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A link between cell movement and gene expression argues that motility is required for cell-cell signaling during fruiting body development

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Nonmotile mutants of Myxococcus xanthus (Myxobacterales) failed to execute the morphogenetic movements required to shape a fruiting body. In addition, nonmotile mutants produced very few spores when plated for fruiting body development at cell densities appropriate for wild-type cells. At higher initial cell densities, the proportion of nonmotile cells that sporulate increased, indicating that one important function of motility in fruiting body development is to increase the local cell density. However, even at 10 times normal cell density, nonmotile cells sporulated at only 1% the wild-type level. This sporulation deficiency of nonmotile mutants accompanies an altered pattern of gene expression, monitored by using transcriptional fusions of lacZ to genes expressed at specific times during fruiting body development. Motility was not required for normal expression of five lac fusions that are expressed within the first 6 hr of fruiting-body development. However, the levels of expression from five lac fusions to later-expressed genes were reduced or abolished in nonmotile strains. β-Galactosidase expression in these late Tn5 lac insertions was increased, and fruiting body development occurred in certain nonmotile strains that can be stimulated to move when mixed with a donor strain. This shows that motility itself is required because the stimulated cells are nonmotile genotypically. The nonmotile mutations had the same effect on developmental β-galactosidase expression from these 10 lac fusions as an insertion mutation in the csg (formerly spoC) gene. csg mutants have a cell-cell interaction defect that blocks fruiting body development at ~6 hr. The similarity in the pattern of developmental expression of motility mutants and csg mutants suggests that motility is required for this csg-mediated cell-cell interaction.

[Key Words: Cell-cell interactions, Myxobacterales; transcriptional fusion; gliding; gene regulation]

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Myxococcus xanthus (Myxobacterales) is a Gram-negative soil bacterium that moves by gliding (Reichenbach and Dworkin 1981; Rosenberg 1984). Gliding cells move in the direction of their long axis, and they move only when they are in contact with a surface. When Myxococcus cells are starved at a high cell density on an agar or plastic surface, the process of fruiting body development is induced. The cells glide to aggregation centers and move over each other to construct mounds that have a species-characteristic size and shape. Within a nascent fruiting body, cells differentiate to resting, ovoid spores (Wireman and Dworkin 1975; Inouye et al. 1979; Zusman 1984). In this paper 'development' will be used as shorthand for 'fruiting body development,' and genes whose expression increases at some time during fruiting body development will be referred to as 'developmentally regulated' genes.

Here, we report that cell motility is required not only to bring cells to an aggregation center but also to activate expression of a particular set of developmentally regulated genes. Many nonmotile mutants of M. xanthus have been isolated. Two independent sets of genes have been found to control cell movement, and the motility governed by each set has been characterized. When the gene set called system A (for 'adventurous') is operative, the cells can move on agar whether or not they are near other cells (Hodgkin and Kaiser 1979a). On the other hand, when the set of genes comprising system S (for 'social') alone is operative, cells move only when they are near one another (Hodgkin and Kaiser 1979b). Strains that carry two mutations, one in system A and one in system S, have an A-S− genotype and are nonmotile. A second type of nonmotile mutant has been isolated in a single mutational step from wild type. Ten such one-step mutations that map to the same genetic locus, called mgl, have been found (Hodgkin and Kaiser 1979b; Stephens et al., in prep.).

Hagen et al. (1978) isolated four groups of sporulation...
mutants asg, bsg, csg, and dsg (formerly spoA, spoB, spoC, and spoD), which behave as if they are defective in developmentally necessary cell–cell interactions that precede sporulation and upon which sporulation depends. These mutants cannot sporulate alone, but they can be rescued extracellularly for sporulation by co-development with wild-type cells or with cells of a different mutant group. These cell-interaction mutants also modify the pattern of developmental gene expression (Kuspa et al. 1986; Kroos and Kaiser 1987). A transposable promoter probe, Tn5 lac [Kroos and Kaiser 1984], has been used to generate transcriptional fusions of lacZ to developmentally regulated genes in Myxococcus [Kroos et al. 1986]. The assay of β-galactosidase activity in these fusion strains provides a measure of gene expression during development. It was found that asg and bsg mutations block or reduce β-galactosidase expression from lac fusions that normally would begin to be expressed in the first few hours of development in wild-type cells. A csg mutation did not affect expression of lac fusions that are normally expressed in the first 6 hr of development but did block or reduce expression of lac fusions that would normally begin to be expressed after 6 hr of development. Here, we report that nonmotile mutants, both of the one-step mgl type and of the two-step A–S– type, exhibit the same disruption of developmental gene expression as csg mutants. Although the requirements for asg and bsg cell–cell interactions early in development appear to be met in the absence of motility, the csg interaction appears to require cell movement.

Results

A nonmotile mutant is defective in fruiting body sporulation

Normal fruiting body development begins with a phase of aggregation followed by sporulation. To test whether active cell movement is needed for fruiting body development, a one-step mutation to nonmotility, mgl-9, was transduced into the fully motile (A+S+) and fruiting-competent genetic background of DK1622, as described in Materials and methods, to construct the nonmotile strain DK3685. The mgl-9 mutant fails to produce mgl protein detectable in Western blots (Stephens el al., unpublished).

In fact, DK3685 and other mgl-9 strains derived from it do fail to aggregate under the standard conditions of development on plates. Patches of starving DK3685 cells at high density on agar remain flat with no sign of aggregation. Nor do these strains form ripples, the traveling ridges of cells that appear early in normal fruiting-body development (Reichenbach 1965, 1966; Shimkets and Kaiser 1982a).

To test whether an aggregated condition could be simulated in mgl-9 strains by raising the concentration of cells, spore levels were determined for patches of developing mgl-9 cells containing a series of increasing densities of cells [Table 1]. Under the standard developmental conditions [20 μl spots of 5 × 10⁸ cells/ml on TPM agar plates], the mgl-9 strain DK3685 formed 0.002% the number of heat-resistant spores formed by its mgl+ parent DK1622 (Table 1). As the initial cell density was raised, by up to 10-fold, the number of spores increased about 500-fold. Thus, a higher initial cell density may circumvent partially the need for aggregation. But even at the highest cell density tested [20-μl spots of 5 × 10⁹ cells/ml], the mutant produced 2 × 10⁴ spores/ml, which is only 1% the number produced by 5 × 10⁹/ml of wild-type cells. A 20-μl spot of 5 × 10¹⁰ cells/ml forms a layer of cells that is about one-quarter as high as a normal fruiting body [Kuner and Kaiser 1982]. The initial cell density was not raised beyond 5 × 10⁹/ml, as it became very difficult to produce uniform cell suspensions at higher densities.

mgl-9 alters developmental gene expression

The results of the sporulation with mgl-9 [Table 1] showed that increasing the cell density artificially did not restore sporulation levels seen in wild type. To find an additional cause of reduced sporulation, developmental gene expression was compared in mgl+ and mgl-9 strains. Ten different transcriptional fusions between developmentally regulated promoters in Myxococcus and the lacZ gene from Escherichia coli, described by Kroos et al. [1986], were made mgl-9 by transduction. The strains are listed in Table 2. These fusion strains were chosen because each one normally begins its major increase in β-galactosidase at a different time in development (expression time). The expression times for the 10 fusions chosen cover different parts of the interval from a few minutes after starvation initiates development to the time when spores begin to mature [Table 3]. Of the 10 fusions chosen, 8 permitted normal development when present in the mgl+ background. The exceptions, fusions Ω4408 and Ω4414, produce defects that are manifested later in development than their ex-
pression times so they are valid indicators of developmental gene expression [L. Kroos, A. Kuspa, and D. Kaiser, unpubl.]. For measurements of gene expression, each mgl- lac fusion strain was plated for development under the standard conditions; cells were harvested at 6, 12, 18, 24, 36, 48, and 72 hr; and the specific activity of β-galactosidase was measured in sonic extracts of each sample to obtain a time profile of enzyme activity.

The maximum values of β-galactosidase activity derived from the time profiles for each lac fusion are summarized in Table 3. Five fusions, listed in the upper part of Table 3, exhibited the same, or higher, maximal specific activities in an mgl-9 strain, as compared with those in an mgl+ strain. These are said to give a 'normal' response, neglecting overproduction for the moment. Two fusions, listed in the middle of Table 3, express β-galactosidase in the mgl-9 background, but at levels that are 25–50% of those in an mgl+ background. These are referred to as 'reduced.' Three fusions listed at the bottom of Table 3, when placed in an mgl-9 background exhibit less than a few percent of the β-galactosidase activity shown by the corresponding mgl+ strain at the time the mgl+ strain has reached its maximum β-galactosidase activity. These fusions are said to have their expression 'abolished' by mgl-9. Examples of the three types of responses are illustrated in the profiles shown in Figure 1: Ω4521 for a normal response (with overproduction), Ω4414 for a reduced response, and Ω4435 for an abolished response.

There is a clear correlation between the time that β-galactosidase activity begins its major rise [i.e., the 'expression time', as defined by Kroos et al. 1986] in wild-type mgl+ strains and the response of the fusion to mgl-9 shown in Table 3. Those lac fusions that are expressed in wild-type cells beginning in the first 5 hr of development give a normal response to mgl-9. The two giving a reduced response begin expression later in mgl+ at 9 and 10 hr. Finally, the three fusions whose expression is abolished by mgl-9 begin expression in mgl+ at 15 hr or later.

Nonmotile mutants of the A-S- type alter developmental gene expression

The pattern of developmental gene expression in the mgl- strain was compared with that in four genetically different A-S- strains to determine whether the pattern correlated with the lack of motility. Figure 2 is a graph of maximum specific β-galactosidase activity observed from 10 different lac fusions, plotted according to their expression times. Data are presented for mgl-9 as wide bars below the midline, and for four different A-S- strains as thin bars above the midline, with the same set of lac fusions. Comparing length of bars above and below the midline, a similar pattern of specific enzyme activities is evident in nonmotile mutants of the A-S- type and the nonmotile mgl-9. In particular, lac fusions normally expressed at 5 hr or before are expressed normally and have a relative specific β-galactosidase activity of about 1, except for the fusion Ω4521 expressed at 1.5 hr, which is overexpressed in both mgl-9 and three of the four A-S- strains. lac fusions whose expression times are 9 and 10 hr are reduced similarly in all four A-S- and mgl-9 strains; fusions normally expressed at 15, 25, and 30 hr show little, if any, β-galactosidase activity in mgl-9 or any of the A-S- nonmotile mutants.

A second kind of experiment that demonstrates the correlation between motility and developmental gene expression utilizes motility stimulation. Cells of genotype cgl- tgl- are nonmotile (A-S-), yet can have their motility temporarily restored by contact with cgl+ tgl+ cells [Hodgkin and Kaiser 1977, 1979a, b]. Stimulation is locus specific; and nonmotile cells, as long as they are cgl- tgl-, can stimulate cgl- tgl- strains [Hodgkin and Kaiser 1977]. For the experiment, stimulatable cells of the genotype cgl tgl Tn5 lac Ω4435 or Ω4401 were employed. The fusions Ω4435 and Ω4401 are normally expressed at 25 and 30 hr in development, and their expression was abolished in mgl-9 or A-S- strains. These cells are nonmotile, as demonstrated by their sharp-edged colonies (Fig. 3a). When they are placed under conditions that induce development, they fail to form...
Table 3. β-Galactosidase expression in mgl* and mgl-9 strains

| Expressionb | Specific activityc | Response to mgl-9 |
|-------------|-------------------|------------------|
| time in mgl* [hr] | β-galactosidase activityd | at maxd | at t maxd | at maxd |
| Ω* | in mgl* | in mgl-9 | in mgl-9 |
| 4408 | 1 | 38 | 23 | 39 | normal |
| 4521 | 1.5 | 140 | 440 | 460 | normal |
| 4455 | 3 | 470 | 570 | normal |
| 4469 | 5 | 430 | 320 | 470 | normal |
| 4273 | 5 | 63 | 38 | 110 | normal |
| 4514 | 9 | 280 | 150 | 150 | reduced |
| 4414 | 10 | 1100 | 320 | 420 | reduced |
| 4403 | 15 | 190 | 8 | 25 | abolished |
| 4435 | 25 | 450 | 18 | 18 | abolished |
| 4401 | 30 | 220 | 2 | 2 | abolished |

*a The site of Tn5 lac insertion is designated by Ω, followed by a number. Strain numbers are given in Table 2.

b An estimate of when β-galactosidase specific activity begins its major increase during development (Kroos et al. 1986).

c Values are the specific activity of β-galactosidase at the specified time minus the specific activity in t = 0 samples (i.e., cells sedimented from growth medium and resuspended in starvation buffer). The units of β-galactosidase specific activity are nmoles ONP/min/mg/protein. Typical values for t = 0 samples can be found in Kroos and Kaiser (1987).

d Values are the highest β-galactosidase specific activities measured during a 72-hr developmental time course for mgl* (column 3), for mgl-9 (column 4) at the time (t max) when mgl-9 reaches its peak, and for mgl-9 (column 5) at its own peak.

e The effect of mgl-9 on developmental β-galactosidase expression from each lac fusion is summarized. Normal indicates β-galactosidase expression began at the same time and reached the same or higher specific activity in the mgl-9 lac fusion strain than in the wild-type lac fusion strain. Reduced indicates that β-galactosidase expression began at the same time but increased less rapidly and reached a lower maximum in the mgl-9 mutant strain than in wild type [see text for the quantitative limits]. Abolished indicates that no developmental expression occurred in the mgl-9 mutant.

f The maximum β-galactosidase specific activity was observed when the 72-hr sample was sonicated with glass beads to disrupt spores (Kroos et al. 1986). Sonication with glass beads did not change the β-galactosidase specific activities of 72-hr samples of mgl- lac fusion strains, as expected, because these samples contain very few spores.

Discussion

mgl mutant cells fail to aggregate, and they produce very few spores when plated for development at cell densities adequate for fruiting body development of wild-type cells. At higher initial cell densities the proportion of mgl-9 cells that sporulate increases (Table 1), indicating that one important function of motility in Myxococcus development is to increase the local cell density, perhaps to establish cell—cell contacts during the aggregation phase of early development. However, even at the highest cell density tested, mgl-9 cells sporulate at only 1% the level seen for wild-type cells at a 10-fold lower density. The mgl-9 mutation also reduces or abolishes the expression of several developmentally regulated genes that are normally expressed after 9 hr, during the postaggregation phase of development. Genes expressed early in development are unaffected by mgl-9.

The finding that mgl-9 prevented the expression of certain developmentally regulated lac fusions raised a question of whether mgl might encode a regulatory protein that happens to control both gliding and developmental gene expression. This possibility was examined by testing the effect of other mutations that block gliding. Four nonmotile mutants, which carry different pairs of mutations in the A and S motility systems, were tested. The two A-S- strains revealed an altered pattern of β-galactosidase expression from lac fusions that was parallel to that of mgl-9. In addition, an experiment employing motility stimulation (Hodgkin and Kaiser 1977) showed that expression of the lac fusions Ω4401 and Ω4435 can occur if the cells are stimulated to move, even when they are genotypically nonmotile. Stimulation also allowed fruiting body aggregates to form, indicating that other developmental functions needed for aggregation that are motility dependent can also occur following stimulation. The requirement for motility during fruiting body development is first manifest between 5 and 9 hr after starvation: All five lac fusions that normally begin β-galactosidase expression at or before 5 hr of development were expressed normally in the nonmotile mgl-9 or A-S- mutants, whereas all five lac fusions
Motility and developmental gene expression

Figure 1. Kinetics of developmental gene expression, comparing the effects of mgl-9 and csg mutations on gene expression. Wild-type (●), mgl-9 (○), and csg :: Ω205 (▲) strains containing Tn5 lac insertion Ω4521 (a), Ω4414 (b), or Ω4435 (c) were plated for development and harvested at various times for determination of β-galactosidase specific activity (nmole ONP/min • mg of protein), as described in Materials and methods. The △ in c indicated the β-galactosidase specific activity released from wild-type samples sonicated with glass beads, a procedure that breaks open spores (Teintze et al. 1985).

Figure 2. Maximum specific β-galactosidase activity reached from each of 10 developmentally regulated lac fusions, each one in five different nonmotile strains. Data for each lac fusion are clustered according to β-galactosidase expression time in wild-type for each fusion, shown on the x axis. Note that the time scale is not linear. Thus, the data at 1 hr are all for fusion Ω4408, at 1.5 hr for Ω4521, at 3 hr for Ω4455, at 5 hr for Ω4469, at 5 hr (second one) for Ω4273, at 9 hr for Ω4514, at 10 hr for Ω4414, at 15 hr for Ω4403, at 25 hr for Ω4435, and at 30 hr for Ω4401. Four different A−S− genotypes are shown above the horizontal midline. The length of each vertical line represents the maximum specific activity in the nonmotile (A−S−) strain relative to that in the wild-type (A+S+) strain (DK1622). The A−S− combinations are shown in the same order for each cluster; the order reading left to right is aglE2 sglB46, aglB1 tgl-1, aglB1 sglC1, and cglB2 sglC1. Maximum specific activity for the mgl-9 nonmotile is shown by the length of the wide bar extending below the midline. Data for mgl-9 were obtained in the same experiment, and their specific activity is expressed relative to the same fusions in DK1622.

that normally begin expression after 9 hr of development, like Ω4401 and Ω4435, had reduced expression or failed to be expressed.

β-Galactosidase expression pattern in nonmotile mutants parallels that in csg cell interaction mutants

Nonmotile cells exhibit a specific disruption of gene expression evident in developmentally regulated lac fusion strains. Kuspa et al. (1986) and Kroos and Kaiser (1987) have examined the effect of three classes of cell-interaction mutants asg, bsg, and csg on the same set of lac fusions. Comparison of these data reveals a parallel between the consequences of loss of motility and the consequences of a null mutation in the cell-interaction locus csg. The β-galactosidase data for mutations in two different asg genes, for an insertion mutation in a bsg gene, and for an insertion in the csg gene are summarized in Table 5, where they can be compared with the corresponding pattern for mgl or A−S− nonmotile mutants. Table 5 indicates whether the expression of β-galactosidase during development for each of the 10 lac fusions is normal, i.e., approximately the same or higher than wild type; reduced, i.e., between 15% and 65% that of wild type; or abolished, i.e., <15% that of wild-type.

No similarity is evident between the nonmotile mutants and asg or bsg mutants, as shown in Table 5. However, the pattern for the nonmotile mutants and for csg is the same. The parallel between nonmotile mutants and csg mutants not only includes the identity of lac fusions that are expressed but also the kinetics of β-galactosidase-specific activity in individual lac fusion
strains. For example, in one set of experiments, higher than normal maximal β-galactosidase specific activities were observed of mgl-9 lac fusion strains containing Ω4455, Ω4469, and Ω4273 [Table 3], higher than normal levels were also observed for csg strains containing these same lac fusions [Kroos and Kaiser]. For these particular fusion strains, β-galactosidase activity in the mgl-9 and csg derivatives paralleled the activity in the wild-type strain until it reached a peak, but it continued to increase in the mutants at a point where activity had fallen in the wild-type strain. For the fusion Ω4521, β-galactosidase expression reached a higher peak in the csg derivative than in the wild-type strain at the time of the wild-type peak, and expression from Ω4521 reached an even higher peak in the mgl-9 derivative [Fig. 1a]. Both the mgl-9 and the csg mutations reduced expression from lac fusion Ω44414 to similar extents [Fig. 1b]. Finally, the level of expression of Ω4435 is abolished in the same way by csg and mgl-9 [Fig. 1c].

Kroos and Kaiser [1987] showed that asg+ and bsg+ functions precede csg+ function on the same regulatory pathway. csg mutants behave as though they are unable to produce a cell interaction necessary to continue development and associated gene expression beyond 6 hr [Kroos and Kaiser 1987]. Cell movement might be required for the csg-mediated cell interaction to generate, transfer, or receive a ‘C-signal.’

It appears that at the high initial cell densities employed standardly in our experiments to study development, motility is not essential until about the same time that the csg+-dependent cell–cell interaction is required. In contrast, the asg+ - and bsg+-dependent cell–cell interactions must occur in the absence of movement, as two lac fusions [Ω4521 and Ω4273] that are partially bsg+-dependent [Kroos and Kaiser 1987] and absolutely asg+-dependent [Kuspa et al. 1986] are expressed normally or are overexpressed in the nonmotile strains [Table 3 and 5].

The suggested link between motility and C signaling may help to explain why csg mutants fail to form ripples [Shimkets and Kaiser 1982]. Ripples are ridge-shaped accumulations of cells that move in the synchronous, pulsating manner of traveling waves and are usually generated early in fruiting-body development [Reichenbach 1965, 1966, Shimkets and Kaiser 1982]. If C-signaling caused cells to move, then a positive feedback loop would be created [movement, C-signaling, movement, etc.], which could result in an oscillation that would produce rippling. The feedback loop would be broken, either by loss of motility or a mutation in csg, and both have been observed to be needed for rippling [Shimkets and Kaiser 1982; Shimkets et al. 1983].

Table 4. Motility stimulation increases development-specific β-Galactosidase activity

| Strain  | Motility genotype | Tn5 lac insertion | unstimulated | stimulated by a nonmotile strain [DK4141] | stimulated by a wild-type strain [DK1622] | stimulated by a csg- strain [DK2634] |
|---------|------------------|------------------|--------------|---------------------------------------------|-------------------------------------------|-------------------------------------|
| DK6155  | cgF1 tgl-3       | Ω4435            | 43           | 110                                         | 245                                       | 219                                 |
| DK6165  | cgE4 tgl-1       | Ω4435            | 27           | 81                                          | 163                                       | 147                                 |
| DK6151  | cgF1 tgl-3       | Ω4401            | 23           | 78                                          | 145                                       | 129                                 |
| DK6161  | cgE4 tgl-1       | Ω4401            | 15           | 47                                          | 96                                        | 105                                 |

Experimental protocol is given in Materials and methods.

* Specific activity in nmoles ONP/min/mg/protein. Values listed are for the maximum β-galactosidase activities measured during the 72-hr developmental time course. To disrupt spores, glass beads were added to the harvested cells, as described in footnote f to Table 3.
Table 5. Expression patterns in cell-interaction and nonmotile mutants

| Expression time (hr) | Ω | asg | bsg | csg | mgl-9 | A-S- |
|---------------------|----|-----|-----|-----|-------|------|
| 4408                | 1  | n   | r   | n   | n     | n    |
| 4521                | 1.5| a   | r   | n   | n     | n    |
| 4455                | 3  | n   | r   | n   | n     | n    |
| 4469                | 5  | n   | r   | n   | n     | n    |
| 4273                | 5  | a   | r   | n   | n     | n    |
| 4514                | 9  | a   | r   | r   | r     | r    |
| 4414                | 10 | a   | a   | r   | r     | r    |
| 4403                | 15 | a   | a   | a   | a     | a    |
| 4435                | 25 | a   | a   | a   | a     | a    |
| 4401                | 30 | a   | a   | a   | a     | a    |

The responses of asg, bsg, and csg mutants are taken from Kuspa et al. (1986) and Kroos and Kaiser (1987). The responses for mgl-9 are from Table 3; responses for A-S- are from Fig. 2. * [n] Normal; [r] reduced; [a] abolished.

Materials and methods

Counting spores during fruiting body development

Cells growing exponentially in CTT liquid medium (Hodgkin and Kaiser 1977) were sedimented at 10,000g for 10 min at 5°C and suspended in TPM buffer [10 mM Tris-HCl (pH 7.5), 1 mM K2HPO4, 8 mM MgSO4] at densities ranging from 5 × 109 to 5 × 1010 cells ml, and 20 µl aliquots were spotted on TPM agar (TPM plus 1.5% agar) in the wells of a 24-well tissue-culture plate (Falcon, Lincoln Park, New Jersey). The number of sonication- and heat-resistant viable spores after 3 days of development at 32°C was determined. Samples were incubated for 2 hr at 50°C and then harvested by adding 0.5 ml of TPM buffer to the well, dislodging the cell mat with a bent loop, and sonicating the suspension for 45 sec. Heating resulted in a 107-fold loss in viability of vegetative cells and no loss of viability of myxospores, and the sonication in a 10-fold loss in viability of vegetative cells and no loss of viability of myxospores (Hagen et al. 1978). Appropriate dilutions of the spore-containing samples were made in TPM buffer and mixed with 2.5 ml of CTT soft agar (0.7%) for plating on CTT agar (1.5%) plates. Plates were incubated for 6 days at 32°C to allow spores to germinate and form colonies, which were then counted.

Construction of mgl-1 Tn5 lac derivatives

The mgl-9 mutations, used to construct nonmotile Tn5 lac derivative, is linked genetically to Tn5 insertion Ω1901 (Sodergren and Kaiser 1983; Stephens and Kaiser 1987). The kanamycin resistance [Km'] marker gene of Tn5 Ω1901 was replaced with a tetracycline resistance [Tc'] marker gene (Avery and Kaiser 1983) so that the mgl-9 mutation could be introduced into Km' Tn5 lac strains by generalized transduction and selection for Tc'. In practice, M. xanthus strain DK3685 containing mgl-9 and Tc' at Ω1901 was constructed as described below and was used as the donor in myxophage Mx8 transductions. A liquid phage stock of Mx8 clp2 was grown on DK3685, as described previously (Kroos and Kaiser 1987), and was used to transduce Tn5 lac insertion-containing strains that have been described (Kroos et al. 1986) to Tc', also described (Avery and Kaiser 1983). The Tc' of transductants was scored by transfer to CTT agar plates containing 12.5 µg/ml oxytetracycline. Nonmotile Tc' transductants arose at the expected frequency [80–85% of Tc' colonies], based on previous studies of genetic linkage between Ω1901 and mgl-9 mutations (Sodergren and Kaiser 1983; Stephens and Kaiser 1987). For each strain constructed, 10–15 nonmotile, Tc' transductants were transferred to CTT agar plates containing 40 µg/ml kanamycin sulfate to test for retention of the Tn5 lac insertion. All nonmotile Tc' transductants were also Km' for all strains constructed, indicating an absence of genetic linkage (i.e., 7–10% cotransduction) between any of the Tn5 lac insertions listed in Table 2 and Tc' at Ω1901 among nonmotile transductants. The nonmotile, Tc', Km' transductants were screened for β-galactosidase activity during growth and development on plates containing 5-bromo-4-chloro-3-indolyl-β-d-galactoside (X-Gal), as described previously (Kroos et al. 1986). For each cross, one transductant that displayed colony color (on the X-Gal plates) typical of the majority of transductants for the cross was chosen for further study and given a strain number (see Table 2). As described previously (Kuspa et al. 1986), in such crosses, transductants with aberrant β-galactosidase expression arise at a low frequency (0.2%) and the colony color screen avoids their isolation.

The M. xanthus donor strain DK3685 containing mgl-9 and Tn5 Tc' at Ω1901 was constructed as follows: DK371 (Hodgkin and Kaiser 1979a), which carries the mgl-9 mutation, was the recipient of an Mx8 transduction (Avery and Kaiser 1983) in which DK1901 carrying Tn5 Ω1901 was the donor; a nonmotile Km' transductant was chosen and was the donor of an Mx8 transduction with the wild-type strain DK1622 as the recipient; a nonmotile Km' transductant was chosen and was infected with P1 :: TnS (bacteriophage P1 bearing a Tc' version of Tn5), as described by Avery and Kaiser (1983), to obtain Tc' at position Ω1901. The resulting nonmotile, Tc' Km' strain, DK3685, has Tc' at the Ω1901 site, as verified by Southern blot hybridization analysis (data not shown), and was used to transduce the mgl-9 mutation into lac fusion strains, as described above.

Construction of A-S- Tn5 lac derivatives

The generalized transducing myxophage Mx4 ts18 ts27 (Campos et al. 1978) was used to introduce Tn5 lac insertions into four different A-S- strains. As the transducing capacity of Mx4 is ~50 kb of DNA, Tn5 lac from the donor is transferred to the recipient by homologous recombination, because recombination is more frequent than transposition, thus preserving the original site of Tn5 lac insertion and its corresponding regulation (Kroos et al. 1986). Plate stocks of Mx4 grown on the 10 donor strains carrying Tn5 lac Ω4273, Ω4401, Ω4403, Ω4408, Ω4414, Ω4435, Ω4455, Ω4469, Ω4514, and Ω4521 (Kroos et al. 1986) were used to transduce A-S- recipients to Km', as described elsewhere (Avery and Kaiser 1983). The A-S- strains used were DK1246 [aagB2, sgl646], DK1250 [aagB1, sgl1], DK1259 [aagB1, sglC1], and DK1261 [aagB2, sglC3], described previously (Hodgkin and Kaiser 1979b). Nonmotile, Km' transductants were selected and used in the subsequent developmental β-galactosidase assays, as described below. For consistency, the set of nonmotile strains having the same Tn5 lac insertion was assayed as a group. For example, wild-type, mgl-9 and each of the four A-S- strains carrying the insertion Ω4414 were all assayed at the same time, starting with the spotting of cultures on TPM agar plates through the β-galactosidase and protein assays, described below.
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Measurement of developmental β-galactosidase expression

Expression of β-galactosidase from Tn5 lac insertion-containing M. xanthus strains was quantitated, as described previously [Kroos et al. 1986]. Cells growing exponentially in CTT liquid medium were sedimented and resuspended in TPM buffer to a density of 5 × 10^6 cells/ml. A 0.1-ml aliquot was added to 0.3 ml of TPM and was stored at −20°C for later determination of β-galactosidase activity in growing (vegetative) cells. This aliquot was the r = 0-hr sample. For development, 20-μl aliquots were spotted on TPM agar plates, the spots were allowed to dry for 1 hr at room temperature, and plates were incubated at 32°C. At various times, cells were scraped from the agar into TPM buffer and stored at −20°C until all samples were collected. The specific activity of β-galactosidase [1 unit = 1 nmole o-nitrophenol (ONP) produced per minute per milligram protein] in each sample was determined after sonication to disrupt the cells.

Stimulation

The two Myxococcus strains, DK1252 [cglF1, tgl3] and DK1255 [cglE4, tgl1], carry mutations in both motility systems [Hodgkin and Kaiser 1979b], which render these strains stimulatable. Into these two genetic backgrounds, Tn5lac insertions Δ4401 and Δ4435 were introduced by Mx4 transduction to generate the four different stimulatable A−S− strains in Table 4. These can be stimulated to move transiently by a donor strain of M. xanthus, which is cgl+, tgl+ [Hodgkin and Kaiser 1979b]. The donor strains tested were DK4141 (mgl-9, which is nonmotile itself, but cgl+, tgl+), DK1622 (wild type), and DK2634 (csg−, a fully motile strain that is defective as a donor for C−signal). None of the three donor strains have Tn5 lac, and they produce no β-galactosidase themselves.

For a stimulation experiment, recipient strains were grown at 33°C in 10 ml of CTT liquid plus kanamycin monosulfate (40 μg/ml, Sigma) to a density of 100–200 Klett U/ml. Cells were sedimented at 12,000g for 10 min and resuspended in a volume of TPM, which would yield a density of 1000 Klett U/ml. Donor strains were treated identically, except that the original culture medium did not contain kanamycin. Cells (recipient strain plus donor strain) were mixed in a 1:1 ratio prior to spotting the mixture on a TPM plate for development. The protocol for induction of development was identical to that described in the preceding section. Mixtures were spotted in 20- and 40-μl aliquots to control for the presence of the donor cells (because the normal developmental conditions are based on 20-μl spots containing a single strain). The protein concentration attributed to the recipient (one-half the total concentration) was used to calculate the specific activity of β-galactosidase, because only the recipient carried a lacZ gene. To ensure that equal amounts of the two strains had been mixed, each of the original TPM cell suspensions, prior to mixing, was also spotted in 10- and 20-μl aliquots on TPM plates to determine the background levels of β-galactosidase and the protein concentration contributed by each strain.

The mixtures of recipient A−S− cells and donor cells were also spotted on CTT plates to detect motility stimulation by light microscopy, using the criteria of Hodgkin and Kaiser [1977, 1979b].

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