotypes of the reference strain JZ007 at 24 h in development and strain JZ334 (*cglF3*::Tn*phoA*) at 24, 70, and 144 h in development. Panels A and B show development at low and high cell densities, respectively. Bar, 50 μm.

Table 2 summarizes the developmental phenotypes of the different double mutants tested under the condition of low cell density.

Figure 4 shows the developmental phenotypes of the particular double mutants containing Tn*phoA* at Ω334 (*cglF3*::Tn*phoA*) and a *cgl* point mutation at *cglB2*, *cglC1*, *cglE1*, or *cglF1*. When tested at low cell density, each of these double mutants failed to produce the distinct mounds of cells characteristic of normal aggregation and fruiting (Fig. 4A). Their fruiting phenotypes were judged to be "severely aberrant" (Table 2). Each also failed to fruit when tested at high cell density (Fig. 4B). The defects were significantly more severe than the defects in the original parental strains containing only a single *cgl* mutation. In these assays, cells were allowed to develop for 8 days. Therefore, the defects did not reflect a limit in the time allowed for fruiting. The results indicated that the products of genes *cglB*, *cglC*, *cglE*, and *cglF1* were critical for normal aggregation and fruiting in the absence of the motility-stimulating factor encoded by the locus Ω334.

Figure 4 also shows the developmental phenotype of the double mutant containing Tn*phoA* at Ω334 (*cglF3*::Tn*phoA*) and an *agl* mutation, *aglA1*. We observed that cells from this strain moved into focal centers and formed compact mounds. When tested at either low or high cell density, this strain behaved like the parental strain JZ334 containing only the single Tn*phoA* mutation at Ω334. Since this strain was not severely aberrant in fruiting, the *aglA* gene product was not critical for development in the absence of the motility-stimulating factor encoded by the locus Ω334.

We also examined the fruiting phenotypes of double mutants containing Tn*phoA* at other loci. One set of double mutants contained Tn*phoA* at Ω306 (*cglB*::Tn*phoA*) together with *cgl* mutation *cglC1*, *cglE1*, or *cglF1*. We observed that each of these double mutants, at both low and high cell densities, exhibited a fruiting phenotype characteristic of the parental mutants containing only a single *cgl* mutation (Table 2). This indicated that the products of genes *cglC*, *cglE*, and *cglF1* were not critical for development in the absence of the contact-gliding CglB motility-stimulating factor.

Figure 5 shows the developmental phenotypes of double mutants containing Tn*phoA* at Ω302 (*cglF2*::Tn*phoA*) combined with another *cgl* mutation (*cglB1*, *cglE1*, or *cglF1*) or combined with an *aglA1* mutation, assayed at low and high cell densities. We observed that the combination of Tn*phoA* at Ω302 and the *cglB1* mutation or the *cglE1* mutation did not lead to a developmental phenotype significantly different from that of the parental strains containing a single *cgl* mutation. However, the combination of Tn*phoA* at Ω302 with the *cglF1* mutation produced large, diffuse, multilobed aggregates of cells at low cell density. These fruits differed in morphology from fruits produced by the parental strains

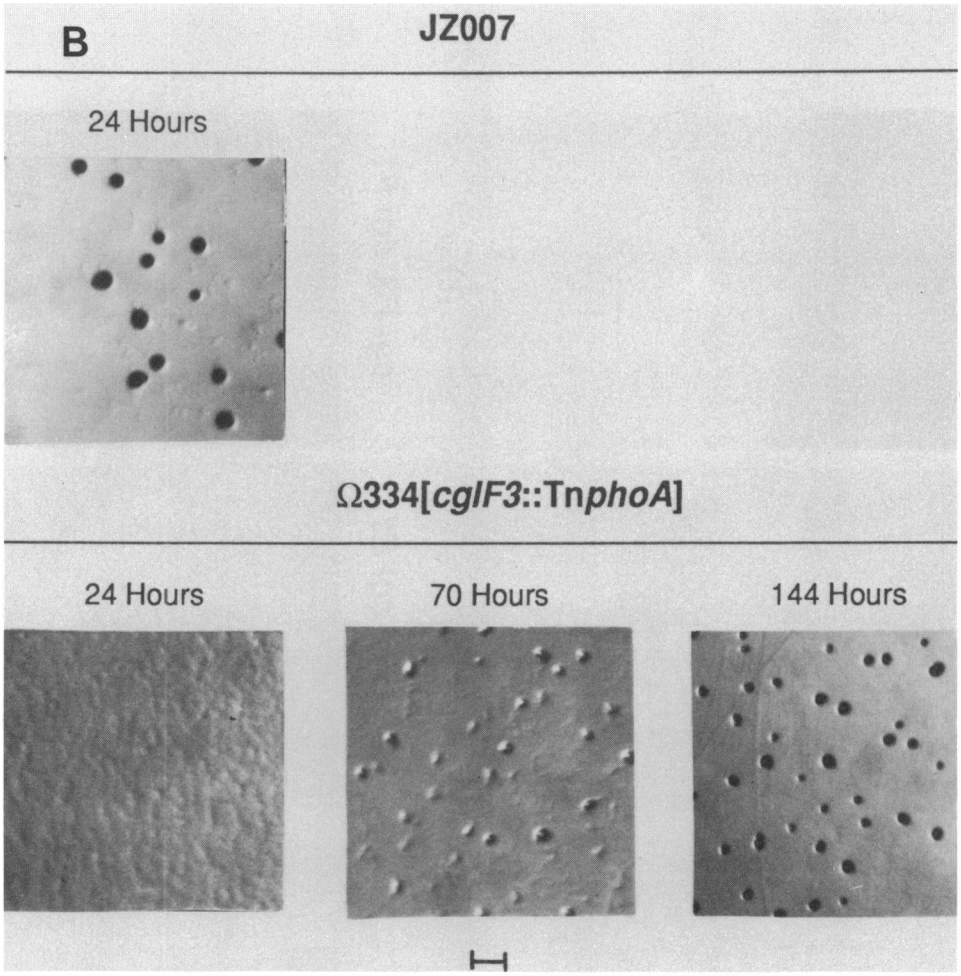

FIG. 2—*Continued*

used to construct the double mutants and were judged to be aberrant (Table 2). At high cell density, fruiting produced the more compact mounds characteristic of the parental strains containing a single mutation.

***cgl* genes tagged with Tn*phoA* produce PhoA enzyme activity during development.** Strains containing Tn*phoA* produce an alkaline phosphatase fusion protein if the *phoA* gene is fused to proper upstream sequences upon insertion of the transposon into the host genome. Since all regulatory sequences have been removed from the beginning of the *phoA* gene (25), expression of the *phoA* gene reflects the initiation of both transcription and translation from upstream host sequences.

Using Tn*phoA* inserted at *cgl* loci as probes for gene activity, we examined the level of active PhoA enzyme produced by *cgl* loci during development. The transposon Tn*5 lac* has been used in *M. xanthus* in a similar approach to examine gene activity during development (19–21, 43). We examined the change in PhoA levels by using submerged-fruiting assays for development. In these assays, cells adhered to a surface (the bottom of wells in a 96-well tissue culture plate) and progressed through development under a liquid. Development was induced by the addition of a starvation medium. At 2-h intervals during development, cells in quadruplicate wells were lysed, and the contents of the wells were assayed collectively for PhoA enzyme activity (Materials and Methods). Although alkaline phosphatase activities have been reported to exist in developing *M. xanthus* cells (29, 42; M. Dworkin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, G27, p. 30), our assays at different time points during development tested small amounts of extract and did not detect any significant alkaline phosphatase enzyme activity in JZ007. Therefore, the PhoA enzyme detected in strains containing the transposon Tn*phoA* did not reflect background expression of an endogenous alkaline phosphatase.

We measured the alkaline phosphatase (PhoA) enzyme produced by strains containing Tn*phoA* at *cgl* loci Ω302 (*cglF2*), Ω306 (*cglB*), and Ω334 (*cglF3*). We observed that the level of PhoA enzyme during development increased approximately four- to fivefold from loci Ω306 and Ω334 and increased approximately two- to threefold from locus Ω302 (data not shown). The increase began approximately at 3 h and continued up to 12 h or beyond. Therefore, the increase in PhoA corresponded to the period during which cells were aggregating and forming immature mounds. Since each of the *cgl-phoA* fusion genes appeared to be expressed during aggregation, we conclude that each of the corresponding wild-type *cgl* genes must be expressed during aggregation.

***cgl* mutations decrease the expression of developmentally induced genes.** Kroos et al. isolated different Tn*5 lac* fusions in *M. xanthus* whose expression increased during develop-

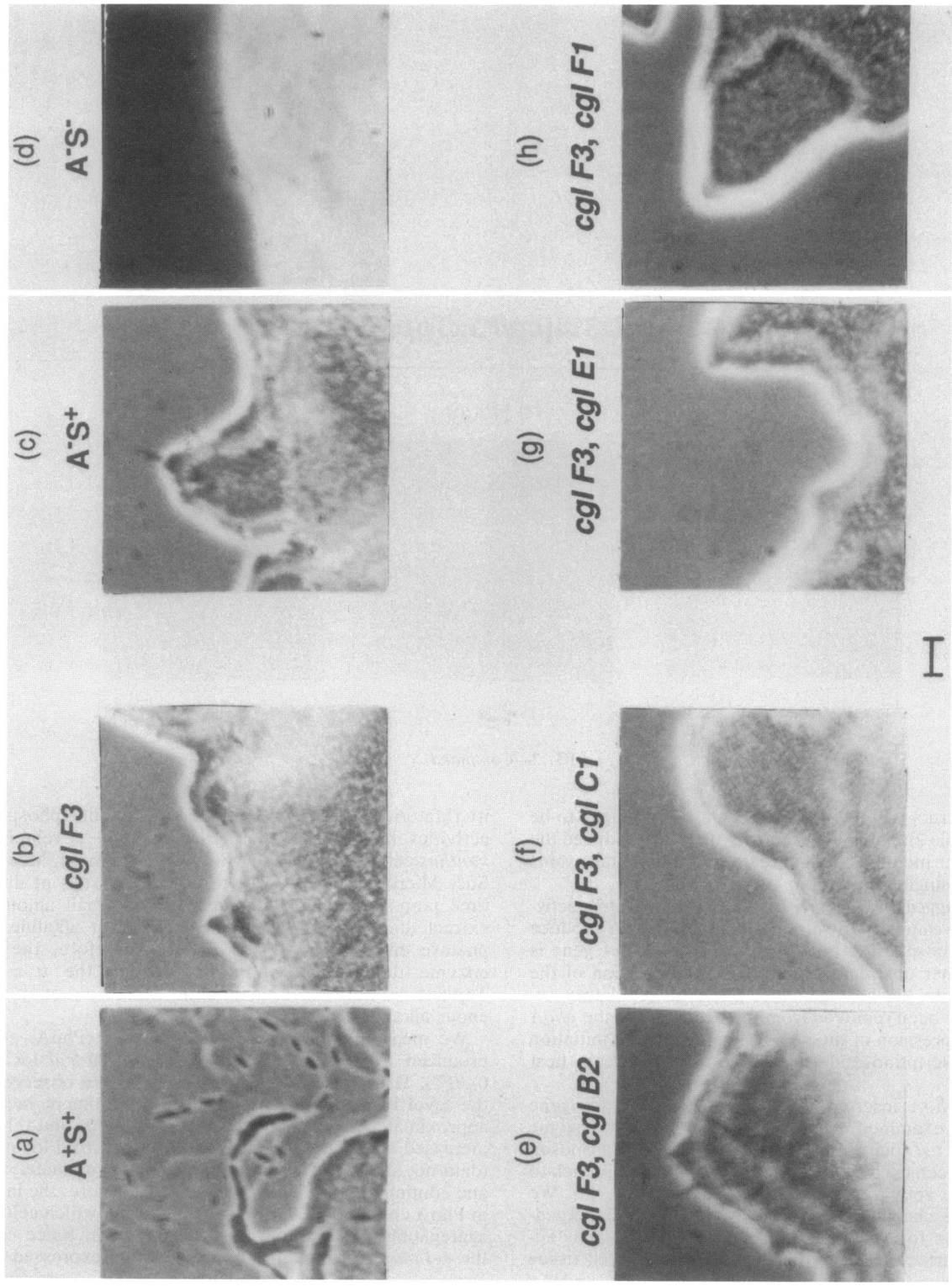

FIG. 3. Motility phenotypes from slide motility assays showing the edges of colonies from strains (a) JZ007, (b) JZ334 (*cglF3*::Tn*phoA*), (c) DK1218 (*cglB2*), (d) DK306 (*mgl sglA1*), (e) JZ377 (*cglF3*::Tn*phoA cglB2*), (f) JZ378 (*cglF3*::Tn*phoA cglC1*), (g) JZ379 (*cglF3*::Tn*phoA cglE1*), and (h) JZ381 (*cglF3*::Tn*phoA cglF1*). Bar, 50 μm.

TABLE 2. Fruiting phenotypes of *cgl* double mutants

Strain	Tn*phoA* insertion	Point mutation	Fruiting phenotype[a]
JZ377	Ω334 (*cglF3*)	*cglB2*	Severely aberrant
JZ378	Ω334 (*cglF3*)	*cglC1*	Severely aberrant
JZ379	Ω334 (*cglF3*)	*cglE1*	Severely aberrant
JZ381	Ω334 (*cglF3*)	*cglF1*	Severely aberrant
JZ372	Ω302 (*cglF2*)	*cglF1*	Aberrant
JZ369	Ω302 (*cglF2*)	*cglB2*	Parental
JZ370	Ω302 (*cglF2*)	*cglE1*	Parental
JZ373	Ω306 (*cglB*)	*cglC1*	Parental
JZ374	Ω306 (*cglB*)	*cglE1*	Parental
JZ376	Ω306 (*cglB*)	*cglF1*	Parental
JZ382	Ω334 (*cglF3*)	*aglA1*	Parental
JZ383	Ω302 (*cglF2*)	*aglA1*	Parental

[a] Fruiting phenotype determined for cells at low density after 8 days of development on TPM starvation agar plates. Parental indicates that the fruiting phenotype of the double mutant resembled that of one or both of the parental single mutant strains.

ment (19–21). The increase in expression could be correlated with a specific period in development. Several of these Tn*5* *lac* fusions could be used as markers for timing the progression of cells through aggregation and fruiting body formation (19–21).

We used these Tn*5* *lac* fusions in order to determine whether *cgl* mutations affected genes whose expression normally increased at specific times during development. We tested Tn*phoA* insertions at Ω302 (*cglF2*::Tn*phoA*), Ω306 (*cglB*::Tn*phoA*), and Ω334 (*cglF3*::Tn*phoA*) for their effects on these genes. We used three Tn*5* *lac* fusions (at sites Ω4521, Ω4455, and Ω4499) whose expression was correlated with very early development (2 to 4 h). We also used the Tn*5* *lac* fusion at Ω4273 whose expression was correlated with aggregation (5 to 6 h), the Tn*5* *lac* fusion at Ω4514 whose expression was correlated with late aggregation (9 to 12 h), and the Tn*5* *lac* fusion at Ω4435 whose expression was correlated with very late development (approximately 25 h). Although these Tn*5* *lac* transposons were inserted into genes that were expressed during development, they did not inactivate genes normally required for development.

Double mutant strains containing particular pairs of Tn*phoA* and Tn*5* *lac* transposons were constructed by growing myxophage Mx4 on each of the Tn*5* *lac* fusion strains and transducing recipient strains containing the Tn*phoA* fusions (see Materials and Methods). To determine β-galactosidase expression from Tn*5* *lac* in the various mutants, each of the strains was tested under conditions of both low and high cell density, and developing cells were characterized visually by monitoring for 10 days the appearance of blue color produced by hydrolysis of the chromogenic β-galactosidase substrate X-Gal present in the plates. The pattern of β-galactosidase expression in these assays was similar for both the low and high cell density conditions.

We observed that expression of the β-galactosidase genes at Ω4514, Ω4455, and Ω4499 (expressed early) was not affected by the presence of the Tn*phoA* insertions at *cgl* loci Ω302, Ω306, and Ω334 (Table 3). The expression of the β-galactosidase gene at Ω4273 (expressed at 5 to 6 h) and at Ω4514 (expressed at 9 h) was not affected by the presence of the Tn*phoA* insertions at *cgl* loci Ω302 and Ω334, but it was affected by the Tn*phoA* insertion at *cgl* locus Ω306. All three Tn*phoA* insertions reduced the expression of the β-galactosidase gene at Ω4435, the fusion normally expressed late in development.

To confirm that a Tn*phoA* insertion was responsible for the decrease in β-galactosidase levels, the Tn*phoA* insertion was replaced by the corresponding wild-type genetic region from the reference strain JZ007. The new *cgl*$^+$ strains were produced by backcrosses via generalized transduction with Mx4 (see Materials and Methods). The Tn*phoA* insertion was replaced with the wild-type genetic region in strain JZ396 (Ω334 [*cglF3*::Tn*phoA*], Ω4435 [Tn*5* *lac*]), in strain JZ389 (Ω306 [*cglB*::Tn*phoA*], Ω4273 [Tn*5* *lac*]), and in strain JZ392 (Ω306 [*cglB*::Tn*phoA*], Ω4514 [Tn*5* *lac*]). We observed that strains JZ403, JZ402, and JZ404, which contained Tn*5* *lac* at Ω4435, Ω4273, and Ω4514, respectively, but not the Tn*phoA* insertion, expressed β-galactosidase like the original parents DK5204 (Ω4435 [Tn*5* *lac*]), DK4290 (Ω4273 [Tn*5* *lac*]), and DK4514 (Ω4514 [Tn*5* *lac*]), respectively (Table 3).

Figure 6 shows quantitatively the effects of Tn*phoA* at *cgl* loci Ω302, Ω306, and Ω334 on the expression of the β-galactosidase gene at Ω4435. Each of the strains was plated on TPM agar under the less stringent condition of high cell density. Cells were harvested at various time points during development, and β-galactosidase specific activity was determined as described by Kroos et al. (21). We observed that each of the Tn*phoA* insertions severely reduced the expression of β-galactosidase from the Tn*5* *lac* fusion at Ω4435. Replacement of the Tn*phoA* insertion at Ω334 by a wild-type genetic region restored β-galactosidase expression to the level characteristic of the normal *cgl*$^+$ strain (DK5204) containing the Tn*5* *lac* fusion at Ω4435.

DISCUSSION

During development, *M. xanthus* cells glide toward aggregation centers and form compact multicellular mounds. Most strains with defects in contact-stimulated gliding moved toward aggregation centers, but at low cell density they formed mounds that were diffuse and irregular. This suggests that contact-stimulated gliding is important for morphogenetic movements.

Mutants carrying mutations in the contact-gliding genes *cglB*, *cglC*, *cglE*, *cglF1*, and *cglF2* produced aberrant mounds, indicating that each of these genes is important during development. *cgl* mutants did not produce the frizzy filaments of aggregating cells characteristic of frizzy (*frz*) mutations. *frz* mutations block a signal transduction pathway that may move cells to foci of aggregation by chemotaxis (30). Since *cglB*, *cglC*, *cglE*, *cglF1*, and *cglF2* mutations did not block the movement of cells toward foci of aggregation, they probably do not affect the mechanisms that attract cells into foci from a distance.

Although single *cgl* mutations caused defects in developmental aggregation, the defects were subtle. Moreover, defects in cells present at the low density of $3 \times 10^6/cm^2$ were absent from cells at the slightly higher cell density of $1 \times 10^7/cm^2$. Therefore, the requirement for individual *cgl* genes in forming compact mounds is partial and extremely sensitive to cell density.

In contrast to the slight defects caused by most *cgl* mutations, the defect caused by the *cglF3* mutation was severe. This mutation caused a striking delay in the timing for aggregation. Whereas the normal strain formed dark, compact aggregates by 24 h of development, the *cglF3* mutant had not formed mounds of aggregating cells by this time. Eventually, this mutant formed mounds that matured into compact aggregates. The condition of higher cell density did not rescue the delay in aggregation. The inability to form aggregates early in development suggests that the *cglF3* gene might affect a mechanism stimulating cells moving to foci

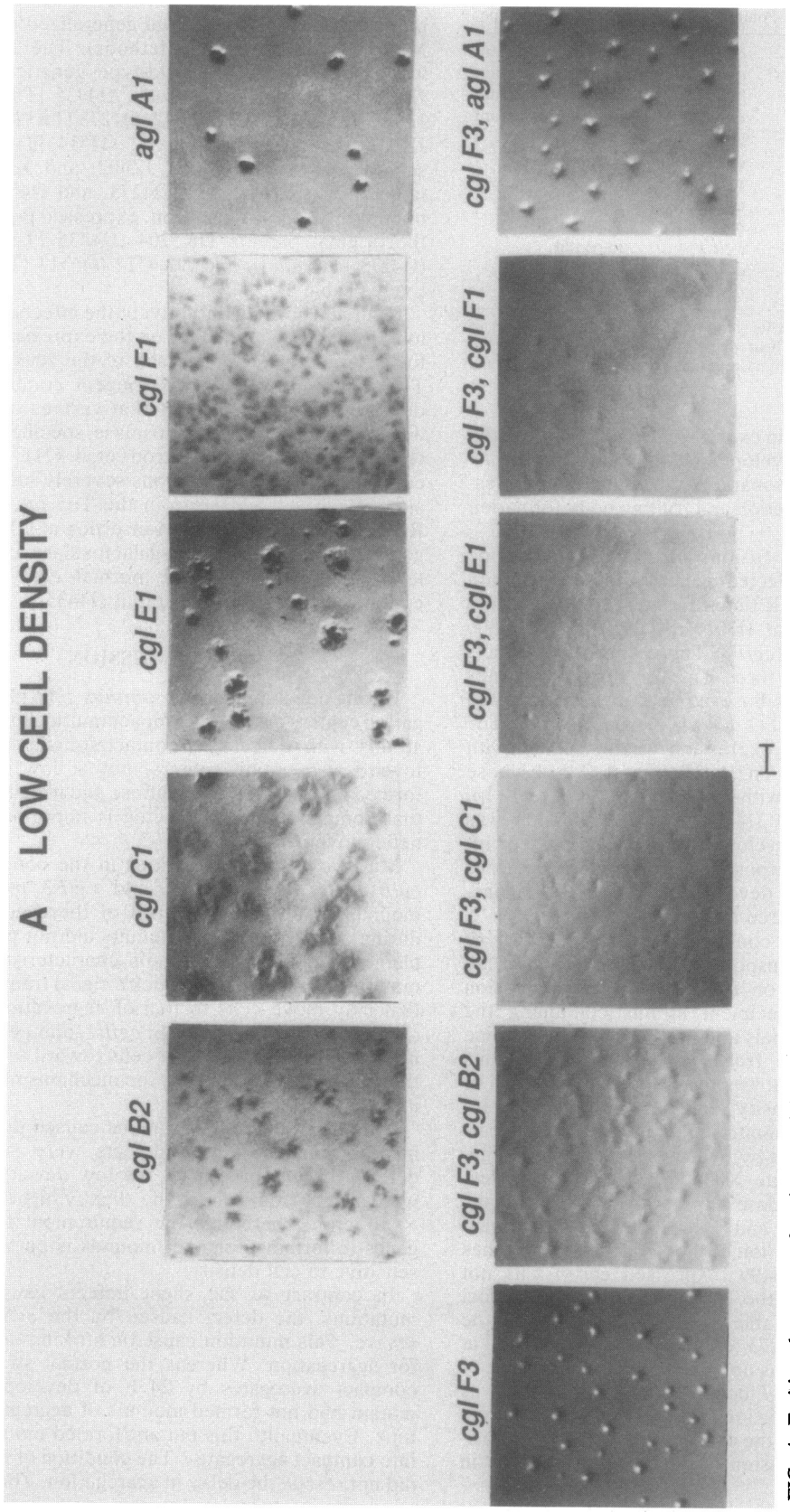

FIG. 4. Fruiting phenotypes of strains containing single *cgl* point mutations and Tn*phoA* insertions and strains containing two *cgl* mutations. Panels A and B show assays at low and high cell densities, respectively, after 8 days of development for *cglB2* (strain DK1218), *cglC1* (strain DK1219), *cglE1* (strain DK1230), *cglF1* (strain DK1234), *aglA1* (strain DK1215), *cglF3* (strain JZ334), *cglF3 cglB2* (strain JZ377), *cglF3 cglC1* (strain JZ378), *cglF3 cglE1* (strain JZ379), *cglF3 cglF1* (strain JZ381), and *cglF3 aglA1* (strain JZ382). Bar, 50 μm.

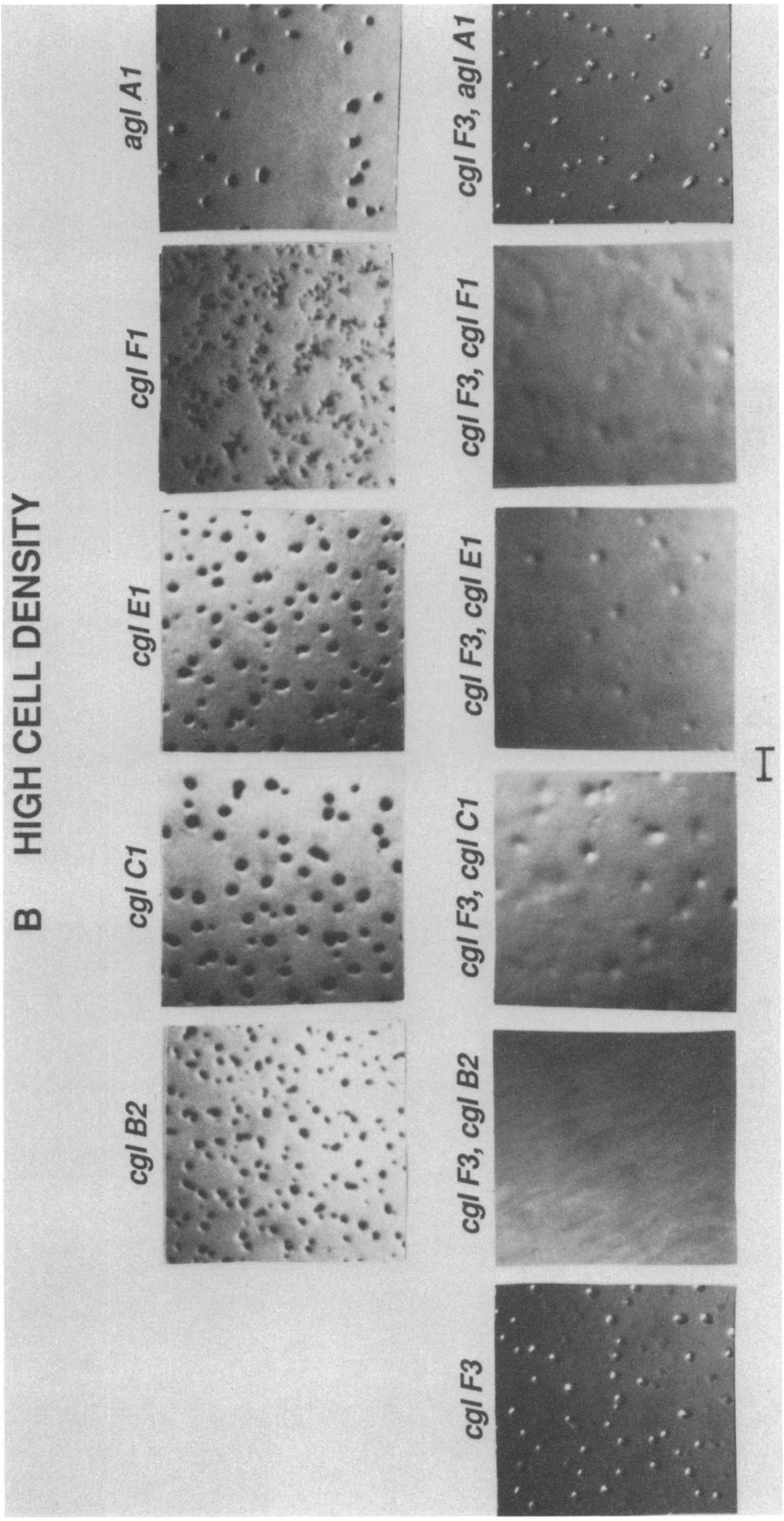

FIG. 4—*Continued*

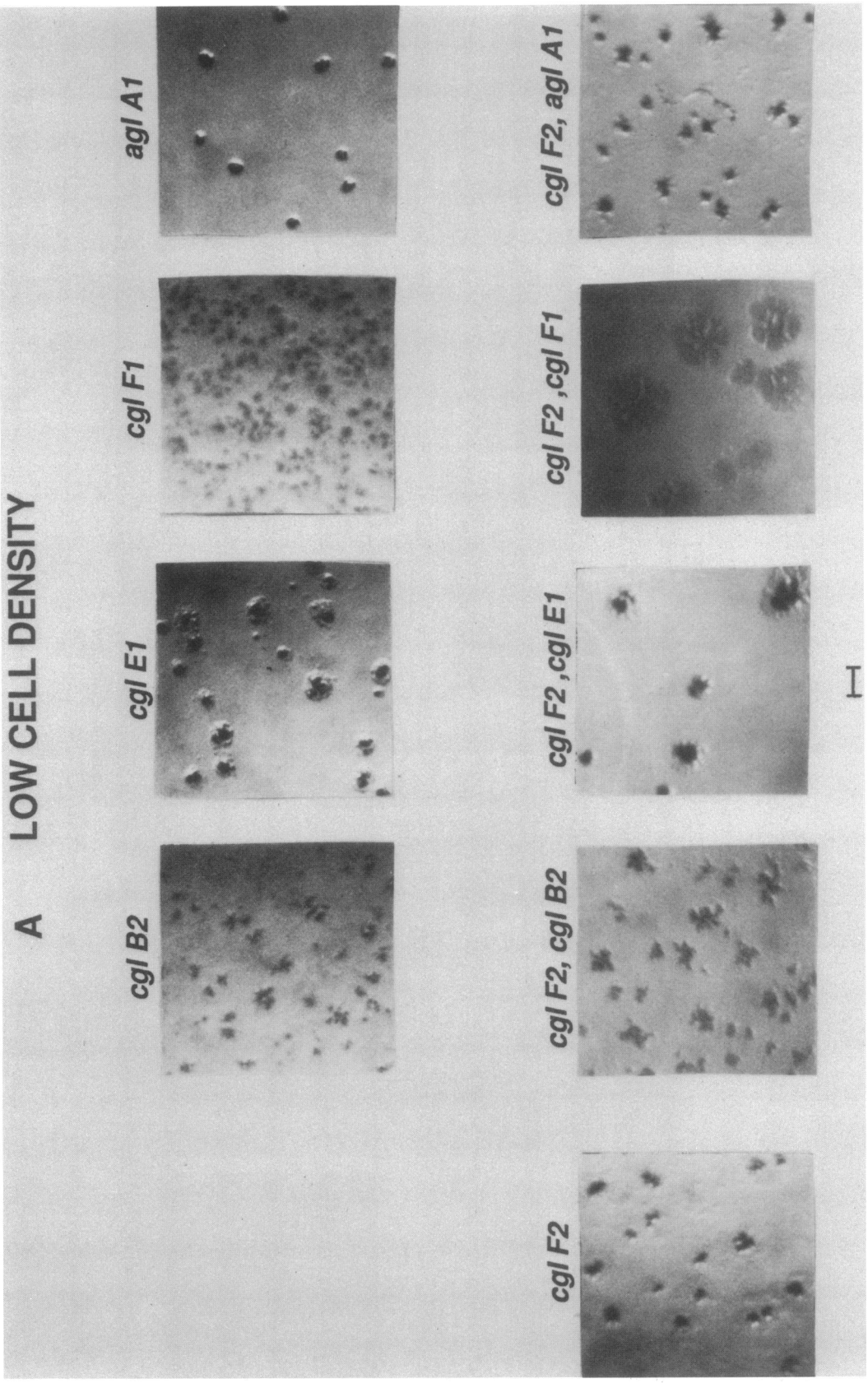

FIG. 5. Fruiting phenotypes of strains containing single *cgl* point mutations and Tn*phoA* insertions and strains containing two *cgl* mutations. Panels A and B show assays at low and high cell densities, respectively, after 8 days of development for *cglB2* (strain DK1218), *cglE1* (strain DK1230), *cglF1* (strain DK1234), *aglA1* (strain DK1215), *cglF2* (strain JZ302), *cglF2 cglB2* (strain JZ369), *cglF2 cglE1* (strain JZ370), *cglF2 cglF1* (strain JZ372), and *cglF2 aglA1* (strain JZ383). Bar, 50 μm.

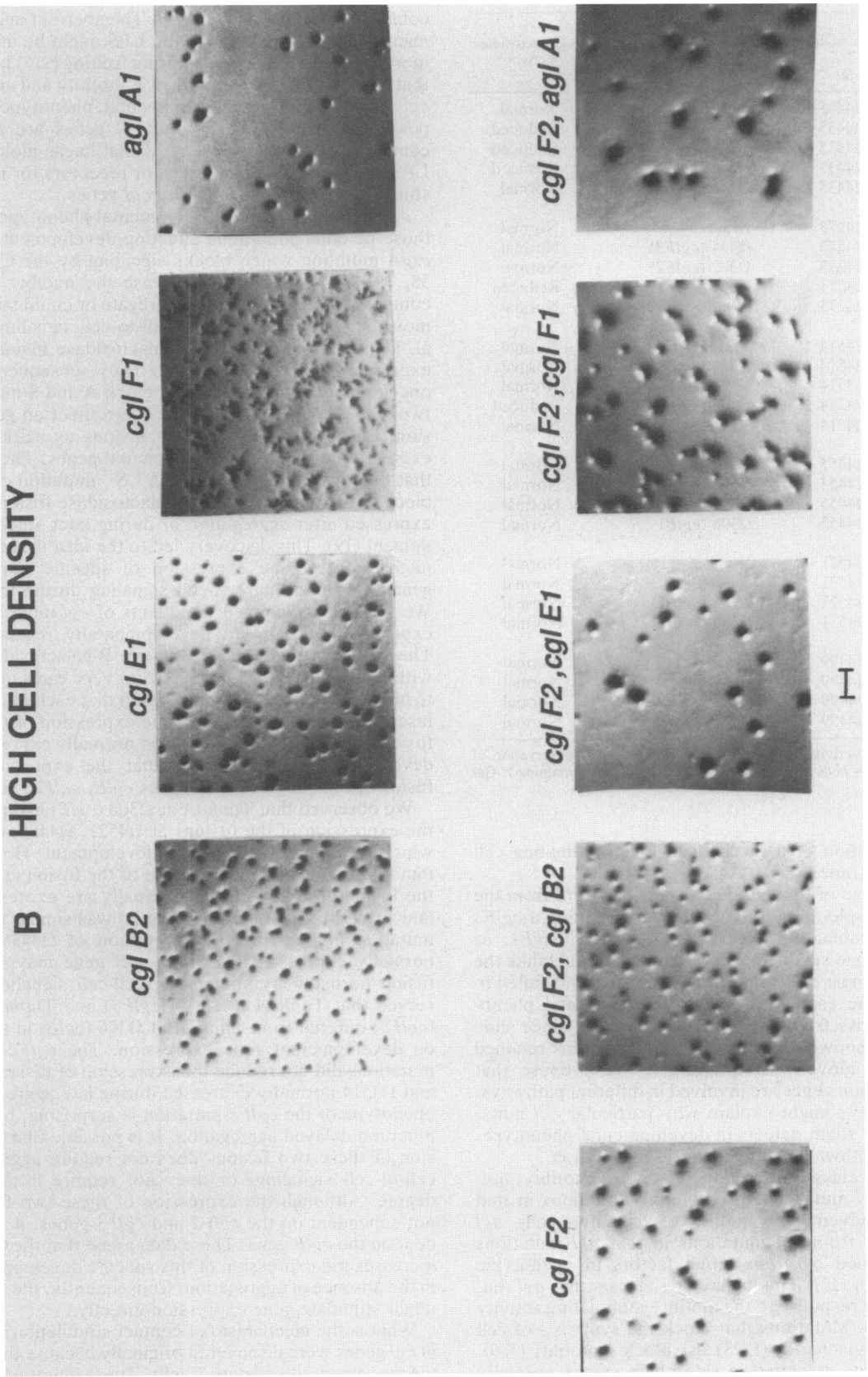

FIG. 5—*Continued*

TABLE 3. Effects of *cgl* mutations on expression of Tn*5 lac* fusions

Strain	Insertions		β-Galactosidase activity[a]
	Tn*5 lac*	Tn*phoA*	
DK5204	Ω4435	None	Normal
JZ396	Ω4435	Ω334 (*cglF3*)	Reduced
JZ397	Ω4435	Ω302 (*cglF2*)	Reduced
JZ398	Ω4435	Ω306 (*cglB*)	Reduced
JZ403	Ω4435	None	Normal
DK4290	Ω4273	None	Normal
JZ387	Ω4273	Ω334 (*cglF3*)	Normal
JZ388	Ω4273	Ω302 (*cglF2*)	Normal
JZ389	Ω4273	Ω306 (*cglB*)	Reduced
JZ402	Ω4273	None	Normal
DK4514	Ω4514	None	Normal
JZ390	Ω4514	Ω334 (*cglF3*)	Normal
JZ391	Ω4514	Ω302 (*cglF2*)	Normal
JZ392	Ω4514	Ω306 (*cglB*)	Reduced
JZ404	Ω4514	None	Normal
DK5206	Ω4455	None	Normal
JZ399	Ω4455	Ω334 (*cglF3*)	Normal
JZ400	Ω4455	Ω302 (*cglF2*)	Normal
JZ401	Ω4455	Ω306 (*cglB*)	Normal
DK4521	Ω4521	None	Normal
JZ393	Ω4521	Ω334 (*cglF3*)	Normal
JZ394	Ω4521	Ω302 (*cglF2*)	Normal
JZ395	Ω4521	Ω306 (*cglB*)	Normal
DK4499	Ω4499	None	Normal
JZ384	Ω4499	Ω334 (*cglF3*)	Normal
JZ385	Ω4499	Ω302 (*cglF2*)	Normal
JZ386	Ω4499	Ω306 (*cglB*)	Normal

[a] β-Galactosidase activity was determined by 10 days of observation of color development by cells developing on TPM agar plates containing X-Gal at 40 μg/ml.

early in aggregation or a mechanism for coordinating cell behavior at this time.

The critical role of the *cglF3* gene was apparent from the analysis of multiple mutations. Mutants containing a *cglF3* mutation in combination with a *cglB2*, *cglC1*, *cglE1*, or *cglF1* mutation had severe defects in aggregation. Unlike the mutant with a single *cglF3* mutation, these mutants failed to recover from the early delay. The developmental phenotypes did not arise from a change in S motility, since slide motility assays showed that double mutant strains retained their ability to move with S motility. We propose that contact stimulation genes are involved in different pathways. Multiple pathways might explain why particular *cgl* mutations cause only slight defects in developmental phenotype. Together, the pathways are critical for aggregation.

At least three classes of mutations affect A motility: *agl*, *cgl*, and *lps*. *agl* mutations resemble *cgl* mutations in that they block the adventurous motility of vegetative cells. *agl* mutations differ from *cgl* mutations in that *agl* mutations cannot be rescued by *trans*-acting factors in phenotypic complementation (13). This behavior suggests that *agl* mutations affect the response to the motility-stimulating activity of the *cgl* genes. Mutations that block the synthesis of cell surface lipopolysaccharide (LPS) also block A motility (7–9). Many *lps* mutants defective in side chain sugars normally attached to the O antigen region of LPS are aberrant in developmental aggregation. Three mutants defective in side chain sugars normally attached to the core region of LPS fail completely to form mounds (9). The aberrant aggregation of many *lps* mutants suggests that LPS might be important for morphogenetic movements during fruiting (9). The discovery that *lps* mutants are defective in A motility and are similar to *cgl* mutants in their developmental phenotype raises the possibility that *lps* genes and *cgl* genes are involved in common pathways. We suggest that sugar moieties within LPS might serve as the targets or receptors for the contact-stimulating factors encoded by *cgl* genes.

cgl mutations confer developmental phenotypes similar to those of other mutations affecting development, such as a *csgA* mutation which blocks signaling by the C-signal (18, 38, 39). *cgl* genes could increase the number of cell-cell contacts within the forming aggregate or could stimulate cell movement so as to increase cell-to-cell signaling. Kroos et al. identified a number of β-galactosidase fusions to genes expressed during development. They subsequently studied one-step *mgl* mutations (which block A and S motility), and two-step A^- S^- mutations, for their effect on gene expression, by using β-galactosidase fusions as markers for the expression of specific developmental genes. They observed that the *mgl* mutation and the A^- S^- mutation combination blocked the expression of β-galactosidase fusions normally expressed after aggregation or during later stages of development (19). This discovery led to the idea that cell motility might increase the expression of specific developmental genes by increasing cell-cell signaling during fruiting (19). We therefore examined the effects of *cgl* mutations on the expression of different developmentally regulated genes. The markers for these genes were β-galactosidase fusions with expression times ranging from very early development to late development. We discovered that each of the Tn*phoA* insertions at *cgl* loci blocked the expression of the β-galactosidase fusion at Ω4435, a fusion normally expressed late in development. This indicates that the expression of this fusion specifically requires genes *cglB*, *cglF2*, and *cglF3*.

We observed that Tn*phoA* at Ω306 (*cglB*) had no effect on the expression of the fusions at Ω4521, Ω4455, and Ω4499, which were expressed early in development. However, this mutation reduced the expression of the fusion at Ω4273 and the fusion at Ω4514, which normally are expressed during late aggregation. The *cglB* mutation was similar to the *csgA* mutation in blocking the expression of Ω4435, which is normally expressed late. The *cglB* gene may induce this fusion normally by stimulating cell-cell signaling. We observed that Tn*phoA* at Ω334 (*cglF3*) and Tn*phoA* at Ω302 (*cglF2*) differed from Tn*phoA* at Ω306 (*cglB*) in their effects on developmental gene expression. The *cglF2* and *cglF3* mutations did not reduce the expression of fusions at Ω4273 and Ω4514 normally expressed during late aggregation. The phenotype of the *cglF3* mutation is surprising, because this mutation delayed aggregation. It is possible that the expression of these two fusions does not require aggregation (or cell-to-cell signaling) or does not require it to the same degree. Although the expression of these two fusions was not dependent on the *cglF2* and *cglF3* genes, it was dependent on the *cglB* gene. These data argue that the CglB factor increases the expression of this specific developmental gene in the absence of aggregation. Consequently, the CglB factor might stimulate gene expression directly.

What is the mechanism of contact stimulation? Mutations in *cgl* genes were discovered originally because they affected the movement of vegetative cells. These mutations block the motility of single cells, which move out a short distance from

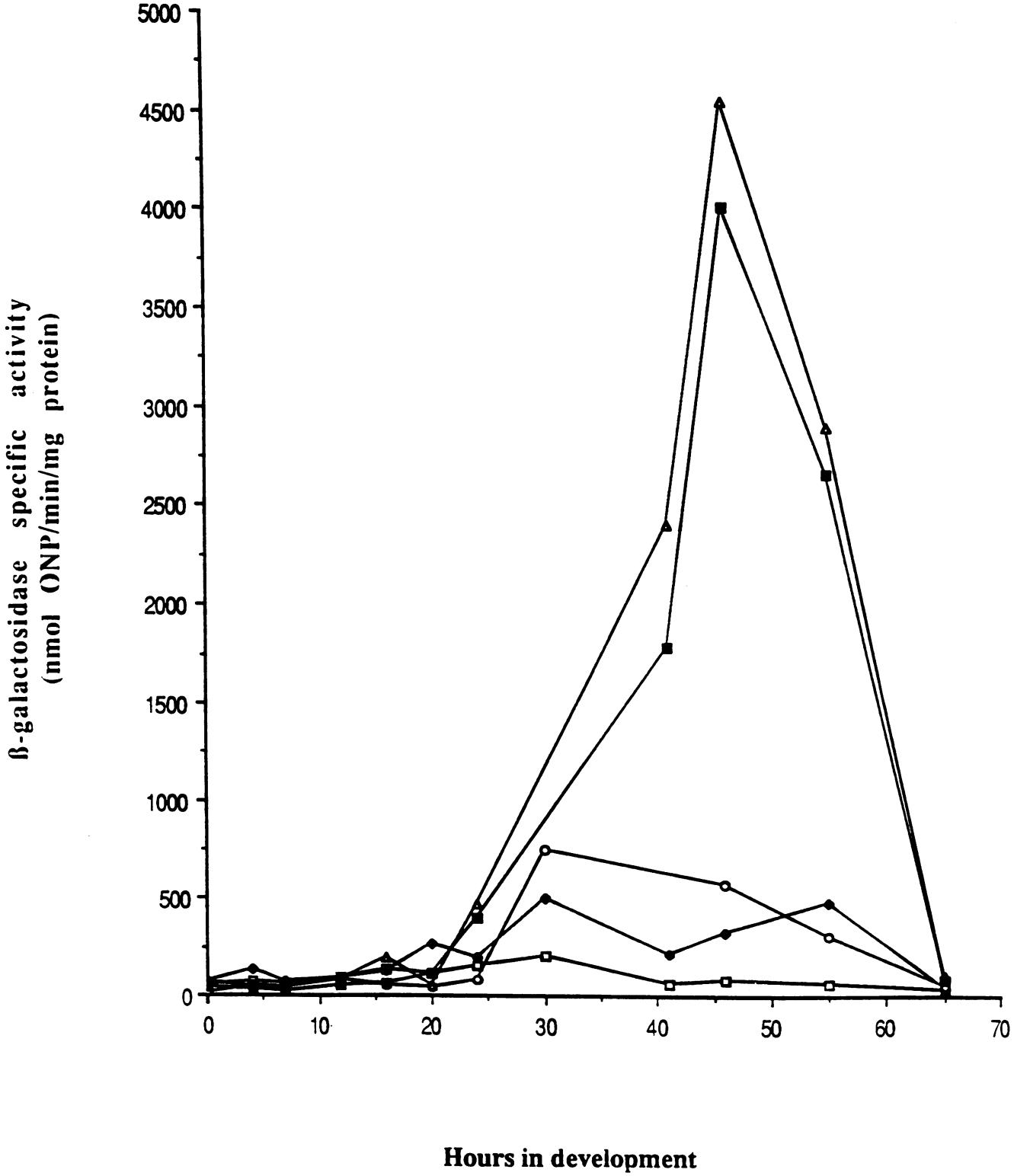

FIG. 6. β-Galactosidase specific activity produced during development by mutants containing Tn*5 lac* at Ω4435 and double mutants containing Tn*5 lac* at Ω4435 and Tn*phoA* at different *cgl* loci. Symbols: □, JZ396 (Ω334 [*cglF3*::Tn*phoA*], Ω4435 [Tn*5 lac*]); ◆, JZ397 (Ω302 [*cglF2*::Tn*phoA*], Ω4435 [Tn*5 lac*]); ○, JZ398 (Ω306 [*cglB*::Tn*phoA*], Ω4435 [Tn*5 lac*]); △, JZ403 (Ω4435 [Tn*5 lac*]); and ■, DK5204 (Ω4435 [Tn*5 lac*]). ONP, *o*-Nitrophenol.

the edge of an expanding swarm by adventurous gliding (17). Mutants defective in A motility are stimulated to move with A motility through contacts between neighboring cells or through signal exchange involving nearby cells (13). If these components are regulatory in nature, they might function either as cell-to-cell signals or as extracellular matrix molecules to control cell movement. We have shown that contact-stimulated gliding is critical to developmental aggregation, which is a process involving oriented cell movement. This raises the possibility that contact stimulation affects the direction of cell movement within the forming aggregate.

In an analysis of aggregation by the related organism *Stigmatella aurantiaca*, Vasquez et al. (41) discovered that cells move cooperatively in vortices and streams. Cells appear to move in circles or spirals, where aggregates begin to form. Raised mounds emerge from early aggregates by changes in the direction of cell movement. O'Connor and Zusman characterized cell movement by *M. xanthus* during aggregation and showed that cells move during early aggregation within a spiral pattern (30). At low cell density, cells may move in circles, not spirals. Normally, cells within the spiral pattern are closely packed and arrayed within a monolayer; monolayers become stacked as the mound forms, and cells within different monolayers move in nonaligned spiral patterns as if the monolayers were independent (30). The arrangement of cells in spirals or sheets and the stacking of monolayers to form terraces could be coincident with the formation of different contacts between cells. These cell-cell contacts could involve molecules specific for end-end or side-side interactions (30).

Since contact stimulation is critical within the forming aggregate, stimulation might direct cell movement within spirals or within monolayers as cells form terraces. Also, contact stimulation might mediate cooperative behavior. Either of these activities might involve specific contacts between cells or contacts between cells and the extracellular matrix. Multiple factors with different specificities could provide the mechanism for forming different types of contacts or signals (13). We have shown that cell movement during aggregation is subject to genetic dissection and that different *cgl* genes are critical for morphogenetic movements. This could provide a way for determining how cell-cell contacts or signals stimulate cell behavior.

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LITERATURE CITED

1. **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:** 8767–8776.
2. **Burchard, R. P.** 1981. Gliding motility of prokaryotes: ultrastructure, physiology, and genetics. Annu. Rev. Microbiol. **35:**497–529.
3. **Burchard, R. P., A. C. Burchard, and J. A. Kloetzel.** 1977. Intracellular, periodic structures in the gliding bacterium *Myxococcus xanthus*. J. Bacteriol. **132:**666–672.
4. **Doetsch, R. N., and G. J. Hageage.** 1968. Motility in prokaryotic organisms: problems, points of view and perspectives. Biol. Rev. **43:**317–362.
5. **Dworkin, M.** 1962. Nutritional requirements for vegetative growth of *Myxococcus xanthus*. J. Bacteriol. **84:**250–257.
6. **Dworkin, M. D., K. Keller, and D. Weisberg.** 1983. Experimental observations consistent with a surface tension model of gliding motility of *Myxococcus xanthus*. J. Bacteriol. **155:**1367–1371.
7. **Fink, J. M., M. Kalos, and J. F. Zissler.** 1989. Isolation of cell surface antigen mutants of *Myxococcus xanthus* by use of monoclonal antibodies. J. Bacteriol. **171:**2033–2041.
8. **Fink, J. M., and J. F. Zissler.** 1989. Characterization of lipopolysaccharide from *Myxococcus xanthus* by use of monoclonal antibodies. J. Bacteriol. **171:**2028–2032.
9. **Fink, J. M., and J. F. Zissler.** 1989. Defects in motility and development of *Myxococcus xanthus* lipopolysaccharide mutants. J. Bacteriol. **171:**2042–2048.
10. **Gill, J., E. Stellwag, and M. Dworkin.** 1985. Monoclonal antibodies against cell-surface antigens of developing cells of *Myxococcus xanthus*. Ann. Microbiol. (Paris) **136A:**11–18.
11. **Gill, J. S., and M. Dworkin.** 1986. Cell surface antigens during submerged development of *Myxococcus xanthus* examined with monoclonal antibodies. J. Bacteriol. **168:**505–511.
12. **Henrichsen, J.** 1962. Bacterial surface translocation: a survey and a classification. Microbiol. Rev. **36:**478–503.
13. **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.
14. **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.
15. **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.
16. **Kaiser, D.** 1979. Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76:** 5952–5956.
17. **Kaiser, D., and C. Crosby.** 1983. Cell movement and its coordination in swarms of *Myxococcus xanthus*. Cell Motil. **3:**227–245.
18. **Kim, S. K., and D. Kaiser.** 1990. C-factor: a cell-cell signaling protein required for fruiting body morphogenesis of *M. xanthus*. Cell **61:**19–26.
19. **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 signaling during fruiting body development. Genes Dev. **2:**1677–1685.
20. **Kroos, L., and D. Kaiser.** 1987. Expression of many developmentally induced genes in *Myxococcus* depends on a sequence of cell interactions. Genes Dev. **1:**840–854.
21. **Kroos, L., A. Kuspa, and D. Kaiser.** 1986. A global analysis of developmentally regulated genes in *Myxococcus xanthus*. Dev. Biol. **117:**252–266.
22. **Kuner, J., and D. Kaiser.** 1982. Fruiting body morphogenesis in submerged cultures of *Myxococcus xanthus*. J. Bacteriol. **151:** 458–461.
23. **Lapidus, I. R., and H. C. Berg.** 1982. Gliding motility of *Cytophaga* sp. strain U67. J. Bacteriol. **151:**384–398.
24. **Lusdorf, H., and H. Reichenbach.** 1989. Ultrastructural details of the apparatus of gliding motility of *Myxococcus fulvus* (Myxobacterales). J. Gen. Microbiol. **135:**1633–1641.
25. **Manoil, C., and J. Beckwith.** 1985. Tn*phoA*: a transposon probe for protein export sequences. Proc. Natl. Acad. Sci. USA **82:**8129–8133.
26. **Martin, S., E. Sodergren, T. Masuda, and D. Kaiser.** 1978. Systematic isolation of transducing phages for *Myxococcus xanthus*. Virology **88:**44–53.
27. **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.
28. **Mitchell, P.** 1956. Hypothetical thermokinetic and electrokinetic mechanisms of locomotion in micro-organisms. Proc. R. Phys. Soc. **25:**32–34.
29. **Munoz, J., F. Gonzalez, M. M. Martinez-Canamero, M. Angeles Goicoechea, A. L. Extremera, and J. M. Arias.** 1989. Deoxyribonuclease and phosphatase activities in myxobacteria. Microbios **58:**43–47.
30. **O'Connor, K. A., and D. R. Zusman.** 1990. Patterns of cellular

interactions during fruiting-body morphogenesis in *Myxococcus xanthus*. J. Bacteriol. **171**:6013–6024.
31. **Pate, J. L., and L. Y. E. Chang.** 1979. Evidence that gliding motility in prokaryotic cells is driven by rotary assemblies in the cell envelopes. Curr. Microbiol. **2**:59–64.
32. **Reichenbach, H.** 1966. Myxococcus spp. (Myxobacterales) Schwarmentwicklung und bildung von protocysten. *In* G. Wolf (ed.), Encyclopedia Cinematographica, film E778/1965. Institut Wissenschaft Film, Göttingen, Federal Republic of Germany.
33. **Reichenbach, H.** 1966. Rhythmsche vorgange bei der schwarmenfatung von Myxobacterien. Ber. Dtsch. Bot. Ges. **78**:102–105.
34. **Reichenbach, H., and M. Dworkin.** 1981. The order Myxobacterales, p. 328–355. *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes: a handbook on habitats, isolation, and identification of bacteria. Springer-Verlag, Berlin.
35. **Shimkets, L. J.** 1987. Control of morphogenesis in myxobacteria. Crit. Rev. Microbiol. **14**:195–227.
36. **Shimkets, L. J.** 1986. Correlation of energy-dependent cell cohesion with social motility in *Myxococcus xanthus*. J. Bacteriol. **166**:837–841.
37. **Shimkets, L. J.** 1986. Role of cohesion in *Myxococcus xanthus* fruiting-body formation. J. Bacteriol. **166**:842–848.
38. **Shimkets, L. J., R. E. Gill, and D. Kaiser.** 1983. Developmental cell interactions in *Myxococcus xanthus* and the *spoC* locus. Proc. Natl. Acad. Sci. USA **80**:1406–1410.
39. **Shimkets, L. J., and D. Kaiser.** 1982. Induction of coordinated movements in *Myxococcus xanthus*. J. Bacteriol. **152**:451–461.
40. **Sodergren, E., and D. Kaiser.** 1983. Insertion of Tn*5* near genes that govern stimulatable cell motility in *M. xanthus*. J. Mol. Biol. **167**:295–310.
41. **Vasquez, G. M., F. Qualls, and D. White.** 1985. Morphogenesis of *Stigmatella aurantiaca* fruiting bodies. J. Bacteriol. **163**:515–521.
42. **Weinberg, R. A., and D. R. Zusman.** 1990. Alkaline, acid, and neutral phosphatase activities are induced during development in *Myxococcus xanthus*. J. Bacteriol. **172**:2294–2302.
43. **Weinberg, R. A., and D. R. Zusman.** 1989. Evidence that *Myxococcus xanthus frz* genes are developmentally regulated. J. Bacteriol. **171**:6174–6186.
44. **Zusman, D. R.** 1982. Frizzy mutants: a new class of aggregation-defective developmental mutants of *Myxococcus xanthus*. J. Bacteriol. **150**:1430–1437.