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Genetics

Transposon tagging of genes for cell–cell interactions in *Myxococcus xanthus*

(Tn*phoA*/cell signaling/motility/cell surface)

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ABSTRACT **The prokaryote *Myxococcus xanthus* is a model for cell interactions important in multicellular behavior. We used the transposon Tn*phoA* to specifically identify genes for cell-surface factors involved in cell interactions. From a library of 10,700 insertions of Tn*phoA*, we isolated 36 that produced alkaline phosphatase activity. Three Tn*phoA* insertions tagged cell motility genes, called *cgl*, which control the adventurous movement of cells. The products of the tagged *cgl* genes could function in trans upon other cells and were localized primarily in the cell envelope and extracellular space, consistent with Tn*phoA* tagging genes for extracellular factors controlling motility.**

Multicellular organisms are dependent on many molecules to communicate messages between cells. These molecules could include developmental factors guiding cell migration, growth factors stimulating cell proliferation and maturation, and hormones for regulation. Even though these molecules differ widely in their specific functions, they share certain fundamental properties because they promote cell communication. Almost without exception, they are exported to the cell surface or beyond.

A genetic approach has been developed in prokaryotes that uses a transposon termed Tn*phoA* (1) to specifically detect genes coding for cell envelope proteins. Tn*phoA* is the transposon Tn*5* with *phoA* the gene for alkaline phosphatase (PhoA) from *Escherichia coli* inserted at the left end of the transposon. All DNA sequences for regulation and export have been deleted from the *phoA* gene. The potential for studying cell surface proteins with Tn*phoA* depends on the critical fact that the *phoA* gene product requires export to the periplasm of cells or beyond to be enzymatically active (2, 3). Therefore, the detection of PhoA enzyme activity requires fusion of *phoA* to upstream sequences that allow export. This could occur if Tn*phoA* inserts into a gene encoding a product normally exported to the cell surface or to the periplasm. Tn*phoA* has been used to determine the membrane localization of specific protein sequences and to detect genes coding for exported proteins (4–7).

We used Tn*phoA* as a probe to identify genes for cell interactions and cell-to-cell signaling in *Myxococcus xanthus*, a prokaryotic model for multicellular behavior. Under specific conditions, *M. xanthus* initiates a complex developmental cycle that requires coordinated cell movement and culminates in the production of a multicellular fruiting body containing spores (8). During vegetative growth, cells feed by moving in large groups known as swarms (9). A swarm moves as a coherent unit, implying that cells have cooperative behavior. Hodgkin and Kaiser (10) have proposed that myxobacteria stimulate each other to move by the exchange of signals between cells in contact.

In this report we describe the use of Tn*phoA* to identify genes encoding molecules involved in cell interactions. By utilizing Tn*phoA*, we identified genes (*cgl* genes) whose products are thought to be involved in cell-to-cell signaling important for the motility of this organism (10). We showed that the *cgl* genes tagged by Tn*phoA* are clustered on the chromosome where the *cgl* loci map, and we found that the Cgl–PhoA fusion proteins (and by extrapolation the corresponding Cgl molecules) are extracellular, where they can act as effector molecules controlling the motility of cells.

MATERIALS AND METHODS

Bacterial Growth. *M. xanthus* strains were grown in liquid CT medium (11) or on CTT plates (12). *E. coli* strains were grown in L broth (13) at 37°C.

Introduction of Tn*phoA* into *M. xanthus*. Tn*phoA* was introduced into *M. xanthus* by conjugation with *E. coli*. The conjugation procedure was adapted from Breton *et al.* (14). *M. xanthus* JZ007 was grown to 1×10^9 cells per ml in liquid CT broth. The *E. coli* strain SM10 (15) harboring the conjugative plasmid pRT291 (16) was grown in L broth to midlogarithmic phase. Transconjugants were selected on a CTT plate containing kanamycin monosulfate at 50 μg/ml and ampicillin at 50 μg/ml, to counterselect the donor *E. coli* (*M. xanthus* is naturally resistant to these levels of ampicillin).

Screen for Strains Producing an Active PhoA. Tn*phoA* mutants were picked into wells of 96-well tissue culture plates containing 100 μl of CT broth supplemented with kanamycin (50 μg/ml) and ampicillin (50 μg/ml) and grown at 32°C, with slow shaking for 3–5 hr. Samples of 1–5 μl from the wells were spotted onto fresh thinly poured CTT plates. The plates were dried briefly under a hood and were incubated at 32°C for 2–3 days. PhoA-producing strains were detected based on a procedure described by Lazzaroni and Portalier (17), using naphthyl acid phosphate, monosodium or monopotassium salt at 7 mg/ml, and dianisidine (tetrazotized, zinc chloride complex, fast blue B salt; Sigma D-3502) at 7 mg/ml.

Oxytetracycline Replacement Strain Generation. The kanamycin-resistance determinant on Tn*phoA* was replaced by the oxytetracycline-resistance determinant on P1:Tn*5–132* as described (18).

Lysate Generation, Generalized Transduction. Mx4 lysates on *M. xanthus* strains were generated by standard techniques at 28°C (19). Transduction of *M. xanthus* strains with Mx4 lysates was performed using standard techniques at 32°C (20).

Molecular Biological Techniques. DNA isolation (19), nick-translation (21), and Southern transfer and hybridization (22) were performed as described.

Slide-Motility Assay and Motility-Stimulation Assay. These assays were adopted from Hodgkin and Kaiser (10). Two microliters containing 5×10^8 cells per ml was spotted onto

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Abbreviations: A, adventurous; S, social; PhoA, alkaline phosphatase.

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microscope slides that had been overlaid with 1 ml of CTT agar [1.5% (wt/vol) Bacto Agar], and the slides were incubated at 32°C in a humidified chamber. Colony edges were examined microscopically over a period of 2–20 hr for the presence of adventurous (A) motile cells.

Cell Fractionation. Cells were grown to logarithmic phase and diluted to 1×10^7 cells per ml. This suspension at 0.3 ml/cm^2 was added to tissue culture flasks (Corning). Flasks were incubated for 24 hr at 32°C with no shaking. Under these conditions, cells grow on the surface of the tissue culture flasks. The supernatant was removed, centrifuged at 10,000 $\times$ *g* for 20 min, and passed through a 0.22-μm pore-size filter (supernatant fraction). The periplasmic fraction was isolated based on a procedure by Nelson *et al.* (23). The membrane fraction was separated from the cytoplasmic fraction based on a procedure by Manoil and Beckwith (24), with an additional centrifugation step at 100,000 $\times$ *g* for 1 hr to ensure a more thorough separation of the two fractions. Phosphoramidon at 10 μg/ml was used in most steps as a protease inhibitor. Isocitrate dehydrogenase (EC 1.1.1.41), a cytoplasmic enzyme in *M. xanthus* (25), was assayed based on a procedure by Watson and Dworkin (25), using 4×10^7 cell equivalents from each fraction.

Quantitation of PhoA Enzyme Activity in Cell Fractions. Approximately 1×10^7 cell equivalents (1.2×10^6 or 5×10^6 cell equivalents for the supernatant cell fractions) were added to wells of a 96-well microtiter plate, in 1 M NaCl/1 M Tris, pH 9.6/2 mM $MgCl_2$/1 mM *p*-nitrophenyl phosphate. The plates were incubated at 37°C until sufficient color developed, for a maximum of 3 hr, and enzyme activity was determined by hydrolysis of the PhoA substrate, *p*-nitrophenyl phosphate, as measured by the absorbance of the wells at 405 nm. The results for the excreted fractions were adjusted to 1×10^7 cell equivalents. Hydrolysis of the substrate was linear with respect to the concentration of the PhoA enzyme for 3 hr.

Determination of Linkage Among Loci. Linkage between loci was determined by detecting loss of antibiotic resistance markers due to homologous recombination after transduction. The distance in kilobases between loci showing linkage was determined using the equation of Wu (26).

RESULTS

Isolation of Tn*phoA* Mutants. We first generated 10,700 strains of *M. xanthus* JZ007 containing the transposon Tn*phoA* at random chromosomal positions. In other systems where Tn*phoA* has been used to identify active PhoA fusions, cells containing Tn*phoA* insertions were screened *in vivo* for PhoA enzymatic activity by including a PhoA substrate in the growth medium (4–7, 16). In these systems, 1–2% of the Tn*phoA* insertion mutants exhibited PhoA enzyme activity (7, 16). Since all of the PhoA substrates we tested were inhibitory to the growth of *M. xanthus*, we devised an *in vitro* scheme to identify mutants that produced enzymatically active PhoA fusion proteins. By using this scheme on the total of 10,700 strains, we identified a set of 36 strains whose products potentially were exported into the cell envelope or extracellular space. This number represented ≈0.3% of the total Tn*phoA* mutant population.

Three of the 36 strains were mutants defective in motility. The three motility mutants, JZ302, JZ306, and JZ315, contained Tn*phoA* at loci designated Ω302, Ω306, and Ω315, using the symbol Ω to refer to the insertion site. These motility mutants and a fourth motility mutant, JZ334, containing Tn*phoA* at locus Ω334 (isolated by Annick Breton, Laboratory de Genetique Microbienne, Universite de Compiegne, Compiegne, France), were studied further because Tn*phoA* at these loci appeared to identify genes encoding extracytoplasmic molecules important in normal cell motility.

Cotransduction of Tn*phoA*, Motility Phenotype, and PhoA Enzyme Activity. Transduction of Tn*phoA* insertions at Ω302, Ω306, and Ω334 into our reference strain JZ007 using the myxophage MX4 (27, 28) showed that the motility defects cotransduced with Tn*phoA*, indicating that the transposons acted as single mutations in causing the motility defects. Analysis of DNA from each strain using Tn*phoA* sequences as a probe confirmed the presence of a single Tn*phoA* element in each strain. Transduction of DNA from JZ315 into JZ007 showed that the motility defect in JZ315 cotransduced with Tn*phoA*. However, this genetic analysis also revealed the presence of two linked Tn*phoA* elements in JZ315. The second Tn*phoA* element in JZ315 did not produce an active PhoA fusion protein nor did it affect motility. Analysis of DNA from JZ315 by DNA hybridization physically showed the presence of the two linked Tn*phoA* elements. Ω315 designates the insertion site for the Tn*phoA* affecting motility in this strain.

Motility Defects in JZ302, JZ306, JZ315, and JZ334. *M. xanthus* exhibits two types of motility, adventurous (A) and social (S). Motility A is the movement of single cells (29), and motility S is the movement of groups of cells (30). Motility behavior can be distinguished microscopically by observing the edge of a swarm. A fully motile (A^+S^+) strain shows both single cells and groups of cells moving at the colony edge (Fig. 1*a*). A mutant lacking motility S shows movement of single cells at the colony edge but not the thick rafts generated by motility S (Fig. 1*b*). A nonmotile mutant lacks motilities A and S, as the colony edge is totally smooth (Fig. 1*c*). Strains defective in their motility A system do not show single cell movement but do display the rafts of cells characteristic of a functional motility S system (Fig. 1 *d–f*). Like the A^-S^+ control mutants, the three Tn*phoA* mutants, JZ302, JZ306,

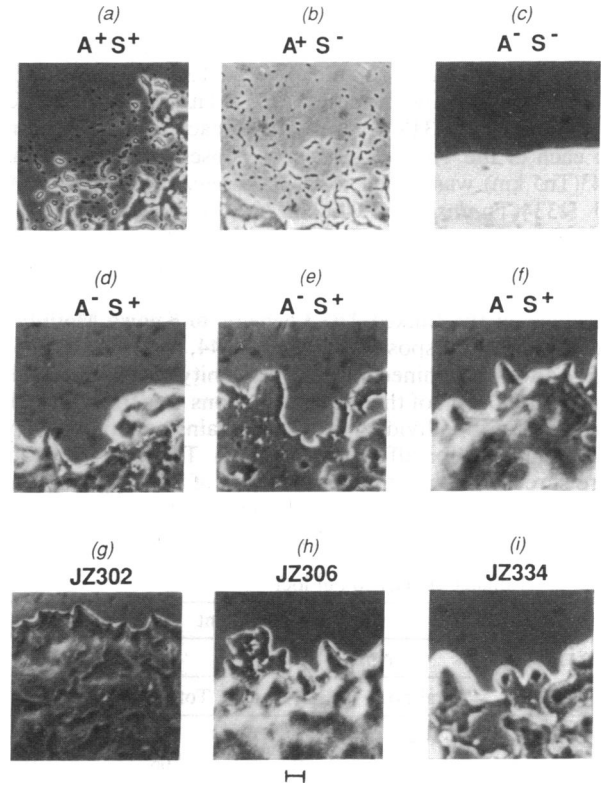

FIG. 1. Motility phenotypes of reference and motility defective strains. Micrographs show colony edges from slide-motility assays. (*a*) JZ007. (*b*) DK1300. (*c*) DK306. (*d*) DK1218 (*cglB*). (*e*) DK1230 (*cglE*). (*f*) DK1234 (*cglF*). (Bar = 50 μm.)

Table 1. Linkage of Tn5 to the loci defined by Tn*phoA*

	Recipient							
	Ω302		Ω306		Ω315		Ω334	
Donor	Total no.	% A	Total no.	% A	Total no.	% A	Total no.	% A
Ω343	158	0	318	3.5	219	2.7	138	6.5
Ω344	166	0	317	28	243	35	234	0.8
Ω346	304	86	114	0	256	0	224	0

Transductants were selected by resistance to kanamycin monosulfate. Total no., total number of transductants; % A, percent with a functional motility A system.

and JZ334, failed to show single cells moving out from the colony edge, indicating a defect in motility A (Fig. 1 *g–i*). All three showed the swarms characteristic of motility S. To critically test whether each transposon specifically affected the motility A system, each Tn*phoA* was transduced by generalized transduction using the myxophage Mx4 into strains with defects in the motility A or S system. When transduced into an A^+S^- strain, each transposon produced a totally nonmotile (A^-S^-) colony. When transduced into an A^-S^+ strain, each transposon failed to change the motility behavior. Therefore, each transposon specifically affected motility A but not motility S. The Tn*phoA* insertion at Ω315 also affected only motility A (data not shown).

Isolates of Tn5 Linked to the Tn*phoA* Insertion Sites. To study the linkage between the Tn*phoA* mutations and known motility A loci (29), we isolated wild-type Tn5 transposons linked to the Tn*phoA* loci. First, the kanamycin-resistance determinant in each Tn*phoA* was replaced by an oxytetracycline-resistance determinant carried on P1:Tn*5–132*. Then, each of the oxytetracycline-resistant mutants was transduced to kanamycin resistance using a Tn5 lysate grown on a pool of 960 Tn5 mutants of JZ007. Transductants were screened visually for restoration of motility A. The loss of PhoA enzyme activity and the loss of the oxytetracycline-resistant phenotype confirmed the replacement of the sequences contained in the Tn*phoA* element. By using this strategy, we identified linked Tn5 at loci designated Ω343, Ω344, and Ω346. By transducing each of the linked Tn5 into each of the Tn*phoA* strains, we observed (Table 1) that Ω343(Tn5,km) was linked to Ω306(Tn*phoA*), Ω315(Tn*phoA*), and Ω334(Tn*phoA*). Ω344(Tn5,km) also was linked to Ω306(Tn*phoA*), Ω315(Tn*phoA*), and Ω334(Tn*phoA*). Ω346(Tn5,km) was linked to Ω302(Tn*phoA*) (km is kanamycin resistance).

Linkage of the Linked Tn5 Elements to Known Motility A Loci. The Tn5 transposons at Ω343, Ω344, and Ω346 linked to Tn*phoA* were examined for their proximity to known motility A loci. First, each of the Tn5 transposons was transferred by transduction to individual recipient strains containing a specific point mutation affecting motility A. Then, transductants were screened visually for restoration of wild-type motility. The restoration of wild-type motility in the transductants would indicate that the wild-type motility (A^+) locus from the donor was linked to the Tn5 selected. We discovered (Table 2) that Tn5 insertions at Ω343 and Ω344 were linked to the *cglB* locus of the motility A system. The Tn5 insertion at Ω346 was linked to the *cglC* locus of the motility A system. Ω343, Ω344, and Ω346 did not show linkage to the *cglD*, *cglE*, or *cglF* loci.

Table 2. Linkage of Tn5 to *cgl* loci

	Recipient			
	cglB		*cglC*	
Donor	Total no.	% A	Total no.	% A
Ω343	101	4.9	150	0
Ω344	45	40	108	0
Ω346	556	0	311	47

Transductants were selected by resistance to kanamycin monosulfate. % A, percent with a functional motility A system. The following strains were used as representatives of each *cgl* class: *cglB*, DK1218; *cglC*, DK1219.

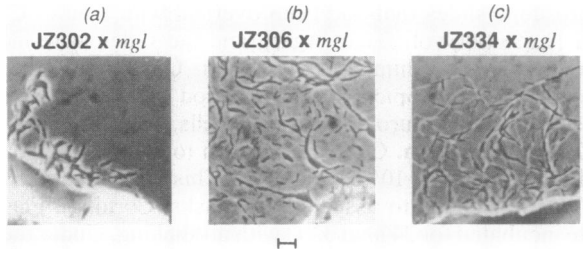

FIG. 2. Stimulation of motility A in Tn*phoA* mutants by an *mgl* mutant. Micrographs show the edge of spots containing various strain combinations from slide-motility assays. The *mgl* mutant used was DK306. (Bar = 50 μm.)

Motility Stimulation Phenotypes of JZ302, JZ306, JZ315, and JZ334. Motility A mutants can be separated into two broad classes: the *agl* (adventurous gliding) class that is comprised of 16 loci and the *cgl* (contact gliding) class, which is comprised of 5 loci, *cglB–cglF* (29). The basis for defining the *cgl* class of motility A mutants is a complementation test devised by Hodgkin and Kaiser (10) using mixtures of two motility A mutants. The *cgl* class of motility A mutants is transiently stimulated to move with motility A when they are in contact or very close proximity with cells that are wild-type for the particular *cgl* locus. The stimulation of motility may occur by a factor produced by a strain that complements the motility defect in the mutant. Hodgkin and Kaiser (10)

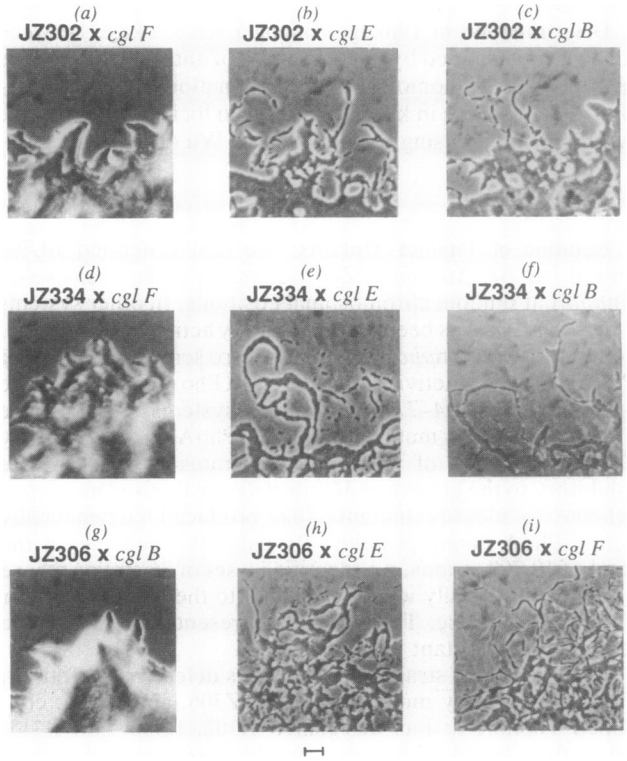

FIG. 3. Stimulation assays between Tn*phoA* mutants and known *cgl* mutants. Micrographs show the edge of spots containing the indicated strain combinations from slide-motility assays. The *cglF* strain was DK1234; the *cglE* strain was DK1230; the *cglB* strain was DK1218. (Bar = 50 μm.)

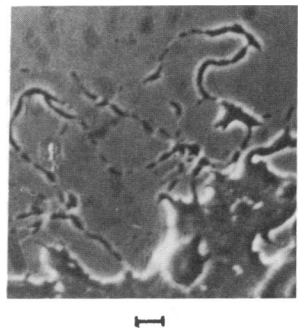

FIG. 4. Stimulation assays between Tn*phoA* mutant JZ302 and the *cglC* strain DK1219. Micrograph shows the edge of a spot containing the indicated strain combination from slide-motility assays. (Bar = 50 μm.)

have proposed that *cgl* mutations might define genes coding for signals that are exchanged between cells.

The stimulation of motility can be induced by a donor *mgl* strain (30). This strain is capable of providing the trans-acting motility-stimulating factors but is unable to move itself. We observed that the *mgl* donor strain (DK306) was capable of stimulating motility A in mixtures with each of the Tn*phoA* mutants (Fig. 2 and data not shown). This indicated that the Tn*phoA* mutants could exhibit motility A when helped. The timing and extent of stimulation were different for each of the four Tn*phoA* motility mutants. The stimulating factor from the donor corresponding to each gene tagged by Tn*phoA*, however, appeared to be trans-acting upon recipient cells.

If stimulation of motility A occurs in a mixture of two *cgl* mutants, the two mutants are classified into different Cgl stimulation groups (B, C, D, E, or F); conversely, if two stimulatable mutants fail to stimulate each other when mixed together, the two mutants belong to the same stimulation group (10). When JZ302 or JZ334 were mixed with a *cglF* mutant, stimulation of motility A failed to occur. Stimulation did occur in mixtures of JZ302 or JZ334 with a mutant of class *cglB, -C, -D,* or *-E*. When either JZ306 or JZ315 was mixed with a *cglB* mutant, stimulation of motility A failed to occur. Stimulation did occur in mixtures of either JZ306 or JZ315 with a mutant of class *cglC, -D, -E,* or *-F* (Fig. 3, and data not shown for JZ315). Fig. 4 shows specifically that stimulation did occur when JZ302 was mixed with a *cglC* mutant. This indicated that the Tn*phoA* insertion at Ω302 did not reside within a *cglC* gene, although it mapped close to the *cglC* locus. In pairwise testing of the Tn*phoA* mutants with each other, stimulation of motility A occurred upon mixing either JZ302 or JZ334 with JZ306 or JZ315. Stimulation did not occur when JZ302 was mixed with JZ334 or when JZ306 was mixed with JZ315. These results confirmed that the insertion mutations at Ω306 and at Ω315 behaved like *cglB* mutations in phenotypic complementation, supporting the mapping data

Table 3. PhoA enzyme activity in cell fractions

Strain	% total activity					PhoA, units
	Cyt	Peripl	Mem	Sup	Extracyt	
JZ302	17	1	6	62	69	2.6
JZ306	2	1	51	37	89	5.8
JZ315	5	2	19	64	85	7.0
JZ334	5	1	46	46	93	4.6
JZ309	10	0	13	77	90	2.2
JZ318	8	1	3	89	93	4.1
JZ324	14	0	4	82	86	4.5
JZ329	22	1	55	36	92	5.8
JZ310	31	0	33	42	75	1.0
JZ312	23	2	33	43	78	5.2
JZ300	39	2	50	10	62	1.4
JZ313	49	6	42	11	59	2.1
JZ322	48	1	32	16	49	3.4
JZ319	47	4	17	15	36	4.4

Total activity is defined as the sum of the activities in the supernatant and whole cell fractions. Cyt, cytoplasm; Peripl, periplasm; Mem, membrane; Sup, supernatant; Extracyt, extracytoplasmic (extracytoplasmic activity is the sum of the periplasm, membrane, and supernatant fractions). For PhoA, data are total units of PhoA (whole cell + supernatant). One unit is defined as the amount of PhoA enzyme in 1×10^7 cell equivalents, which results in an increase of 0.1 absorbance unit/30 min at 405 nm.

that Ω306(Tn*phoA*) and Ω315(Tn*phoA*) were near the *cglB* locus on the genetic map (Fig. 5). The insertion mutations at Ω302 and at Ω334 behaved like a *cglF* mutation in complementation. Since Ω302(Tn*phoA*) and Ω334(Tn*phoA*) were unlinked to the *cglF* locus as well as to each other, they define two *cgl* loci of stimulation class F. The two loci are defined as *cglFB* [Ω302(Tn*phoA*)] and *cglFC* [Ω334(Tn*phoA*)]. The original *cglF* locus defined by the *cglF1* mutation (10) is now renamed *cglFA*.

Localization of the Cgl–PhoA Fusion Proteins in Cell Fractions. We fractionated cells and assayed PhoA activity in the various cell fractions (Table 3). For JZ302, a large amount of the activity appeared in the membrane and supernatant fractions, although a significant percentage also appeared in the cytoplasmic fraction. For JZ306, JZ315, and JZ334, essentially all of the enzyme activity appeared in the membrane and supernatant fractions.

A control was necessary to assess the degree of cross-contamination between different subcellular fractions during the fractionation protocol. We, therefore, determined the levels of isocitrate dehydrogenase in the different cell fractions, since isocitrate dehydrogenase has been shown to be an enzyme existing in the cytoplasm in *M. xanthus* (25). In all cases, we observed that at least 96% of the isocitrate dehydrogenase enzyme activity appeared in the cytoplasmic fraction, while the remainder of the activity appeared in the membrane fraction. This implied that cytoplasmic enzymes

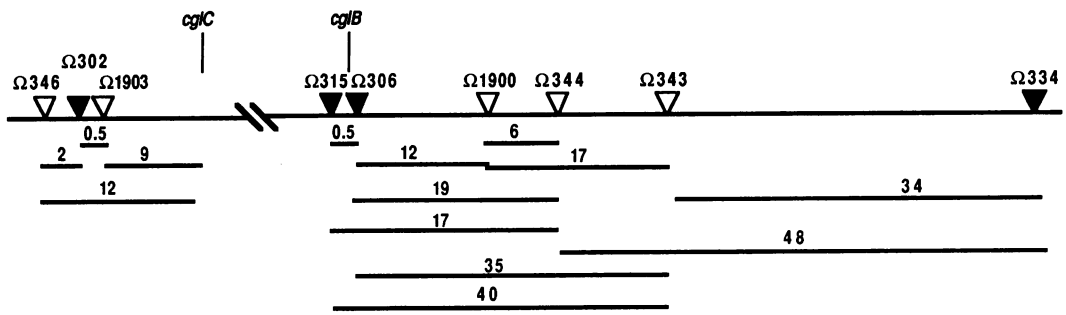

FIG. 5. Genetic map of the *cgl* region. Solid triangles represent Tn*phoA* insertions. Open triangles represent Tn*5* insertions. The numbers below the map represent the distance, in kilobases, between the corresponding markers calculated from the transduction data using the Wu equation (26).

did not appear in the membrane or supernatant fractions by a general process of cross-contamination or cell lysis.

We also chose 10 other strains from the original collection of 36 strains and tested these for the localization of PhoA activity. All strains tested produced significant amounts of enzyme activity that appeared in the membrane and supernatant fractions. A subset of these strains (JZ309, JZ318, and JZ324) showed activity localized predominantly in the supernatant fractions. These data indicated that Tn*phoA* specifically tagged genes encoding exported proteins. Tn*phoA* tagging must be qualified as an enrichment strategy, however, because a number of tagged genes produced significant PhoA activity appearing in the cytoplasmic fraction. This raises the possibility that particular PhoA fusions (perhaps those expressed at a high level) produce detectable enzymatic activities without being exported.

Linkage Map of Region. The genetic transduction data allowed construction of a genetic map of the region containing the Tn*phoA* insertions (Fig. 5). Additional transduction experiments showed that Ω1900, a Tn*5* insertion identified by D. Kaiser's laboratory and linked (42%) to *cglB* (31), was linked (47%) to Ω306, was linked (33%) to Ω343, and was linked (72%) to Ω344. Ω1903, another Tn*5* insertion isolated by D. Kaiser's laboratory and linked (62%) to *cglC* (31), was linked (98%) to Ω302 and was linked (90%) to Ω346. Two Tn*5* insertions, Ω1919 and Ω1931, isolated by D. Kaiser's laboratory and linked to the *cglF* locus defined by Hodgkin and Kaiser (30), did not show linkage to Ω302, Ω306, Ω334, Ω344, or Ω346. Ω315 was shown to be linked (99%) to Ω306. All other combinations of insertions represented on the map did not exhibit linkage. *cglB* and *cglC* have been shown (31) to be linked to each other through *cglD*. The distance between the *cglB* and *cglC* locus must, therefore, be at most two Mx4 genome equivalents (i.e., 110 kilobases).

DISCUSSION

By using Tn*phoA* we identified and physically tagged 36 genes in *M. xanthus* whose products are potentially exported into the cell envelope or extracellular space. To show the power of Tn*phoA* in identifying genes encoding extracytoplasmic molecules involved in cell interactions, we focused on four Tn*phoA* mutants with defects in motility. We observed that the four mutants were defective in motility A and that Tn*phoA* specifically tagged genes (*cgl* genes) involved in contact gliding.

Mutants defective in *cgl* genes can be phenotypically stimulated to move with motility A when they are in contact or in close proximity with wild-type cells. Consequently, it has been proposed that the products of the *cgl* genes may be exchangeable structural components of the motility mechanism or, alternatively, regulatory molecules that communicate messages for stimulating cell movement (10). If Cgl factors are regulatory, they may function as cell-to-cell signal molecules, or extracellular matrix molecules, for guiding the direction or speed of cell movement. Evidence showing that Cgl factors fused with PhoA are localized in the cell envelope or extracellular space supports the phenotypic complementation evidence that these molecules must be on the cell surface or extracellular. Phenotypic complementation evidence also suggests that these factors act in trans upon other cells. These factors transfer information between adjacent cells to the degree that they stimulate cell motility. This action represents biological activity, not mere physical binding to the surface of an adjacent cell. The stimulation of motility is very strong, which might favor a signaling mechanism. If these factors are structural parts of the motility mechanism and acquired from adjacent cells by protein scavenging, they nevertheless behave simultaneously as very strong signals.

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