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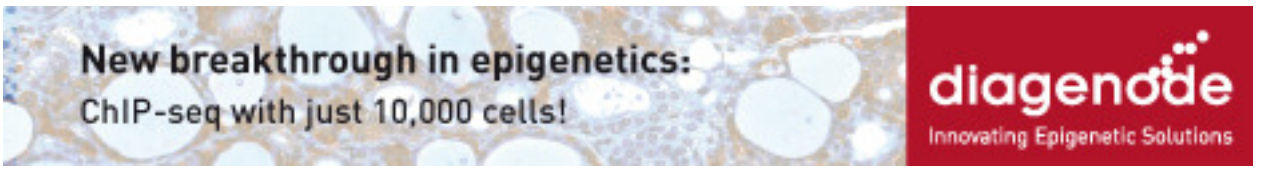

# Cell motility is required for the transmission of C-factor, an intercellular signal that coordinates fruiting body morphogenesis of *Myxococcus xanthus*

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**There are striking similarities between the developmental phenotypes of two different mutant classes of *Myxococcus xanthus*. The first class, *mglA* mutants, are nonmotile under all conditions tested. The second class, *csgA* mutants, are motile but belong to a class of signal-defective developmental mutants that cannot develop alone but will develop when mixed with intact wild-type cells. Nevertheless, both *csgA* and *mglA* mutants fail to aggregate properly or to sporulate when induced to form fruiting bodies. An *mglA* mutation and a *csgA* mutation affect expression of a panel of *lacZ* fusions to developmental genes in the same way, indicating that nonmotile cells and *csgA* cells arrest development at a similar stage. One explanation for the similarity of developmental phenotypes between these mutants is that motility is required for the *csgA*-mediated cell interaction. In support of this hypothesis, we report that C-factor, a protein purified from nascent wild-type fruiting bodies based on its ability to rescue *csgA* mutant fruiting body development, also rescues sporulation and expression of β-galactosidase from developmentally controlled *lacZ* fusions in *mglA* strains, apparently without restoring their motility. Wild-type levels of active C-factor can be purified from *mglA* cells, yet intact *mglA* cells do not rescue *csgA* cells upon cell–cell mixing. Intact wild-type cells are unable to restore the sporulation and β-galactosidase expression of *mglA* mutants. These results support the hypothesis that donor and responder cell motility is required for C-factor transmission between cells during development. We propose that proper intercellular C-factor transmission may require a critical spatial orientation of cells achieved only after cells move into the dense, aligned organization of a nascent fruiting body.**

[*Key Words*: Cell–cell signaling; Myxobacteria; signal transduction; gliding motility; morphogenesis; sporulation]

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Cell interactions establish cell fates in the development of multicellular organisms. Well-studied examples include antigen presentation in the vertebrate immune response (Hood et al. 1984), ommatidial developmental in *Drosophila melanogaster* (Tomlinson 1989), and vulva formation in *Caenorhabditis elegans* (Sternberg and Horvitz 1989). In each case, a short-range cell interaction is restricted by the relative positions of nearby or adjacent signal-producing and signal-responding cells. Cell interactions that require movement of cells into the proper signaling positions also appear to govern multicellular development and cellular differentiation of the gram-negative bacterium *Myxococcus xanthus*, an organism whose prokaryotic genetic system and cellular organization offer experimental advantages (Rosenberg 1984).

Upon starvation at a high cell density on a solid surface, rod-shaped *M. xanthus* cells glide to aggregation centers, where they construct a multicellular structure called a fruiting body. During early aggregation, cells move as traveling waves like ripples on the surface of water (Reichenbach 1965). Within a nascent fruiting body, cells differentiate into dormant, ovoid spores (Wireman and Dworkin 1975). Construction of a fruiting body from ~100,000 cells requires ~20 hr from the removal of nutrient to the onset of sporulation. Maturation of environmentally resistant spores occurs over the next several days.

Control of *M. xanthus* fruiting body development by cell–cell interactions is implicated by the existence of four classes of nonautonomous developmental mutants (Hagen et al. 1978; LaRossa et al. 1983; Janssen and Dworkin 1985; Kroos and Kaiser 1987). *asg*-, *bsg*-, *csg*-, and *dsg*-class mutants cannot sporulate alone but can sporulate when mixed with wild-type cells or with mutants from a different class. Rescue of sporulation occurs extracellularly and not from genetic exchange.

The maximum value of β-galactosidase activity pro-

duced by transcriptional fusions of *lacZ* to developmentally regulated genes and the time when the major increase in β-galactosidase synthesis begins (the expression time) provide measures of developmental promoter activity in *M. xanthus* (Kroos and Kaiser 1984; Kroos et al. 1986). Assay of β-galactosidase activity in *asg*, *bsg*, *csg*, or *dsg* strains containing *lacZ* fusions indicates that developmental gene expression is disrupted in these cell-interaction mutants but can be restored by extracellular complementation (Gill and Cull 1986; Kuspa et al. 1986; Kroos and Kaiser 1987; Cheng and Kaiser 1989). Each group arrests development at a different stage, judged by morphology and by gene expression within the panel of fusions.

Members of the *csg* group, which result from mutation of a single locus called *csgA* (Shimkets et al. 1983; Shimkets and Asher 1988), have been examined in detail. Unlike wild-type cells, which aggregate into compact mounds by 12 hr on a defined starvation agar medium, *csgA* mutants form more diffuse mounds and ridges of cells only after ~18 hr. Under the more stringent starvation conditions possible in submerged culture (Kuner and Kaiser 1982), *csgA* cells fail to construct any detectable multicellular structures. *csgA* cells also fail to ripple or to sporulate (Shimkets and Kaiser 1982). Expression of *lacZ* fusion genes transcribed >6 hr into development is reduced or abolished in a *csgA* mutant. Thus, *lacZ* fusions expressed after 6 hr are called "C-dependent" to distinguish them from *lacZ* fusions expressed before 6 hr, which are "C-independent" (Kroos and Kaiser 1987).

Recent experiments have suggested that cell motility is required for the *csgA*-directed cell interaction. Gliding of *M. xanthus* is controlled by two independent sets of genes (Hodgkin and Kaiser 1979). Cells defective in motility system A (for adventurous) fail to glide independently of one another. Cells defective only in motility system S (for social) can move when they are completely isolated but cluster infrequently as small rafts of cells, even when they are close together. Nonmotile strains may result from double mutations, one inactivating the A system and one inactivating the S system, or from a single mutation at the *mglA* (for mutual gliding function) locus, which has been cloned and sequenced (Stephens et al. 1989). Like *csgA* null mutants, nonmotile cells resulting from mutation in *mglA* are defective in aggregation, rippling, and sporulation (Kroos et al. 1988). Most strikingly, an *mglA* mutation and a *csgA* mutation affect expression of a panel of *lacZ* fusions to developmental genes in the same way (Kroos et al. 1988).

Sequence studies of the *csgA* gene indicate that it could specify a 17.7-kD protein and that *csgA* transcription increases threefold during the first 24 hr of development (Hagen and Shimkets 1990). We recently purified from extracts of nascent fruiting bodies a membrane-associated protein of $M_r$ 17,000. This protein, named C-factor, rescues *csgA* mutant aggregation, sporulation, and gene expression to wild-type levels when added at 1 nM. The size, amino-acid sequence, pattern of expression, and the recognition of C-factor by purified anti-*csgA* antibodies indicate that C-factor is the product of the *csgA* gene (Kim and Kaiser 1990a,b).

If the developmental defects of nonmotile mutants arise from failure to complete the *csgA*-mediated cell interaction, it may be possible to relieve these defects with purified C-factor. In this study we report that added C-factor can restore both sporulation and β-galactosidase production from developmentally expressed *lacZ* fusions to *mglA* mutants. Experiments characterizing this rescue of *mglA* sporulation and gene expression suggest that C-factor signaling occurs over a short range, perhaps limited to directly adjacent cells. We propose that cell movement establishes a spatial pattern of cells crucial for subsequent C-factor transmission.

## Results

### *Purified C-factor restores* mglA *developmental sporulation and gene expression*

Neither aggregates nor refractile spores are formed by *mglA* mutants, as shown in Figure 1a. *csgA* mutants also fail to form detectable multicellular aggregates or spores (Fig. 1d). The similar effects of loss of motility and loss of a *csgA* null mutation on aggregation, sporulation, and developmental gene expression patterns (see below) suggest that nonmotile cells may be defective in the same cell-signaling interaction as *csgA* mutants. If there is a signaling defect, *mglA* cells may fail to produce the *csgA*-mediated signal, to transmit it, or to respond to it. To distinguish these cases, wild-type cells or 1 nM purified C-factor were added to *mglA* cells. These treatments allowed *csgA* cells to form normal levels of darkened, 100-μm-diameter fruiting bodies containing many spores and surrounded by a halo of individual spores (cf. Fig. 1e,f, with d; Kroos and Kaiser 1987; Kim and Kaiser 1990a).

Codevelopment of wild-type cells with *mglA* mutant cells increased *mglA* sporulation as much as 14-fold over background in one case but never to more than several percent of wild-type levels (cf. data columns 1 and 3 in Table 1). Unlike the input nonmotile cells in these mixing experiments, the input wild-type cells sporulated at normal levels (Table 1) in fruiting bodies that were small but of expected number (cf. Fig. 1c,f). A mixture of *csgA* cells (DK5210) with *mglA* cells did not increase *mglA* sporulation over background levels (Table 1, data column 4). The nonmotile strains in these experiments contained developmentally regulated *lacZ* fusions whose β-galactosidase expression was either reduced or abolished as a consequence of nonmotility. The data in Figure 2 show that the low level of β-galactosidase expression in *mglA* mutants is not raised by codevelopment of *mglA* mutants with wild-type strain DK1622. In control experiments, wild-type cell addition raised *csgA* sporulation and developmental gene expression to essentially wild-type levels (Table 1, data column 3; Fig. 2).

In contrast to these results from cell mixing, addition of purified C-factor was found to restore both *mglA* de-

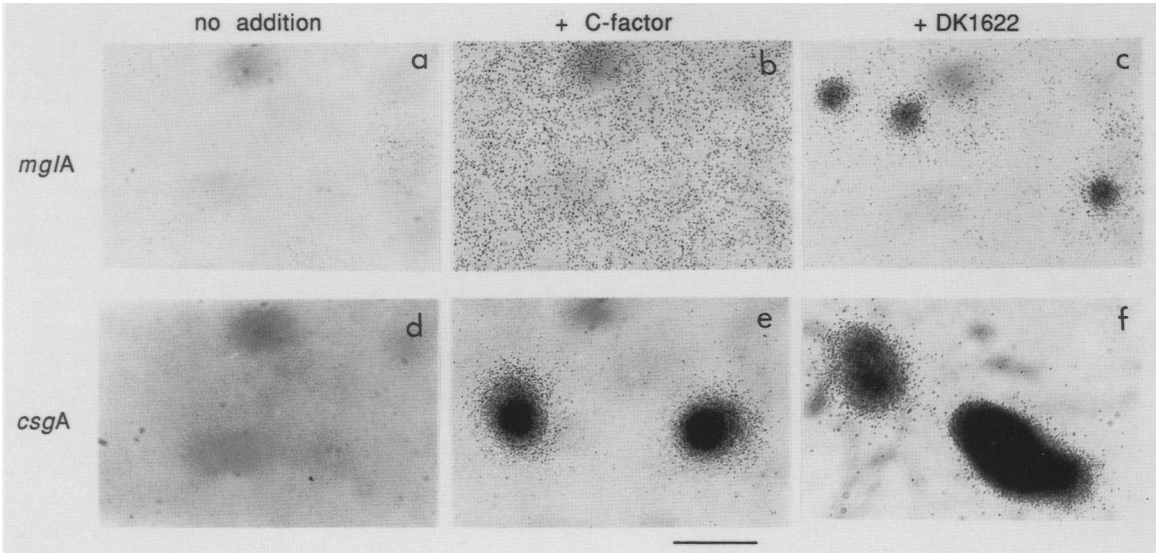


**Figure 1.** C-factor rescues sporulation of *mglA* and *csgA* mutants. *mglA* or *csgA* responder cells ($2.5 \times 10^8$) were resuspended in a developmental buffer (see Materials and methods). (*a*) *mglA* mutant cells fail to form detectable aggregates or refractile spores. (*b*) Dark dots are individual refractile *mglA* spores formed after a 3-day incubation of 1 unit of purified C-factor with *mglA* strain DK4167. (*c*) Spore-filled fruiting bodies of slightly reduced size resulting after mixture of wild-type cells with an equal number of *mglA* cells. (*d*) *csgA* mutant cells fail to form detectable aggregates or refractile spores. (*e*) Spore-filled fruiting bodies formed after a 3-day incubation of 1 unit of purified C-factor with *csgA* strain DK5253. (*f*) Spore-filled fruiting bodies resulting from mixture of wild-type cells with an equal number of *csgA* cells. Bar, 200 μm.

velopmental sporulation and gene expression to wild-type values. Table 1, data (column 2) shows that 1 unit of C-factor rescues *mglA* mutant sporulation as efficiently as it rescues *csgA* sporulation. In these experiments, C-factor caused *mglA* mutants to form spores without multicellular aggregation, as shown in Figure 1b. Individual *mglA* spores are distributed throughout the cell mat, and there is no aggregation, whereas similarly treated *csgA* cells form darkened, spore-filled aggregates (cf. Fig. 1b,e). Refractile ovoid spores were detected 24–30 hr after C-factor addition in both *mglA* and *csgA* strains. A C-factor dilution series demonstrated that *mglA* cells and *csgA* cells respond to the same concentration of C-factor (Fig. 3).

We examined the effect of C-factor on three different *lacZ* fusions: one (Ω4414), whose expression of β-galactosidase was reduced in the *csgA* or *mglA* background, and two (Ω4403 and Ω4435), whose expression was abolished in both mutant backgrounds. The data in Figure 4 show that C-factor addition restored the level of *mglA*-

**Table 1.** *C-factor rescues developmental sporulation of* mglA *and* csgA *mutants*

| | | Untreated | | + C-factor | | + DK1622 | | + DK5210 | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Genotype | spores/ml | wild type (%) | spores/ml | wild type (%) | spores/ml | wild type (%) | spores/ml | wild type (%) |
| DK1622 | wild type | $2.5 \times 10^6$ | (100) | | | | | | |
| DK5279 | Ω4414 | $5 \times 10^4$ | 2 | | | $5 \times 10^4$ | 2 | | |
| DK4172 | *mgl-9*, Ω4414 | $5 \times 10^4$ | 2 | $2 \times 10^5$ | 8 | $5 \times 10^4$ | 2 | $5 \times 10^4$ | 2 |
| DK4177 | *mgl-9*, Ω4403 | $3 \times 10^4$ | 1.2 | $2.5 \times 10^6$ | 100 | $1.2 \times 10^5$ | 5 | $4 \times 10^4$ | 1.6 |
| DK4167 | *mgl-9*, Ω4435 | $7 \times 10^3$ | 0.28 | $2.5 \times 10^6$ | 100 | $1 \times 10^5$ | 4 | $1 \times 10^4$ | 0.4 |
| DK5287 | *csgA*, Ω4414 | $<5 \times 10^2$ | <0.02 | $1 \times 10^5$ | 4 | $1 \times 10^5$ | 4 | | |
| DK5270 | *csgA*, Ω4403 | $<5 \times 10^2$ | <0.02 | $3.5 \times 10^6$ | 140 | $3 \times 10^6$ | 120 | | |
| DK5253 | *csgA*, Ω4435 | $<5 \times 10^2$ | <0.02 | $3 \times 10^6$ | 120 | $3.5 \times 10^6$ | 140 | | |

During C-factor addition or during cell mixing experiments, the total number of input cells was $2.5 \times 10^8$. One unit of C-factor was added to the indicated responder strains in submerged culture (Kuner and Kaiser 1982). Sporulation tests were performed as described in Kroos and Kaiser (1987). Two types of extracellular complementation tests were performed. In the first type, either $Km^r$ *csgA* or $Km^r$ *mglA* strains were mixed and allowed to develop with approximately equal numbers of $Km^s$ wild-type strain DK1622 (under data column + DK1622). In the second type, $Km^r$ *mglA* cells were mixed and allowed to develop with approximately equal numbers of $Km^s$ *csgA* strain DK5210 (under data column + DK5210). Input DK1622 sporulated at normal levels when mixed with *csgA* or *mglA* cells. Alone, DK1622 formed $2.5 \times 10^6$ spores/ml, and this reference value was used to calculate values in the columns marked wild type (%). (For further details, see Materials and methods.)

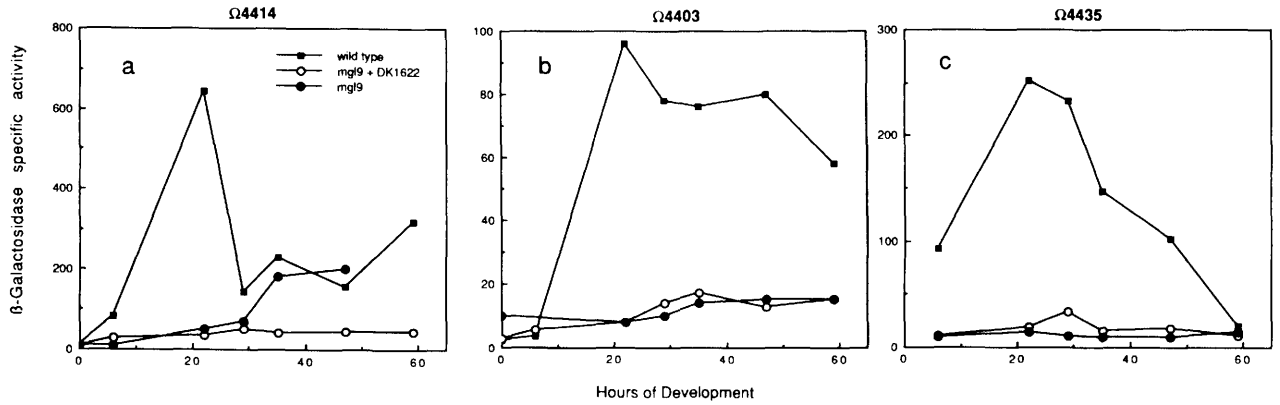


**Figure 2.** Codevelopment with wild-type cells fails to rescue developmental gene expression of *mglA* mutants. *mglA* and *csgA* strains containing Tn*5lac* insertions were plated on developmental media and harvested at various times for determination of β-galactosidase specific activity (nanomoles ONP/min · mg protein), as described in Kroos and Kaiser (1987). *mglA* cells mixed with an equal number of wild-type DK1622 cells (○) and unsupplemented *mglA* cells (●) containing Tn*5lac* insertions (*a*) Ω4414, (*b*) Ω4403, and (*c*) Ω4435 illustrate the effects of the *mglA* mutation on developmentally regulated expression of β-galactosidase. DK1622 does not express β-galactosidase. Addition of an equal number of wild-type cells to *csgA* cells (■ designates wild type) restored *csgA* developmental β-galactosidase expression to wild-type levels.

dependent β-galactosidase expression to approximately wild-type values in all three *mglA* fusion strains; however, the time of maximum β-galactosidase activity in these experiments was later than in wild type.

Unlike the two other Tn*5lac* insertions tested, Ω4414 disrupts the function of a developmentally essential gene that appears to be epistatic to *csgA* function. Strains carrying Ω4414, which are otherwise wild type, produce C-factor and form compact translucent mounds but sporulate at only 2% of wild-type levels and cannot be rescued by extracellular complementation (Kroos et al. 1986, 1990; Kim and Kaiser 1990a). If C-factor restores development to an *mglA* mutant at the point blocked by the *mglA* mutation, then β-galactosidase production of an *mglA* mutant carrying Ω4414 should be rescued; however, C-factor should not bypass the sporulation defect produced by the Ω4414 insertion. In contrast, if C-factor reinitiates *mglA* development at a terminal stage beyond the Ω4414 block, then sporulation without β-galactosidase production from Ω4414 may be observed.

Indeed, β-galactosidase production in strain DK4172, which is *mglA* and contains Ω4414, is restored to wild-type levels by C-factor (Fig. 4). But C-factor addition to this double mutant brings sporulation only to the levels produced in strain DK5279, which contains the Ω4414 insertion but is otherwise wild type (Table 1). This observation, together with restoration of all other C-dependent gene expression suggests that external C-factor allows development to pass through what otherwise would be the developmental block in an *mglA* mutant.

### mglA *mutants produce active C-factor*

Rescue of *mglA* development with purified C-factor argues that the cellular elements essential for interpreting *csgA*-mediated cell signaling are present in nonmotile *mglA* cells. To test whether production of C-factor is defective in *mglA* mutants, we asked whether *mglA* donor cells could rescue development of *csgA* mutants. Intact *mglA* cells, when mixed with *csgA* responder cells in equal proportion, failed to rescue *csgA* sporulation (Table 2, data column 3) or C-dependent gene expression (Fig. 5) to wild-type levels. Previously, we showed that antibodies raised and affinity-purified against a *lacZ–csgA* fusion protein produced in *Escherichia coli* also react with active C-factor purified from wild-type *M. xanthus* cells (Kim and Kaiser 1990b). Purified anti-*csgA* antibody was found in Western blots to react with C-factor purified from *mglA* cells (data not shown). Purified extracts made from *mglA* source cells conditioned on defined developmental media were also found to rescue *csgA* sporulation and aggregation and *mglA* sporulation (Table 2, data column 2; Fig. 6). C-factor from *mglA* cells rescued *csgA* and *mglA* developmental defects with the same timing, efficiency, and dosage dependence as C-factor from wild-type cells. This rescuing activity, when partially purified, was found to have approximately the same specific activity (within the twofold range of the dilution series) as activity derived from developing wild-type source cells.

## Discussion

The failure of *mglA* mutants, which are nonmotile, to develop beyond the *csgA*-signaling step suggests that motility is required for that cell interaction (Kroos et al. 1988). We predicted from this hypothesis that substances restoring *csgA* development may also restore *mglA* development. This prediction has been fulfilled; the experiments reported here demonstrate that C-factor, a 17-kD membrane-associated protein purified based on its ability to rescue *csgA* development, can also restore *mglA* sporulation and developmental gene expression.

The similar reactions of *csgA* and *mglA* mutants to purified C-factor argue that the cellular elements essen-

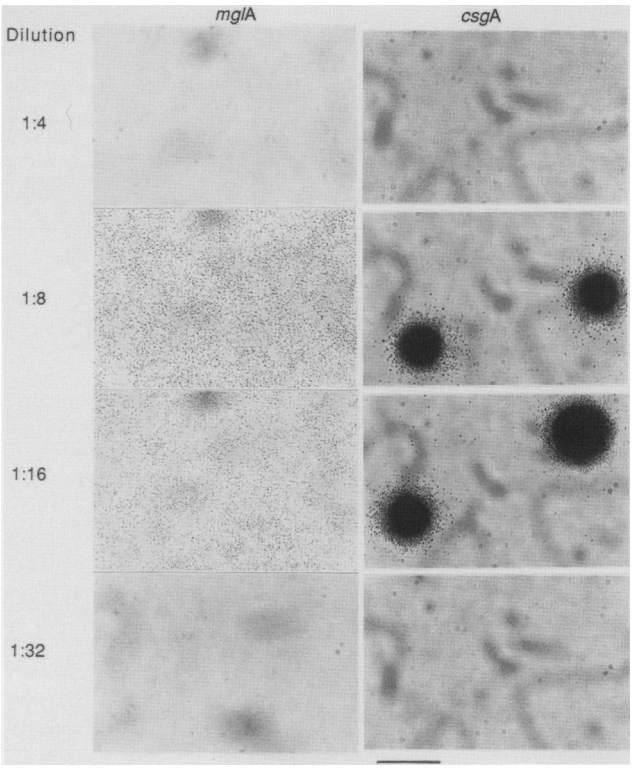


**Figure 3.** Optimal response of *mglA* and *csgA* mutants occurs at the same concentration of purified C-factor. C-factor rescued *csgA* aggregation and sporulation at a concentration of ~1–2 nM (activity observed as spore-filled fruiting bodies rescued here at 1 : 8 and 1 : 16 dilution; Kim and Kaiser 1990a,b). The same dose-response relationship was observed with *mglA* responder cells (activity observed as dark, refractile spores). Concentrations of C-factor approximately twice this optimum showed little or no detectable rescuing activity. Bar, 200 μm.

tial for response to C-factor are present both in *mglA* and *csgA* mutants. Optimal rescue of gene expression and sporulation of *mglA* and *csgA* mutants occurred at 1–2 nM; lower (or greater) concentrations had no detectable effect on responder cells. The kinetics and the extent of sporulation in response to C-factor addition was similar in *mglA* and *csgA* cells.

A number of experiments presented here demonstrate that *mglA* cells not only respond to C-factor but also produce C-factor. The conditions and chromatographic steps used to purify C-factor from wild-type cells (Kim and Kaiser 1990b) allowed equally efficient purification of C-factor from *mglA* cells, indicating close chemical and physical similarities. Affinity-purified antibodies that react with C-factor purified from wild-type cells react with C-factor isolated from *mglA* cells. Activity recovered from an *mglA* source rescued *csgA* aggregation and sporulation with the same timing and at the same concentration as C-factor derived from wild-type cells. Within the limits of our ability to assay C-factor activity, it appears that the abundance of C-factor in *mglA* cell extracts and wild-type cell extracts is similar, if not identical.

If, as we suspect, cell movement is required for *csgA*-mediated signaling but not for production of the *csgA* gene product or signal detection, it remains possible that motility is instead necessary for signal transfer. Two experiments described here indicate this possibility. Wild-type cells form spore-filled fruiting bodies when mixed with *mglA* cells yet fail to restore *mglA* sporulation or gene expression to wild-type levels (Table 1; Figs. 1c and 2). Apparently, the endogenous C-factor on wild-type cells, which must be present in normal amounts because it allows development of the wild-type cells, does not efficiently rescue the admixed *mglA* mutant cells. Second, although extracts made from conditioned *mglA* cells allowed wild-type levels of *csgA* responder sporulation and aggregation, intact *mglA* cells failed to rescue *csgA* development (Table 2; Fig. 5).

A hypothesis that *mglA* function is required to process C-factor to an active state may explain the inability of *mglA* cells to restore *csgA* development but fails to explain the inability of wild-type cells to fully rescue *mglA* sporulation or gene expression. The results suggest, rather, that efficient C-factor transmission occurs over a short range, perhaps only between immediately neighboring cells. This view is also supported by our earlier finding that C-factor is not readily diffusible but normally is tightly associated with the cell (Kim and Kaiser 1990a,b).

A model for motility-dependent *csgA* signaling that is consistent with the available data proposes that cells must achieve a proper spatial orientation before signaling can occur. For example, efficient *csgA* signaling in vivo may require a cell density so high that it can be achieved only by movement of cells into close-packed arrangements. Indeed, scanning electron microscopic observations reveal relatively ordered palisades of rod-shaped *M. xanthus* cells within a nascent fruiting body (Kuner and Kaiser 1982; O'Connor and Zusman 1989). Also consistent with this model is the earlier finding that increasing initial *mglA* cell density by sedimentation will partially rescue *mglA* sporulation (Kroos et al. 1988). Recently, we have shown that simple manipulations of cell position producing ordered parallel alignment of nonmotile cell groups restores C-dependent nonmotile cell sporulation and developmental gene expression (Kim and Kaiser 1990).

At what stage is development reinitiated in *mglA* cells by addition of external C-factor? Two experiments argue that purified C-factor restores development at or close to the point at which it is arrested because of mutation of *mglA*. First, the late sporulation defect caused by Tn*5 lac* insertion at Ω4414 is not bypassed by C-factor. Second, purified C-factor restored β-galactosidase expression by all C-dependent *lacZ* fusions tested. The progress of a cell through its development is, in effect, monitored by this set of *lacZ* fusions. Restored expression from early, as well as late, C-dependent fusions indicates that development never arrests at the stage that would be otherwise blocked by the *mglA* mutation.

C-factor rescue of sporulation without aggregation (in nonmotile mutants) and of aggregation with sporulation

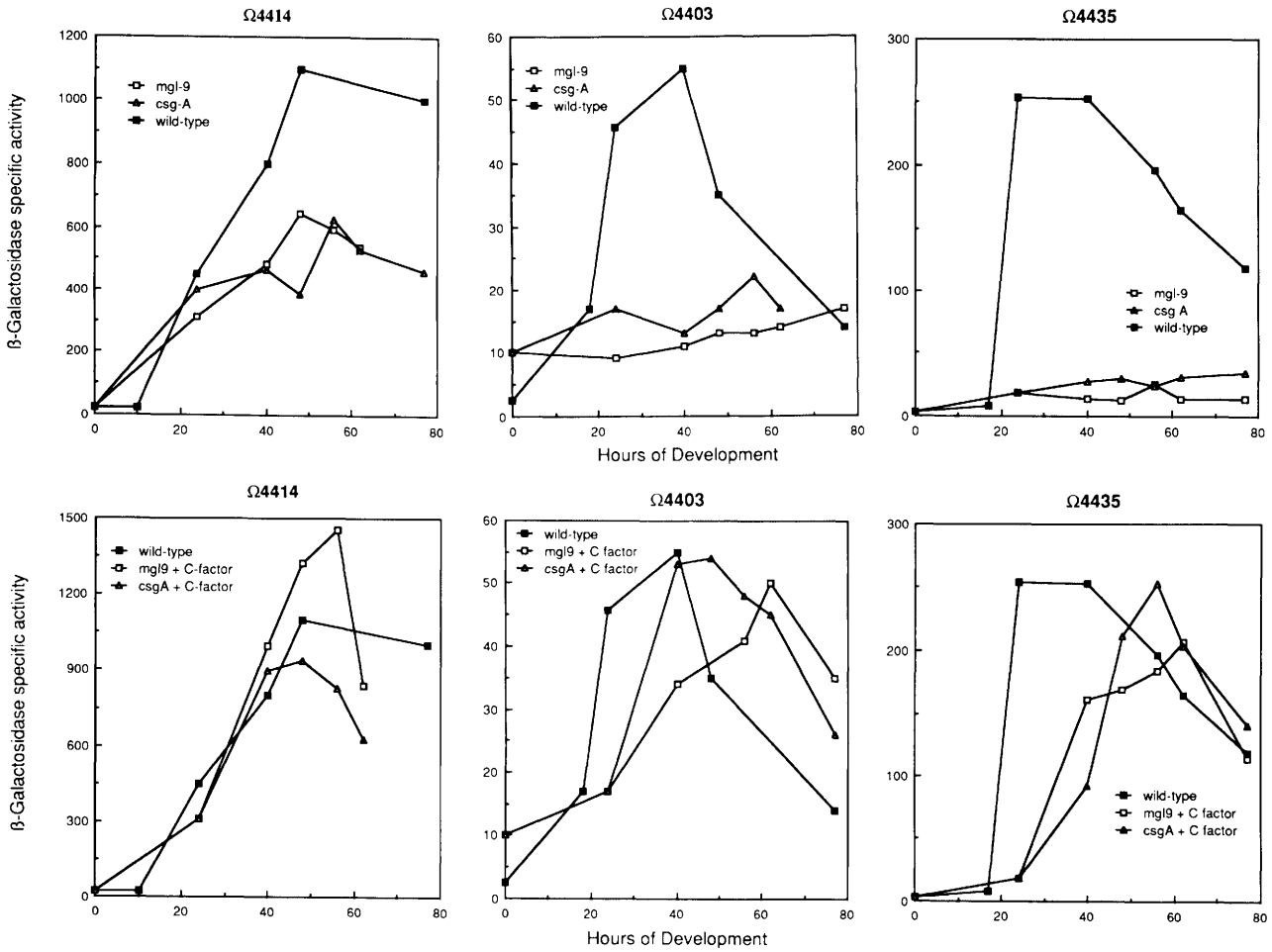


**Figure 4.** C-factor rescues developmental gene expression in *mglA* mutants. *csgA*, *mglA*, or wild-type strains containing Tn*5lac* insertions were plated on developmental media and harvested at various times for determination of β-galactosidase specific activity. (*Top*) Effects of *mglA* and *csgA* mutations on developmentally regulated β-galactosidase expression. Addition of 1 to 2 units of C-factor to *csgA* strains or *mglA* strains (*bottom*) restored developmental β-galactosidase expression to wild-type levels. The same set of wild-type data points was used in *top* and *bottom* groups.

(in *csgA* mutants) raises the question of the primary function of C-factor in normal fruiting body development. Previous studies of developmental mutants that fail to aggregate but sporulate normally contrasted with mutants that aggregate normally but fail to sporulate led to the proposal that aggregation and sporulation follow two independent, parallel pathways during fruiting body formation (Morrison and Zusman 1979). However, *asg*, *bsg*, *csg*, and *dsg* mutants fail to sporulate or aggregate, implying a pathway with functions common to both aggregation and sporulation (Hagen et al. 1978; Kroos et al. 1986; Cheng and Kaiser 1989; Kuspa 1989). The results of this study suggest that the *csgA*-dependent cell interaction, mediated through C-factor, regulates both aggre-

**Table 2.** *C-factor derived from* mglA *source cells rescues developmental sporulation of* csgA *and* mglA *mutants*

| Strain | Relevant genotype | Untreated | | + *mglA*-derived C-factor | | + DK3685 | |
|---|---|---|---|---|---|---|---|
| | | spores/ml | wild type (%) | spores/ml | wild type (%) | spores/ml | wild type (%) |
| DK1622 | wild type | $2.5 \times 10^6$ | (100) | | | $2.5 \times 10^6$ | (100) |
| DK5253 | *csgA* | $<5 \times 10^1$ | <0.002 | $3 \times 10^6$ | 120 | $<5 \times 10^1$ | <0.002 |
| DK5270 | *csgA* | $<5 \times 10^1$ | <0.002 | $2 \times 10^6$ | 80 | $<5 \times 10^1$ | <0.002 |
| DK4167 | *mglA* | $7 \times 10^3$ | 0.28 | $2.5 \times 10^6$ | 100 | | |

During C-factor addition or during codevelopment of *csgA* and *mglA* cells, the total number of input cells was $2.5 \times 10^8$. One unit of C factor was added to the indicated responder strains in submerged culture. Sporulation tests were performed as described in Kroos and Kaiser (1987). In extracellular complementation tests, $Km^r$ *csgA* or $Km^r$ *mglA* cells were mixed and allowed to develop with approximately equal numbers of $Km^s$ *mglA* strain DK3685. DK1622 formed $2.5 \times 10^6$ spores/ml and this reference value was used to calculate values in the columns marked wild type (%). (For further details, see Materials and methods.)

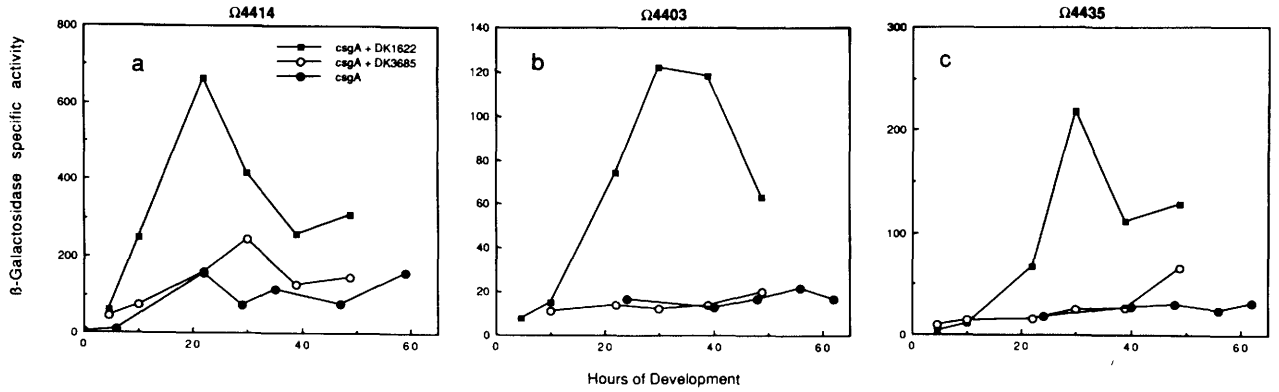


**Figure 5.** Codevelopment with *mglA* cells fails to rescue developmental gene expression of *csgA* mutants. *csgA* strains containing Tn*5lac* insertions (*a*) Ω4414, (*b*) Ω4403, and (*c*) Ω4435 were mixed with an equal number of wild-type strain DK1622 (■), an equal number of *mglA* strain DK3685 (○), or plated alone (●). Cells were harvested at various times for determination of β-galactosidase specific activity.

gation and sporulation. We suggest that C-factor may be the agent that couples aggregation and sporulation.

*csgA* mutants appear to be defective in both the aggregation and sporulation processes, because *csgA* mutants fail to form normal multicellular aggregates, fail to form synchronous, pulsatile multicellular ripples, and fail to sporulate. To the extent that restored aggregation implies cell movement, our finding that C-factor allows *csgA* cells to build aggregation centers that form normal fruiting bodies is consistent with the hypothesis that C-signaling allows or enhances coordinated cell movement. Considered together with our ability to rescue sporulation of nonmotile cells (in the absence of aggregation), this result implies that C-factor may have two distinct functions during development: one in sporulation and another in aggregation.

It is possible that C-factor also helps to direct the shape of a fruiting body. Fruiting body shape must be under genetic control because fruiting body shape is sufficient to distinguish the genera of myxobacteria (McCurdy 1974; Reichenbach and Dworkin 1981; Kaiser 1989). Evidence for a morphogenetic role for C-factor comes from our finding that wild-type cells or codeveloping *csgA* and wild-type cells in submerged culture form predominantly elongated fruiting bodies, whereas fruiting bodies resulting from C-factor addition to *csgA* cells are nearly perfectly rounded (cf. Fig. 1e,f, with Fig. 3, right). Exogenous C-factor may generate circularly symmetric fruiting bodies because it is homogenously distributed in solubilized form. The normal cellular localization of C-factor during development is not yet known. It will be interesting to investigate the possibility that endogenous C-factor, or an as yet unidentified receptor/substrate, is distributed asymmetrically in normal fruiting bodies.

Examples of long-range diffusible signals that direct the differentiated fate of cells from a distant source include *bicoid* protein in *Drosophila* (Driever and Nüsslein-Volhard 1989), retinoic acid in the chick limb bud (Thaller and Eichele 1987), and cAMP in *Dictyostelium* (Devreotes 1983). Short-range developmental cues that direct differentiation over smaller distances have also been described. In *Drosophila*, the products of the *sevenless* and *bride of sevenless* genes are used to determine the R7 cell fate (Tomlinson 1989). In the vertebrate immune system, direct contact of cells through the products of the major histocompatiblity complex (MHC) locus restricts antigen presentation and subsequent humoral and cytotoxic responses (Hood et al. 1984). In *M. xanthus*, cell movement is required to establish proper cell contacts necessary for efficient intercellular trans-

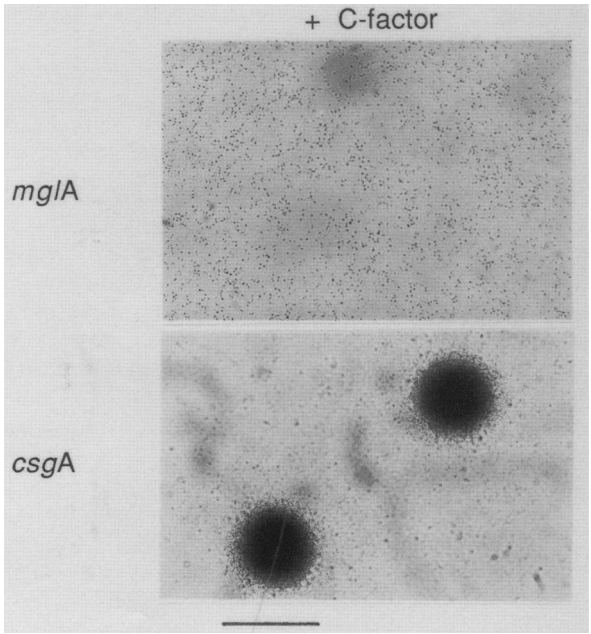


**Figure 6.** C-factor from *mglA* cells rescues defects of *mglA* and *csgA* developmental mutants. (*Top*) Dark dots are individual refractile *mglA* spores formed after a 3-day incubation of 1 unit of C-factor with *mglA* strain DK4167. (*Bottom*) Spore-filled fruiting bodies formed after a 3-day incubation of C-factor with *csgA* strain DK5253. Fruiting bodies and spores resulting from C-factor addition were first detected at ~40 hr after nutrient deprivation initiated development. Bar, 200 μm.

mission of C-factor. Genetic, biochemical, and cell mosaic experiments possible in *M. xanthus* should provide an analysis of this paracrine signal at both cellular and molecular levels.

## Materials and methods

### *Bacterial strains*

All *M. xanthus* strains used in this study were derived from DK1622 (wild type). The Tn*5lac* transposon insertions are designated by the Greek letter Ω followed by a four-digit numeral (Kroos et al. 1986). C-factor was prepared from DK5204, a kanamycin-resistant ($Km^r$), developmentally competent strain that contains Tn*5lac* at position Ω4435, or from DK4167, an *mglA* null strain that contains Tn*5lac* at position Ω4435. The *csgA* mutation used to construct *csgA* Tn*5lac* derivatives was created by insertion of Tn*5*-132 at position ΩLS205 in the *csgA* gene (Shimkets and Asher 1988). Construction of strains that were *csgA* and contained the Tn*5lac* insertions Ω4414 (DK5287), Ω4403 (DK5270), and Ω4435 (DK5253) has been described (Kroos and Kaiser 1987). Construction of DK5210, a tetracycline-resistant kanamycin-sensitive ($Tc^r$ $Km^s$) *csgA* strain, has also been described (Kroos and Kaiser 1987).

Strains that contain the *mgl-9* allele fail to produce *mglA* gene product, as judged by Western analysis (P. Hartzell, unpubl.). Construction of *mgl-9* strain DK3685 and *mgl-9* strains that contain Tn*5lac* Ω4414 (DK4172), Ω4403 (DK4177), and Ω4435 (DK4167) is described in Kroos et al. (1988).

### *Preparation and purification of C-factor*

Cells growing exponentially in CTT liquid medium (Hodgkin and Kaiser 1977) were sedimented at 10,000*g* for 10 min at 4°C and suspended in TPM buffer [10 mM Tris-HCl, 1 mM $K_2HPO_4$/$KH_2PO_4$, 8 mM $MgSO_4$ (pH 7.5)] at a density of $5 \times 10^9$ cells/ml. Approximately 50-μl aliquots were spotted on TPM agar (TPM plus 1.5% agar) in 25 × 25-cm tissue culture plates (Nunc) and incubated at 32°C for 18 hr. Agar, rather than submerged culture, was chosen for conditioning cells because the yield of cells per square centimeter was greater on agar. Cells were scraped with a razor blade into MC7 buffer [10 mM MOPS, 1 mM $CaCl_2$ (pH 7.0)] at a calculated concentration of $1.5 \times 10^{10}$ cells/ml. These cells were suspended by vortex mixing and were flash-frozen in liquid nitrogen and stored at −80°C. Methods for purification of C-factor have been described previously (Kim and Kaiser 1990b).

### *C-factor activity*

C-factor was assayed for its ability to restore fruiting body formation and sporulation to *csgA* mutant cells or sporulation of *mglA* mutant cells. The *csgA* or *mglA* responder strain used is described in the text. Each sample of C-factor was dialyzed against 4 liters of 10 mM MOPS, 1 mM $CaCl_2$, 4 mM $MgCl_2$, and 50 mM NaCl (pH 7.2) (buffer A) for 12–18 hr at 4°C. Aliquots of the dialyzed samples to be assayed were serially diluted in two-fold steps, typically six to eight such steps. Each dilution (400 μl) was warmed to 32°C and added to responder *csgA* or *mglA* cells that had been starved in submerged culture (Kuner and Kaiser 1982) for 6 hr. At this time, when the morphological defects of *csgA* mutants are first manifest, the buffer A overlying the confluent mat of adherent cells is gently removed and prewarmed fractions to be assayed are added. One unit of activity (Table 1) is defined as the amount of C-factor that restores wild-type level fruiting body formation ($300/2.5 \times 10^8$ input cells) and/or sporulation ($2 \times 10^6/2.5 \times 10^8$ input cells) to *csgA* mutants developing in submerged culture.

### *Other methods*

C-factor purification was analyzed by SDS-PAGE by use of 15% acrylamide and 0.12% *bis*-acrylamide (Laemmli 1970; Pfeffer 1987). Measurement of developmental β-galactosidase expression was performed as described by Kroos and Kaiser (1987), and results summarize data from at least two independent experiments. One unit of β-galactosidase specific activity is equal to 1 nmole of *o*-nitrophenol (ONP) produced per minute per milligram of protein. Heat-resistant, sonication-resistant, $Km^r$ spores were quantified, as described by Kroos et al. (1986). Fruiting bodies were scored visually at 6× magnification with a dissecting microscope (Wild-Heerbrug). The presence of ovoid, refractile spores within fruiting bodies at the bottom of a microtiter well was confirmed with a Leitz inverted light microscope at 40× magnification. Western blotting analysis was performed by using primary rabbit anti-*lacZ*–*csgA* antibodies provided by Dr. L. Shimkets and by the methods described by Ey and Ashman (1986). Secondary goat anti-rabbit IgG antibodies conjugated to alkaline phosphatase were from Bio-Rad.

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