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Genetics of Gliding Motility in *Myxococcus xanthus* (Myxobacterales): Genes Controlling Movement of Single Cells

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Summary. *M. xanthus* is a gliding bacterium whose motility is subject to intercellular control. Strain DK101 of *M. xanthus* gives rise to 6 distinct types of nonmotile mutants and transduction of motility between mutants, mediated by the generalized transducing phage Mx8, identifies the gene loci that underlie the six types. Five of the types, B, C, D, E, and F, are conditional mutants that can be stimulated to move by wild-type cells or by cells of a different mutant type. Mutants of each stimulation type lie in separate and distinct loci, *cglB*, *cglC*, *cglD*, *cglE* and *cglF*. The sixth mutant type can stimulate any of the five other types to move, never moves itself, and is produced by mutations in at least 17 loci.

Introduction

The fruiting myxobacteria are a group of gram-negative bacteria that exhibit primitive multicellular development (McCurdy, 1974). Myxobacteria move by gliding on a surface (Henrichsen, 1972), rather than by the flagellar swimming employed by most motile bacteria. Gliding is a smooth progression of rod-shaped cells in the direction of their long axis, with occasional stops or reversals of direction. The rate of movement by gliding is slow: less than 10 μm/min for *Myxococcus* on agar.

Growing myxobacterial cells do not move independently, but form loose and ever-changing multicellular associations. For example, thousands of vegetative cells that arise by germination of spores from a cyst of *Chondromyces apiculatus* form a single, beelike "swarm", which subsequently moves as a coherent unit. Time lapse motion pictures (Kühlwein and Reichenbach, 1968) show that cells in a swarm move individually and may even migrate away from the swarm edge, but they usually return quickly so that the whole swarm migrates as a unit. Colonies of *Myxococcus* on a fully supplemented agar exhibit swarming behavior: they are flat and spreading with irregular borders made of peninsulas and islands of cells moving away from the colony center (cf. Fig. 1). Dworkin (1973) and Rosenberg et al. (1977) have suggested that multicellular associations have survival value bacause they allow cooperative utilization of extracellular digestive enzymes from many cells. This "wolf pack" effect is consistent with the ecological role of myxobacteria, which live by degrading macromolecular organic material in the soil.

We began a genetic analysis of motility in *M. xanthus* because it seemed likely that mutants could be obtained both in the mechanism and in the control of movement in this organism. Mechanism mutants might shed light on the gliding process; control mutants might reveal how movement is regulated and how the movements of many cells are coordinated in swarming and in fruiting. It also seemed likely that efficient selection for motility could be applied, facilitating genetic analysis. Several generalized transducing phages of *M. xanthus* have been isolated (Campos et al., 1978; Martin et al., 1978) thus genetic crosses can be performed.

Some motility mutants of *M. xanthus* have already been reported. Burchard (1970) described a mutant which exhibits gliding only in groups of two or more cells, which he called semimotile, and from which he was able to isolate a completely nonmotile strain. MacRae and McCurdy (1976) have described four nonmotile mutants, isolated using very stringent criteria for nonmotility. Finally, we have reported nonmotile mutants that can move transiently following contact with other mutant or wild-type cells (Hodgkin and Kaiser, 1977).

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This paper describes the isolation and characterization of many mutations that prevent the gliding of single cells.

Materials and Methods

Strains. Strains are listed in Table 1. The parent strain used in this work was DK101, a motile strain and a spontaneous mutant of *M. xanthus* FB (Dworkin, 1963). The yellow phase variants of strains, rather than the tan variants, have been used whenever possible in an attempt to avoid effects of phase on motility (Wireman and Dworkin, 1975). Mx8, a generalized transducing phage active in *M. xanthus*, is described by Martin et al. (1978). A motile non-fruiting strain isolated by C. Manoil from FB, DK510, was used to propagate and to assay Mx8.

Table 1. Catalogue of strains

Strain	Genotype		Phenotype	Origin
DK100	+	*sglA*$^+$	m	*M. xanthus* FB strain YS (Wireman and Dworkin, 1975)
DK101	+	*sglA1*	m	A spontaneous mutant of FB
DK302	*aglJ1*	*sglA1*	nm(ts)	EMS mutagenesis of DK101
DK305	*aglN1*	*aglA1*	nm	UV Mutagenesis of DK101
DK306	*mgl-1*	*sglA1*	nm	
DK307	*cglB1*	*sglA1*	nm	
DK308	*mgl-2*	*sglA1*	nm	
DK309	*mgl-3*	*sglA1*	nm	
DK310	*mgl-4*	*sglA1*	nm	
DK314	*mgl-5*	*sglA1*	nm	
DK315	*mgl-6*	*sglA1*	nm	
DK321	*cglB2*	*sglA1*	nm	
DK323	*cglC1*	*sglA1*	nm	
DK324	*mgl-7*	*sglA1*	nm	
DK326	*aglF1*	*sglA1*	nm	
DK327	*aglG1*	*sglA1*	nm	
DK329	*aglE2*	*sglA1*	nm	
DK331	*cglB3*	*sglA1*	nm	
DK333	*cglB4*	*sglA1*	nm	
DK335	*cglB5*	*sglA1*	nm	
DK339	*aglD1*	*sglA1*	nm	
DK343	*aglC7*	*sglA1*	nm	
DK344	*cglB6*	*sglA1*	nm	
DK345	*cglD1*	*sglA1*	nm(ts)	
DK348	*cglB8*	*sglA1*	nm(ts)	
DK352	*cglB10*	*sglA1*	nm	
DK355	*cglB12*	*sglA1*	nm	
DK356	*aglD2*	*sglA1*	nm	
DK357	*cglB13*	*sglA1*	nm	NTG Mutagenesis of DK101
DK358	*aglF2*	*sglA1*	nm	
DK359	*aglE1*	*sglA1*	nm	
DK360	*cglE1*	*sglA1*	nm	UV Mutagenesis of DK101
DK363	*aglL1*	*sglA1*	nm	
DK364	*aglM1*	*sglA1*	nm	
DK365	*aglK1*	*sglA1*	nm	
DK366	*mgl-8*	*sglA1*	nm	
DK368	*aglF3*	*sglA1*	nm	
DK369	*aglH1*	*sglA1*	nm	
DK370	*cglF1*	*sglA1*	nm	
DK371	*mgl-9*	*sglA1*	nm	
DK510		*sglA1*	m	(non-fruiting mutant) DK101
NM	*aglE3*	*sgl-7*	nm	Burchard (1970)

m = motile, nm = nonmotile, ts = temperature sensitive

Nomenclature. We have tried to conform to the recommendations of Demerec et al. (1966) for bacterial genetics. All genes involved in gliding have been given names containing *gl* as the second two letters, to avoid confusion with genes for metabolic functions such as *glc*, *glu*, *glt*, *gln*, etc. Four gene names are used in this paper: *agl* ("adventurous" or single cell gliding); *cgl* (conditional or contact stimulated gliding); *mgl* (mutual function for gliding); *sgl* ("social" or cell group gliding). Upper case is used to designate a phenotype, e.g. Cgl designates the phenotype of a strain that carries a mutation at one of the *cgl* loci.

Abbreviations. m.o.i., multiplicity of infection; p.f.u., plaque-forming units; EMS, ethyl methanesulfonate; NTG, N-methyl-N′-nitro-N-nitrosoguanidine; UV, ultraviolet light.

Media. The complex, Casitone-containing media, CTT, and $^1/_2$ CTT have been described previously (Hodgkin and Kaiser, 1977). YT agar consists of 1% (w/v) tryptone, $^1/_2$% yeast extract, and 1% agar.

Mutagenesis and Mutant Isolation. Single colonies of the source strain were inoculated into CTT broth, grown to a density of 5×10^8 cells per ml, exposed to mutagen (EMS, NTG, UV or ICR-191), washed, diluted into CTT broth, grown 2 to 6 doublings to allow expression of recessive mutations, and finally plated for single colonies on CTT agar. The plates were incubated at 33° and the colonies inspected for mutant types. The protocols are described in Hodgkin and Kaiser (1977). ICR-191 was a gift of R. Peck.

Phage Growth and Transduction. Mx8 phage stocks were grown and assayed on YT plates. The indicator strains for assay were DK306 and DK510. For motility transduction, phage stocks were diluted in CTT broth and irradiated with 360 J/m^2 of UV light from a mercury lamp. Phage and exponentially growing bacteria were mixed in known numbers in CTT broth made 1 mM in $CaCl_2$. Adsorption occurred during 10–20 min at 25° C, then the bacteria were sedimented and resuspended in approximately 50 µl of CTT broth. This suspension was distributed in 55 drops on $^1/_2$ CTT plates, unless otherwise specified, and the plates were incubated for 5 days at 33° C. Spots were examined for flares of motile cells using a phase contrast microscope with a long working distance condenser.

Motility Stimulation Test. Certain nonmotile strains can be stimulated to move when mixed with other strains, as described by Hodgkin and Kaiser (1977). To test a given strain, a culture is grown in suspension to a density of about 5×10^8 cells/ml, and mixed with standard test strains at the same concentration. We have used DK306 (*mgl-1*) as a nonmotile universal stimulator and appropriate *cgl* mutants as test recipients for the B, C, D, E, and F stimulations. When large numbers of mutants are to be tested, the mixing is carried out in microtiter trays. A 2 µl drop of each mixture is placed on each of two CTT plates using an inoculating loop, and the plates are examined several times between 12 and 24 h of incubation at 33° C and 28° C to detect stimulation, even if transient.

Results

1. Isolation and Characterization of Nonmotile Mutants

It has been generally observed that *M. xanthus* FB (Dworkin, 1963) gives rise to exceedingly few nonmotile mutants (Burchard, 1970; MacRae and McCurdy, 1976). However, in the early stages of this work a spontaneous mutant of *M. xanthus* FB, called DK101, was isolated that was able to give rise to nonmotile mutants at a frequency of about 0.1% after mild EMS mutagenesis (Hodgkin and Kaiser, 1977). The capacity of DK101 to yield nonmotile mutants at appreciable frequencies is due to a mutation *sglA1*. We propose to defer analysis of *sglA1* and other mutations with similar effects to the following paper (Hodgkin and Kaiser, 1979). By using DK101 as the standard of reference for nonmotile mutants derived from it, effects of *sglA1* tend to cancel out since both DK101 and its nonmotile mutants carry *sglA1*.

Colonies of DK101 are thin, flat, and surrounded by an irregular spreading fringe of moving cells, mostly single cells (Fig. 1). In contrast, colonies formed by nonmotile mutants are tight, sharp edged, heaped up and consequently smaller. Nonmotile mutants were isolated by visually screening more than 250,000 colonies of mutagen-treated DK101 for these differences.

About 400 nonmotile mutants were isolated and among them seven different types could be distinguished by their stimulation properties (Hodgkin and Kaiser, 1977). Cgl mutants can be stimulated to move and can be assigned to one of five Cgl classes: CglB, CglC, CglD, CglE, and CglF. Stimulatable mutants of different types can stimulate each other, while those of the same type cannot. Agl and Mgl mutants are not themselves stimulatable but they can stimulate all 5 Cgl types. Mgl mutants are distinguishable from Agl mutants because they show a more extreme nonmotile phenotype than Agl (or Cgl) mutants, as described in Section 4. Table 2 summarizes the distribution of types obtained with four different mutagens, employed so as to minimize the possible effects of mutational hot spots.

From the total, a collection of 87 mutants, chosen for stability, independence, and variety, was retained for further analysis. In choosing mutants we were

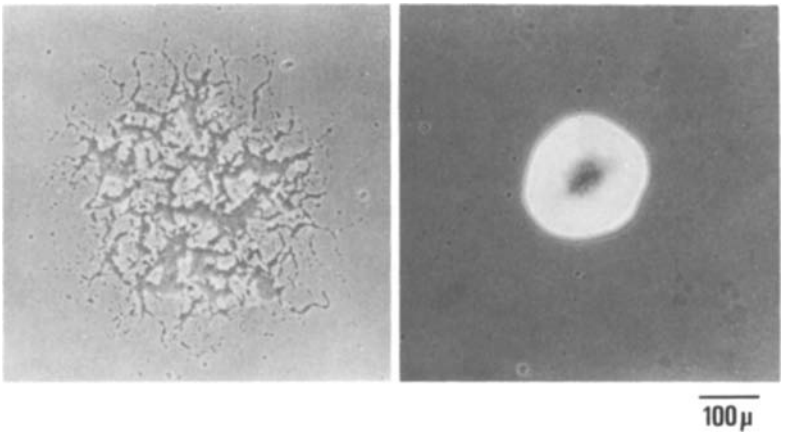

Fig. 1. Colonies of motile (at left, strain DK101) and nonmotile (at right, strain DK306) *M. xanthus* on CTT agar, 48 h at 33°

Table 2. Induction of nonmotile mutants in DK101 and frequency of mutant phenotypes

		No Mutagen	EMS	NTG	UV	ICR-191
Number of experiments		7	12	6	65	5
Total colonies screened (approximate)		28,000	25,000	10,000	185,000	19,000
Total mutants picked		2	21	9	261	115
Phenotypes[a]:	Mgl	–	–	–	10	–
	Agl	2	20	8	219	107
	CglB	–	1	1	23	5
	CglC	–	–	–	4	1
	CglD	–	–	–	3	–
	CglE	–	–	–	1	2
	CglF	–	–	–	1	–

[a] Phenotypes were determined by stimulation tests as described in the text and Methods.

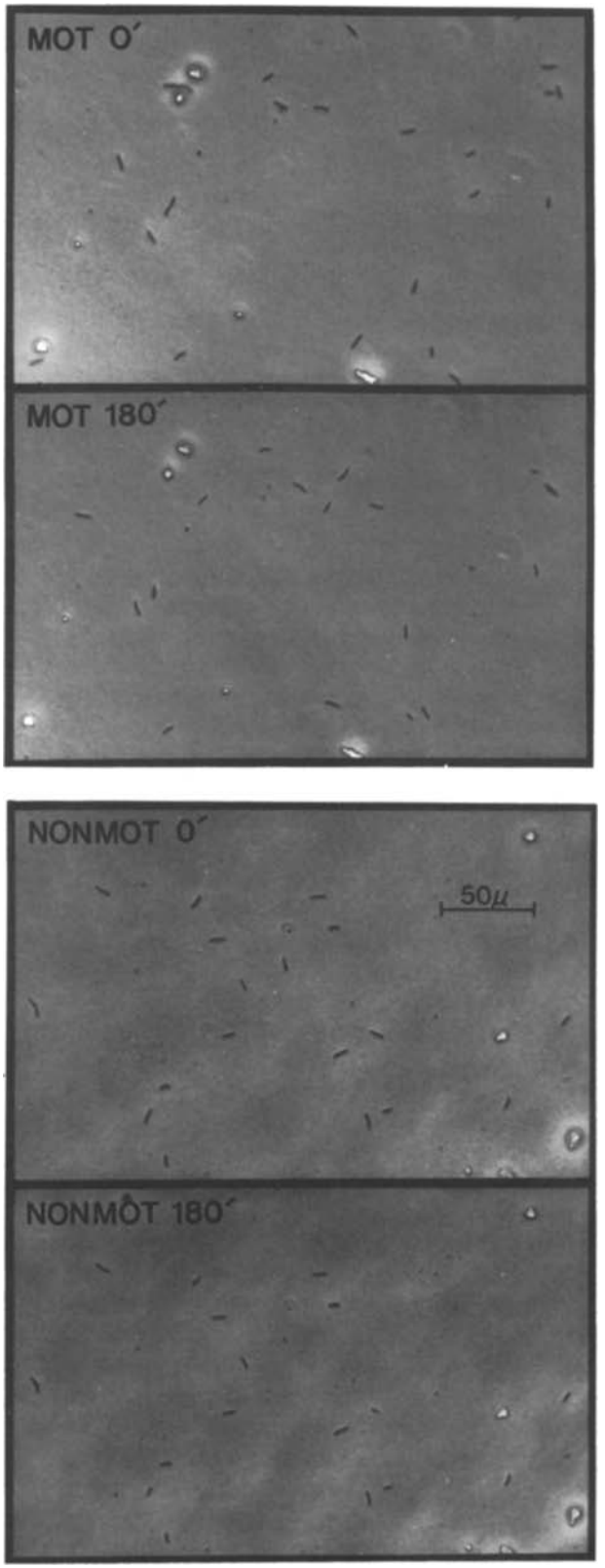

Fig. 2. Movement of single cells on agar. In the upper two frames (labeled "Mot"), the same field of DK101 cells is shown at the beginning and end of a 180 min interval. A nonmotile mutant, DK321 (*cglB2*), is shown in the lower two frames (labeled "Non-mot"), photographed at the beginning and end of a 180 min interval. In both experiments 5 μl of a suspension containing 4×10^6 cells/ml and carbon grains was placed on $^1/_2$ CTT agar, allowed to dry and photographed at intervals

biased towards Cgl and Mgl phenotypes because they are more distinctive, so only 46 of the mutants retained were of the Agl type although *agl* mutants accounted for about 90% of all nonmotile mutants initially isolated.

The absence of a fringe of cells around colonies of the mutants suggested that single cells were unable to move. This was tested directly by microscopic examination of cells deposited on agar at such low density that on average cells were several cell lengths apart. Under these conditions single DK101 cells do move. The two upper frames of Fig. 2 (labeled "MOT") show the same field of cells photographed at the beginning and end of a 175 min interval and almost all the cells move from their initial positions. Using carbon grains, which had been deposited initially with the cells, as reference points, it is possible to align initial and final photographs accurately. Examination of many fields of DK101 cells (Table 3) showed that more than half the cells moved more than a cell length from their initial position within 3 h. A similar analysis of the nonmotile mutant DK321 (carrying *cglB2*) presented in the lower frames of Fig. 2 (labeled "NONMOT") shows no significant displacement of any cell. One or more representatives of each phenotypic class of mutants have been tested in the same way with results summarized in Table 3.

Table 3. Movement of single cells

Strain	Genotype[a]	# Cells Traced[b]	Time Interval	# Cells Moved[c]
DK101	+	229	125′, 180′	151
DK302	*aglJ1*	154	180′, 210′	1
DK305	*aglN1*	139	210′	1
DK306	*mgl-1*	104	160′, 180′	0
DK314	*mgl-5*	205	180′	0
DK321	*cglB2*	255	180′, 200′	0
DK323	*cglC1*	159	220′	0
DK345	*cglD1*	232	180′	1
DK360	*cglE1*	137	180′	0
DK370	*cglF1*	218	180′	0

[a] All strains carry *sglA1* (Table 1).

[b] Cells grown in liquid culture at 33° were diluted and deposited with carbon grains on $^1/_2$ CTT agar in a closed chamber (either a small petri dish or a chamber on a microscope slide). The cultures were incubated at 26±1° and photographed at intervals. Photographic negatives were projected and the cells were traced, using the carbon grains to superpose photographs of the same field taken at different times.

[c] A cell is counted as moving if it is displaced at the end of the interval by one or more cell lengths from its initial position.

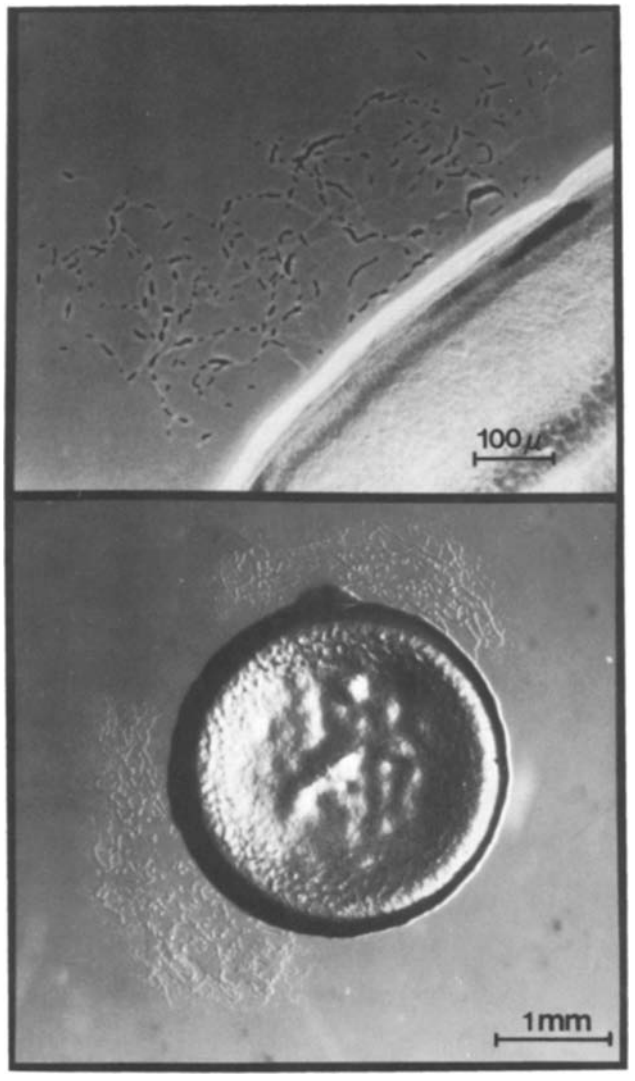

Fig. 3. Flares of motile bacteria produced by transduction of the nonmotile mutant, DK306, by phage Mx8 grown on strain DK101. (Top) A young flare at high magnification. Individual cells are evident. Only part of the spot of nonmotile cells is visible at lower right. Incubation 3 days at 33°. (Bottom) An entire cell spot with flares visible at 1 o'clock and 7 o'clock. Incubation 5 days at 33°

None of the mutant cells moved to a significant extent. To test whether some cells might be executing oscillatory movements that brought them back to their initial position, they were examined at 30 min intervals between the initial and final times, but again no displacement was detected. Therefore, these mutants are nonmotile both as single cells and as masses of cells in a colony.

2. *Quantitating the Transduction of Motility*

The morphological difference between colonies of motile and nonmotile strains permits detection of a few motile cells among many which are nonmotile, for if a mixture of motile and nonmotile cells is allowed to grow on solid nutrient medium in a compact spot, then motile cells can migrate outwards and multiply to form fan-shaped "flares". Flares are readily observed by microscopic examination, or after longer incubation with the unaided eye, because they extend beyond the smooth edge of a spot of nonmotile cells (Fig. 3).

Table 4. Reconstruction experiments: Efficiency of selection for motility

Nonmotile bacteria	Medium	Number of spots with flares when mixed with		Colonies DK101 alone
		No bacteria	DK101	
DK306 (*mgl-1*)	CTT	0	11,15	47,34
DK307 (*cglB1*)	$^1/_2$ CTT	0	6	80
DK323 (*cglC1*)	$^1/_2$ CTT	0	34	80

In each experiment 5×10^7 nonmotile bacteria were mixed with an estimated 50–100 bacteria of strain DK101, and the mixture was distributed in 55 drops on nutrient plates as in the standard transduction protocol. The same quantity of DK101 was plated alone on the same medium to determine by colony count the number of viable cells.

Transduction or mutation from nonmotility to motility can be detected and measured by counting flares. Reconstruction (Table 4) shows that motile cells can be detected when they are present at a ratio of about $1:10^6$ nonmotile cells. Under these conditions and on the average about one motile cell in 4 gives rise to a flare. Apparently stimulation does not affect the sensitivity of the test, because the same frequency of flares was observed in presence of the non-stimulatable nonmotile mutant *mgl-1* as in presence of the stimulatable nonmotile mutant *cglC1*. Some nonmotile mutants such as DK307 reduce the efficiency of detection of motile cells for unknown reasons.

To quantitate the transduction of motility, a given mixture of nonmotile bacteria and bacteriophage are distributed in 55 small drops of at most 10^6 cells per drop on nutrient agar plates, as described in Methods. The plates are incubated and the bacteria in the drop grow into a confluent spot about 3 mm in diameter (Fig. 3). Because it is not possible to distinguish a flare that has been initiated by a single motile cell from a flare initiated by two or more, the number of spots with motile flares are counted, irrespective of the number of flares per spot. The score per plate, therefore, ranges between 0 and 55. An estimate of the total number of transductant flares could be

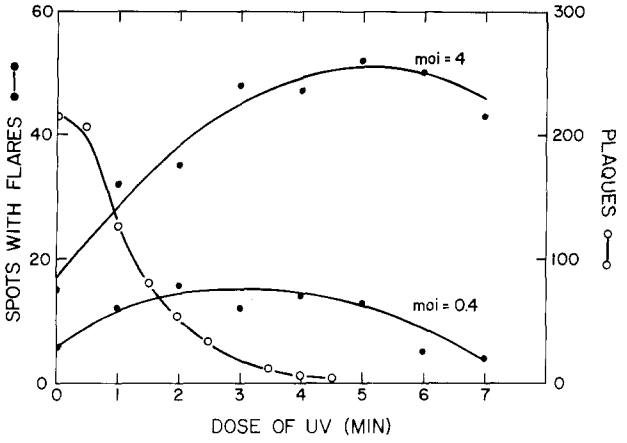

Fig. 4. Effect of UV irradiation of transducing phage on the efficiency of transduction. Phage grown on strain DK100 were diluted in CTT broth to 10^9 p.f.u./ml (plaque-forming units per ml), and irradiated at 120 Joules per m^2 per min with UV light from a mercury lamp. Equal samples were withdrawn at one minute intervals and added to the nonmotile mutant DK306 (*mgl-1 sglA1*) at multiplicities of infection (m.o.i.) of 4 and 0.4 particles/cell. Multiplicity refers to the titer before irradiation. The standard transduction protocol (Methods) was followed, except that for each point 10^8 recipient bacteria were used and the mixtures were spotted on CTT plates. In a parallel experiment, phage were diluted to 2×10^3 p.f.u./ml in CTT broth and irradiated in the same manner. To measure the loss of plaque forming ability, 0.1 ml samples were withdrawn at $^1/_2$ minute intervals and plated on lawns of strain DK510

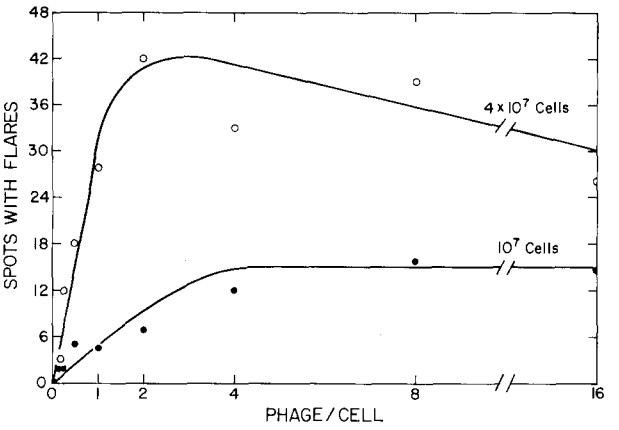

Fig. 5. The effect of multiplicity of infection and number of recipient cells on the number of flares. Transducing phage were grown on the motile strain DK100; recipient bacteria were strain DK343 (*aglC7 sglA1*); and the standard transduction protocol, Methods, was used. Multiplicity refers to the titer before irradiation

made, assuming a Poisson distribution of flares: $N = 55\ ln\ (55/(55-s))$ where s is the score and N the total number of flares. We have not applied this correction in quoting scores, because in general we have used numbers of phage and bacteria that give scores of 30 or less per plate, and in this range s underestimates N by less than 50%.

Experiments were conducted to optimize and validate the transduction procedure. UV irradiation of transducing phage has been found to increase the frequency of general transduction by P22 (Garen and Zinder, 1955). UV irradiation of an Mx8 stock also increased the frequency of motility transduction as measured by the number of spots with flares when the multiplicity of infection was 4 (Fig. 4). Irradiation did not increase significantly the rate of reversion of motility mutants in control experiments in which a nonmotile mutant was treated with an irradiated transducing stock that had been propagated on the same mutant. Consequently, UV irradiation of the phage stock was made part of the standard transduction protocol (Methods).

The number of motile transductants, as measured by the number of spots with flares, was found to be roughly proportional to the number of irradiated phage added at low multiplicities of infection (Fig. 5). But at high multiplicities the number of transductants remained constant (10^7 cells) or decreased (4×10^7 cells), as if phage surviving UV irradiation killed more of the recipient bacteria when high multiplicities were used. This effect is useful in that it tends to reduce variation in transduction frequency caused by differences in phage concentration. When four times as many recipient bacteria are used, roughly four times the number of transductants were observed at low multiplicities (Fig. 5).

Some mutants produce many flares spontaneously, some or all of which contain revertants. Controls in which bacteria are not treated with phage tend to exaggerate this background because there is no killing of the bacteria by phage as there is in the transduction mixture; and, therefore, more cells are present to show reversion. For this reason controls are occasionally not accurately scorable. An alternative control, in which a strain is treated with a transducing lysate of the same strain gives a much lower background in these cases.

Some strains have markedly higher or lower phage sensitivity than other strains and this increases or decreases the number of transductants irrespective of linkage. Therefore, in each set of crosses a control donor is used whose mutation shows no linkage to the mutations under test to allow estimation of the score expected when there is no linkage. Similarly a control recipient strain is included to permit comparison of different transducing phage stocks in the same experiment. In Table 5, for example, the control recipient strain is DK306 (*mgl-1* is not detectably linked to *cglB*) and the control donor is DK510 (DK510 is $cglB^+$). "Tight" linkage is taken to be

indicated by scores which are a tenth or less of the score obtained with the control strains. Smaller deviations indicate looser linkage. There are several sources of variability: variation in humidity and aeration during incubation which alter motility, inaccurately known phage stock concentrations, and statistical fluctuation. Consequently, we do not regard deviations of less than 50% as significant unless repeatedly observed. When reciprocal transductions (i.e. reversal of donor and recipient) deviate by more than 50%, the looser linkage is taken.

We have assigned mutants to specific loci on the basis of this linkage analysis, and given gene names to these loci, although we do not know whether each locus corresponds to a single cistron or to a group of linked cistrons. In summary, the selection of motile transductants is efficient enough to allow transductional analysis of motility, but difficulties are encountered with some mutant strains, and the method is not fully quantitative. Detailed analysis of linkage data, sufficient to detect very loose linkage, has not yet been attempted.

3. *Mapping Cgl Mutants*

Cgl mutants can be stimulated to move by contact with cells of a different type. Thus, mixtures of CglB and CglC cells show extensive movement, because each cell type can be stimulated by the other. Mixtures of CglB and Agl, or CglB and Mgl, show less movement because only one cell type is being stimulated to move. Previous crosses (Hodgkin and Kaiser, 1977) showed that Mgl, AglB, CglB and CglC mutants carried mutations which were either undetectably linked or, in the case of *cglB* and *cglC* mutations, very loosely linked. Seventeen CglB mutants have now been tested and all mutations are tightly linked, including some that are temperature sensitive. Data for ten of them are shown in Table 5. All CglC mutants (5/5) carry mutations which are tightly linked at a different locus, and the mutations in all CglE mutants (3/3) are linked at a third locus (data not shown).

CglD mutants are poor recipients for motility transduction for unknown reasons, and all three isolates are temperature sensitive conditional mutants, showing extensive movement at 25° C. A search for derivatives of *cglD1* that would be nonmotile at 25° C was made by mutagenizing DK345 and screening colonies grown at 25° C. Many nonmotile mutants were found, mostly double mutants having CglD Agl, CglD CglB, and CglD CglC phenotypes, but also some that behaved as CglD mutants without temperature sensitivity. One of these was an improved recipient for motility transduction, and it was possible using this host to demonstrate linkage between the three CglD isolates.

Table 5. Linkage between *CglB* mutants

Recipient bacteria	None	Transductional Donor		
		DK510 (motile)	DK307 (*cglB1*)	DK321 (*cglB2*)
DK306 (*mgl-1*)	0	26	35	38
DK331 (*cglB3*)	0	16	1	0
DK333 (*cglB4*)	0	34	0	1
DK335 (*cglB5*)	0	30	0	0
DK344 (*cglB6*)	0	38	0	0
DK348 (*cglB8*)	0	10	0	0
DK352 (*cglB10*)	0	31	0	0
DK355 (*cglB12*)	0	31	0	1
DK357 (*cglB13*)	0	34	0	0

In each cross 5×10^7 bacteria were mixed with 3×10^8 phage. Scores are number of spots with flares. The column headed none had no phage added.

The loci *cglB*, *cglC* and *cglD* show loose linkage implying an order *cglB-D-C* (Hodgkin and Kaiser, 1977). Double mutants of the type *cglD cglC* do give a few motile transductants when treated with a transducing lysate grown on DK101, implying cotransduction of the loci *cglD* and *cglC*. However, no motile transductants were obtained for mutants of the type *cglD cglB* although they would have been expected. None of the *cgl* loci were found linked to the *mgl* locus. It is possible that some *cgl* loci may be found to be linked to *agl* loci in future crosses although tests of about half of the pairwise combinations have not yet resulted in detection of linkage.

4. *Nonstimulatable Mutants*

The majority of nonmotile mutants are not themselves stimulatable, though they can stimulate all *cgl* mutants. Thus, they behave as if defective in reception or response to stimulation. Among the more than 400 nonmotile mutants isolated in the initial screen (Table 2) two sorts of nonstimulatable mutants could be recognized. One sort, though nonmotile under standard conditions, did show slight motility at low temperature or on plates with low nutrient levels (Hodgkin and Kaiser, 1977); they are called *agl* mutants. The other sort was nonmotile under all conditions tested and are called *mgl* mutants. Nine *mgl* mutants were crossed and all mutations were found to be linked to each other (Table 6), with some pairs like *mgl-4* and *mgl-9* giving appreciable recombination. Thus, there may be a single large *mgl* gene or a cluster of adjacent ones.

Table 6. Linkage between *mgl* mutants

Recipient bacteria	None	Transductional Donor						
		DK510 (*motile*)	DK306 (*mgl-1*)	DK309 (*mgl-3*)	DK310 (*mgl-4*)	DK314 (*mgl-5*)	DK315 (*mgl-6*)	DK324 (*mgl-7*)
DK307 (*cglB1*)	0	27	27	45	48	37	34	39
DK306 (*mgl-1*)	0	21	1	0	4	4	1	0
DK308 (*mgl-2*)	0	31	0	0	7	4	1	0
DK310 (*mgl-4*)	0	26	1	1	0	7	0	0
DK366(*mgl-8*)	0	44	2	2	6	2	0	3
DK371 (*mgl-9*)	0	36	3	3	8	1	1	3

Conditions and scores as Table 5

Table 7. Multiplicity of *agl* loci

Recipient bacteria	None	Transductional Donor					
		DK356 (*aglD2*)	DK369 (*aglH1*)	DK359 (*aglE1*)	DK365 (*aglK1*)	DK363 (*aglL1*)	DK364 (*aglM1*)
DK339 (*aglD1*)	0	2	39	46	55	51	55
DK356 (*aglD2*)	0	0	16	26	52	42	55
DK369 (*aglH1*)	0	5	0	29	44	17	49
DK365 (*aglK1*)	0	8	4	1	0	24	32
DK363 (*aglL1*)	0	10	18	45	51	0	55
DK364 (*aglM1*)	0	29	36	8	46	42	0

Conditions and scores as Table 5

Table 8. Linkage between *agl* mutations

Recipient bacteria	None	Transductional Donor				
		DK100[a] (motile)	DK359 (*aglE1*)	DK329 (*aglE2*)	NM (*aglE3*)	DK305 (*aglN1*)
DK306 (*mgl-1*)	1	47	33	41	33	31
DK359 (*aglE1*)		32	1	2	1	8
DK329 (*aglE2*)	0	18	2	1	0	6
NM (*aglE3*)	0	44	4	1	0	15
DK365 (*aglK1*)		41	8	13	8	6
DK305 (*aglN1*)		39	11	35	22	2

Conditions and scores as Table 5.

[a] Since DK100 is $sglA^+$ and all recipients except NM are *sglA1*, $sglA^+$ as well as agl^+ transductants arise in these crosses. But $sglA^+$ and agl^+ transductants are morphologically different (Hodgkin and Kaiser, 1979) and only agl^+ transductants were scored.

Forty-six mutants of the Agl type were retained from approximately 350 nonstimulatable mutants isolated in the initial screen. Linkage tests on these have shown that mutations at a large number of unlinked loci can generate Agl mutants. Four of the 46 were too unstable to allow genetic analysis and one was Mx8 resistant. Thirty-eight of the remaining 41 carry mutations which can be assigned to 16 separate loci. The three unassigned mutants have not been tested for linkage to representatives of all the 16 loci, and may represent an additional three loci. The assignments are based on a large number of linkage tests, one set of which is shown in Table 7. Most of the seven mutations in the mutants involved show no linkage to each other. However, there appears to be tight linkage between the *agl* sites in DK356 and DK339 and this was confirmed by a second cross, not shown, hence they are tentatively assigned to the same locus, called *aglD*. Mutant sites in DK359 and DK365 are linked; a second cross (Table 8) confirms the linkage and suggests that it is loose, hence they are assigned to different loci. Almost all the assignments of mutants to specific loci are based on at least two concurrent crosses (for example, the data of Table 8 for the locus *aglE*, and the data of Table 9 for the locus *aglF*).

Some cases of loose linkage are observed which probably represent gene clusters: one example is the

Table 9. Linkage between *aglF* mutations

Recipient bacteria	None	Transductional Donor			
		DK364 (*aglM1*)	DK326 (*aglF1*)	DK358 (*aglF2*)	DK368 (*aglF3*)
DK327 (*aglG1*)	0	55	18	53	41
DK326 (*aglF1*)	0	55	0	1	3
DK358 (*aglF2*)		46	6	2	5
DK368 (*aglF3*)	0	38	0	0	0

Conditions and scores as Table 5.

Table 10. Distribution of *agl* mutations into loci and their phenotypes

Locus	Isolates	Phenotype[a]
aglA	2	Smooth colony edge; occasional motile cells at 25°
B	3	
C	7	
D	4	
E	2	Somewhat unstable
F	4	Somewhat unstable
G	1	Unstable
H	2	Smooth colony edge, transduction difficult: excess slime?
J	1	Very unstable at low temperature
K	3	
L	1	Excess slime?
M	1	Excess slime?
N	1	Very unstable at low temperature
P	1	Excess slime?
Q	1	
R	4	

[a] In presence of *sglA1*.

linkage of *aglE*, and *aglK* seen in Tables 7 and 8. Associations suggested by other data are *aglN* with *aglE* and *aglK*; *aglQ* with *aglJ* and *aglB*; and *aglH* with *aglR* and *aglD*. There are also cases of very loose linkage which are difficult to assess; for example, *aglM* may be linked to the *E-N-K* cluster, and *aglG* to the *H-R-D* cluster.

The net result of the analysis of Agl mutants is shown in Table 10: 38 mutations can be assigned to 16 loci. Some Agl mutants can be distinguished by their phenotype and these are noted in Table 10. A number of mutants show movement under some conditions or after prolonged incubation; others seem to produce excess slime, as judged by colony morphology, and may correspond to the "K variants" of *Archangium* described by Grimm and Kühlwein (1973). It is possible that excess slime may inhibit movement, although the role of slime production in gliding motility is not clear (Doetsch and Hageage, 1968).

Discussion

A colony of *M. xanthus* DK101 is bordered by a fringe of cells, mostly single isolated cells, which expands outward as the colony grows. Mutants lacking the fringe arise at a frequency of about 10^{-3} following mutagenesis. We have isolated such mutants and have tested the capacity of representatives of each mutant type to move on agar as single isolated cells. All proved to be nonmotile by this test.

Two basic kinds of nonmotile mutants are discernable. One, the Cgl mutants, can be stimulated to move transiently by mixing them with wild type or other mutants. The other kind, represented by the Mgl and Agl mutants, cannot be stimulated. Both kinds of mutants are capable of stimulating Cgl mutants, though only heterologous Cgl mutants can stimulate each other.

Among Cgl mutants there is a striking correspondence between stimulation phenotype and map position. Each of the five types, CglB, CglC, CglD, CglE, and CglF, controls a different stimulation because each can be stimulated to move by contact with cells of a different type, but not by cells of the same type. For example, a CglB and a CglC mutant can stimulate each other, but two CglB mutants cannot stimulate each other. The mutations in all 17 independent CglB mutants tested are very closely linked to each other, defining a single locus, *cglB*. Within the limits of the tests performed, we have found no sites of other mutant types within *cglB*. These tests include all combinations of *cgl* mutants and half of the possible pairwise crosses between representatives of all *cgl* and *agl* loci. No cis-trans tests have been applied so the *cglB* locus may be one gene or a cluster of adjacent genes. In a similar way CglC mutants define a unique locus, *cglC*, CglD mutants define the locus *cglD*, the CglE mutants, *cglE*, and the single known CglF mutant, *cglF*. There is loose linkage between *cglB*, *cglC*, and *cglD*. The one to one correspondence between locus and phenotype argues that stimulation is characteristic of the locus and not of particular mutations within it.

Does stimulation play a role in the swarming of motile cells? Single, isolated DK101 cells can move (Table 3), showing that recent contact with other cells is not required for movement. Nevertheless, stimulation may increase, maintain or modify movement. This is possible because (i) stimulation is efficient: The amount of movement observed at the edge of a spot containing a mixture of two stimulatable nonmotile mutants resembles that at the edge of a spot of motile cells and (ii) motile cells are capable of stimulating *cgl* mutants (Hodgkin and Kaiser, 1977). The phenomenon of stimulation also raises the ques-

tion whether it reflects the transfer of substances between cells. Transfer is consistent with the observation that stimulation requires that cells be close enough together to touch (Hodgkin and Kaiser, 1977).

Mutants that are unable to respond to stimulation define 17 distinct loci, the *mgl* locus and 16 different *agl* loci, which are organized into at least three clusters. The distribution of numbers of mutants among the *agl* loci can be compared with a Poisson distribution over various total numbers of hypothetical loci. The observed distribution matches most closely that expected for 20 loci, consequently, most of the *agl* loci in *Myxococcus* may have already been detected. However, *agl* mutants that belong to loci having an intrinsically unstable phenotype would not have been retained and ones that are lethal would not have been isolated.

Burchard et al. (1977) have reported that *mgl-1* has a structural alteration in a submembrane fiber which they suspect might be part of the gliding machinery. However, *mgl-4* and *mgl-5* were also examined and showed no change.

Here it is argued that the *cgl* and *agl* loci control the movement of single cells. In the following paper (Hodgkin and Kaiser, 1979) evidence will be presented that at least 8 loci, called *sgl* loci (including *sglA*) and one *tgl* locus control the movement of groups of cells, and that *mgl* function is needed for both single and group cell movement.

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