C-Factor Has Distinct Aggregation and Sporulation Thresholds during *Myxococcus* Development

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C-factor, the protein product of the *csgA* gene, acts as a short-range morphogenetic signal. It is required for fruiting body development of the gram-negative bacterium *Myxococcus xanthus*. Aggregation, sporulation, and expression of a set of genes that are C-factor dependent, all of which fail in *csgA* mutant cells, are completely restored by addition of purified C-factor. We report here that, depending on its concentration, C-factor can elicit two distinct morphogenetic and transcriptional responses from *csgA* cells. Low levels of C-factor bring about aggregation and expression of an early C-dependent gene, whereas higher levels lead to the same effects plus expression of a late C-dependent gene and spore formation. C-factor positively regulates its own transcription. An approximately fourfold net increase in *csgA* transcription and C-factor levels during development was measured. We propose that autoregulation and the two distinct activity thresholds allow C-factor to act as a timer, first triggering aggregation, then sporulation, thereby producing the appropriate developmental order.

Interactions between cells are crucial for cell differentiation in most multicellular organisms. Building multicellular structures and establishing differentiated cell fates during fruiting body morphogenesis of the gram-negative bacterium *Myxococcus xanthus* strictly depends on cell-cell interactions. Following starvation, thousands of rod-shaped cells in an initially undifferentiated sheet glide to aggregation centers. There, cells form patterns of aligned terraced palisades that eventually amass to build a mound-shaped fruiting body (15, 20). Early in aggregation, ridgelike accumulations of cells move coordinately and synchronously over the substrate like ripples on a water surface (21, 26). Some cells die, while other cells within a nascent fruiting body differentiate to ovoid spores that are resistant to heat and desiccation (30). About 10^5 cells participate in constructing a fruiting body. In submerged culture, *M. xanthus* aggregates are first detected between 8 and 10 h after removal of nutrient. Six to 10 h later, sporulation begins. Refractile dormant spores eventually fill and darken the mature fruiting body. Reviews on myxobacteria and their development can be found in reference 22.

Control of *M. xanthus* fruiting body development by cell-cell interactions is implied by the coordinate synthesis of developmentally regulated proteins within a culture (5, 13) and demonstrated by the properties of four classes of nonautonomous mutants. These mutants cannot develop alone but can develop upon admixture with wild-type cells (3, 6, 7, 12, 16, 18). *csg* (c signal) mutants are one class of such developmental mutants. All existing members of the *csg* class have resulted from mutation in a single gene called *csgA* which has been cloned and sequenced (4, 24, 25). *csgA* mutants fail to complete aggregation, to ripple, or to sporulate (26). *csgA* mutants show an altered pattern of developmental gene expression as monitored by transcriptional fusions of *lacZ* to developmentally regulated genes. In *csgA* mutants, expression of *lacZ* fusions in the first 6 h of development is normal (C-independent expression) but lev-

els of β -galactosidase expression from fusions expressed after 6 h are reduced (early C-dependent expression) or abolished (late C-dependent expression). All the developmental defects of *csgA* mutants that have been tested are overcome by admixture of wild-type cells (12).

C-factor, a 17-kDa membrane-associated protein from extracts of starved wild-type cells, was purified by its ability to rescue development of *csgA* mutants (8). C-factor has been identified as the protein product of the *csgA* gene (7). At 1 nM, purified C-factor is sufficient to (i) stimulate movement of *csgA* mutants from disordered cell sheets into aligned cell palisades of a nascent fruiting body, (ii) trigger differentiation of *csgA* cells to spores, and (iii) restore C-dependent gene expression in *csgA* cells to wild-type levels (7, 8). These observations suggest that C-factor is able to directly initiate both aggregate formation and sporulation, the two major morphogenetic events in *M. xanthus* fruiting body development.

How does C-factor regulate such different events as aggregation and sporulation? We report here that in response to C-factor at subnanomolar levels, *csgA* cells express early C-dependent genes and aggregate but fail to proceed further in development. However, at a second, higher C-factor concentration, *csgA* mutants continue to develop past the aggregate stage, express both early and late C-dependent genes, and finally sporulate. Measures of *csgA* transcription and net C-factor production suggest that C-factor accumulates during development and stimulates its own synthesis. We propose that a rising C-factor concentration and two distinct activity thresholds allow C-factor to arrange aggregation and sporulation in their proper order.

MATERIALS AND METHODS

Bacterial strains. All *M. xanthus* strains used in this study were derived from DK1622 (wild type). Tn5*lac* transposon insertions are designated by the Greek letter Ω followed by a four-digit numeral (13). C-factor was prepared from DK5204, a kanamycin-resistant (Km^r), developmentally competent strain which contains Tn5*lac* at position Ω 4435.

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FIG. 1. Aggregation and sporulation are independent morphogenetic events triggered by C-factor. (a) Spore-filled fruiting body formed after 3 days by a mixture of wild-type strain DK1622 and an equal number of *csgA* strain DK5246 cells. (b) Spore-filled fruiting body formed after 3 days of incubation of C-factor with *csgA* strain DK5246. (c) Dark dots are refractile *mglA* spores seen after 3 days of incubation of C-factor with *mglA* strain DK4167. (d) Multicellular mound-shaped aggregate formed after 3 days of incubation of C-factor with *csgA* strain DK5287 which cannot sporulate due to autonomous mutation caused by insertion of *Tn5lac* at position $\Omega 4414$. In these experiments, 1 unit of C-factor was added 6 h after starvation initiated development. Bar equals 50 μm .

Construction of strains which were *csgA* and which contained the *Tn5lac* insertions $\Omega 4414$ (DK5287), $\Omega 4499$ (DK5246), and $\Omega 4435$ (DK5253) has been described previously (12). *Tn5lac* insertion in the amino-terminal half of the *csgA* open reading frame in LS269 creates a transcriptional fusion between *csgA* and *lacZ* and causes a *csgA* mutation (4).

Strains which contain the *mgl-9* allele fail to produce *mglA* protein as judged from Western immunoblot analysis (4a). Construction of *mgl-9* strain DK4167 which contains *Tn5lac* at position $\Omega 4435$ is described in reference 10.

Preparation and purification of C-factor. Methods for purification of C-factor have been described previously (7, 8).

C-factor activity. C-factor was assayed for its ability to restore fruiting body formation and sporulation to *csgA* mutant cells or sporulation to *mglA* mutant cells by a modification of a method previously described (8). The *csgA* responder strain or *mglA* responder strain used is described in the text. One unit of activity is defined as the amount of C-factor that restores wild-type-level fruiting body formation (300 per 2.5×10^8 input cells) and/or sporulation (2.5×10^6 per 2.5×10^8 input cells) to *csgA* mutants developing in submerged culture. C-factor was diluted 20 to 30 times in a linear series of 0.05-unit steps and assayed to determine C-factor concentration near 1 unit. For additions of 0.6 unit of total C-factor activity, three consecutive prewarmed aliquots of 0.2 unit in 20 μl were added at 6, 12, and 18 h of development to responder cells starving in 400 μl of submerged culture buffer (8). For addition of 0.8 unit of C-factor, a fourth aliquot of 0.2 unit in 20 μl was added at 24 h of development. The final volume of buffer over each cell mat was brought to 500 μl by the addition of the appropriate volume of prewarmed submerged culture buffer at 32 h of

development. As described in the text, 1 unit of C-factor in 100 μl was added to responder cells either entirely at 6 h of development or in five consecutive aliquots of 0.2 unit in 20 μl at 6, 12, 18, 24, and 32 h of development.

Other methods. C-factor purification was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 15% acrylamide and 0.12% bisacrylamide (8, 17). Developmental β -galactosidase expression was measured as described in reference 12, and results summarize data from at least two independent experiments. One unit of β -galactosidase specific activity is equal to 1 nmol of *o*-nitrophenol produced per min per mg of protein. Heat-resistant, sonication-resistant, Km^r spores were quantified as described in reference 13. Fruiting bodies were scored visually 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 $\times 40$ magnification.

RESULTS

Aggregation and sporulation are separately initiated by C-factor. In response to starvation, many rod-shaped *M. xanthus* cells aggregate into steep-sided mounds which first appear translucent in bright-field microscopy. Later, cells begin to differentiate into ovoid spores which fill and darken the mound-shaped mature fruiting body (Fig. 1a). *csgA* mutants, which fail to produce C-factor, are defective in both aggregation and sporulation. Purified C-factor, a single 17-kDa protein, rescues both defects (Fig. 1b) (7). Either C-factor directs two morphologically different events, or it might only be required to establish a multicellular aggregate which then generates a separate sporulation signal. To begin to distinguish between these possibilities, we added purified

FIG. 2. Aggregation and sporulation are initiated at different threshold concentrations of C-factor. *csgA* strain DK5253 alone, with added C-factor, or with an equal proportion of wild-type strain DK1622 was photographed in submerged culture at the indicated times over 80 h. Panels under the heading "none" show that untreated *csgA* cells fail to aggregate. Panels under the heading "+ 0.8 unit C-factor" show formation of *csgA* aggregates after four additions of 0.2 unit of C-factor at 6-h intervals within the first 24 h of starvation. Panels under the heading "+ 1.0 unit C-factor" show formation of translucent aggregates and then darkened spore-filled fruiting bodies by *csgA* cells after five additions of 0.2 unit of C-factor at 6-h intervals within the first 32 h of starvation. Panels under the heading "+ wild-type cells" show formation of translucent aggregates and then darkened spore-filled fruiting bodies by *csgA* cells mixed with an equal number of wild-type cells. The levels of heat-resistant *csgA* spores formed after 6 days of the indicated treatments are presented below the panels. Bar equals 100 μ m.

C-factor to two different developmental mutants both defective in C-factor signaling.

Nonmotile cells resulting from mutation of the *mglA* gene (28) arrest development at a stage similar to *csgA* mutants (10), apparently because their inability to move prevents them from forming the intercellular contacts necessary for C-factor transmission. Addition of 1 unit of purified C-factor to nonmotile cells restored their ability to differentiate to spores in the absence of detectable cell movement or aggregation (Fig. 1c). In a complementary experiment, C-factor was added to a developmental mutant strain that carried both an aggregation defect caused by a *csgA* null allele and a cell-autonomous sporulation defect caused by insertion of *Tn5lac* at position $\Omega 4414$ (14). One unit of purified C-factor restored aggregation in this double mutant, but the cells failed subsequently to sporulate (Fig. 1d; compare with Fig. 1a and b). These two experiments demonstrated that C-factor can initiate aggregation without sporulation, as well as sporulation without aggregation. Perhaps, then, the normal association of sporulation with aggregation reflects the ability of C-factor to induce both events.

C-factor thresholds for aggregation and sporulation are

different. Aggregation and sporulation are very different processes. They occur at different times and they can be separately induced by C-factor. To test whether these two processes might have different quantitative requirements for C-factor, we added purified C-factor to *csgA* mutant cells over a range between 0 and 1 unit (see Materials and Methods). Whether added in divided doses or as a single dose, additions of C-factor totaling between 0 and approximately 0.6 unit never resulted in detectable cell aggregation or sporulation. Translucent multicellular aggregates did form at 100% of wild-type levels when a total of 0.8 unit of C-factor was added in four aliquots of 0.2 unit over 24 h. Sporulation, however, only reached 0.1% of wild-type level (Fig. 2). In general, the size of aggregates formed was similar to that of aggregates formed by wild-type cells. Because of the low level of sporulation, these aggregates did not appear as dark as spore-filled fruiting bodies. Addition of C-factor to *csgA* cells at concentrations greater than approximately 0.8 unit resulted in numbers of both aggregates and spores comparable to those produced after admixture of wild-type cells (Fig. 2). For as yet unknown reasons, aggregates and spores resulting from C-factor addition to *csgA* cells appear

TABLE 1. Purified C-factor activity added to *csgA* cells is recovered following developmental rescue^a

Source of developmental supernatant	Spores/ml	% Wild-type sporulation	Fruiting bodies
Untreated <i>csgA</i> cells	$<2 \times 10^2$	<0.008	0
Untreated wild-type cells	1×10^3	0.04	0
<i>csgA</i> cells + 1 unit of C-factor	2.5×10^6	100	300

^a One unit of C-factor was added to a submerged culture of *csgA* strain DK5253 6 h after starvation initiated development. Wild-type numbers of fruiting mounds and ovoid, refractile spores were detected after 40 h. The overlying culture buffer (500 μ l) was removed at 72 h of development and sterilized by passage through a 0.45- μ m-pore-size Millipore filter. C-factor activity in this material and in supernatants similarly obtained from 72-h-old unsupplemented submerged cultures of DK5253 and wild-type strain DK1622 was assayed as described previously (8). Spore titers and fruiting body numbers represent the average of two experiments.

about 24 h later than seen during wild-type development (8) (Fig. 2).

The activity of exogenous, solubilized purified C-factor was quite stable when added to *csgA* mutants in these experiments. To test for inactivation, we added 1.0 unit of purified C-factor to buffer overlying starving *csgA* cells in submerged culture. After 72 h, *csgA* responder cells had formed wild-type numbers of fruiting bodies and spores. The submerged culture buffer overlying the rescued fruiting bodies was recovered and added to a second submerged culture of *csgA* mutants. *csgA* cells formed wild-type numbers of round fruiting bodies and heat-resistant spores (Table 1) from this addition. In a control experiment, addition of buffer overlying wild-type cells which had developed for 72 h did not rescue *csgA* fruiting body formation or sporulation (Table 1).

C-dependent developmental transcription initiates at different C-factor thresholds. Transcriptional fusions of *lacZ* to developmentally regulated genes in *M. xanthus* have been generated with the transposable promoter probe Tn5*lac* (11, 13). β -Galactosidase activity in these fusion strains measures expression of those particular transcription units during development. A mutation in *csgA* does not affect any *lacZ* fusion expressed during the first 6 h of development. However, levels of β -galactosidase synthesis from *lacZ* fusions that should be expressed after 6 h of development, during the time of aggregation and sporulation, are reduced or abolished in *csgA* mutants (C-dependent expression).

Expression of the C-dependent *lacZ* fusion $\Omega 4499$ normally initiates in *csgA*⁺ strains at about 6 h of development, coincident with the beginning of aggregation. In contrast, the C-dependent *lacZ* fusion $\Omega 4435$ normally initiates expression between 12 and 20 h of development, the time when sporulation is first detected (7, 12, 19). Admixture of wild-type cells to *csgA* mutants containing the $\Omega 4499$ or $\Omega 4435$ fusion restored expression of these C-dependent fusions to normal levels (12) (Fig. 3). Addition of 1 to 2 units of purified C-factor also restored β -galactosidase expression by these fusions to normal levels (7) (Fig. 3). *csgA* cell aggregation and sporulation were also rescued to wild-type levels by adding 1 unit of C-factor (7, 8).

When 0.8 unit of C-factor was added to *csgA* strains containing either the $\Omega 4499$ or the $\Omega 4435$ fusion, cells aggregated to the translucent mound stage but did not sporulate (Fig. 2). At this concentration of exogenous C-fac-

FIG. 3. Early and late C-dependent *lacZ* fusions initiate expression at different C-factor concentrations. (a) *csgA* strain DK5246 contains a C-dependent *lacZ* fusion at position $\Omega 4499$ that initiates β -galactosidase (β -gal) expression 6 h after development begins. (b) *csgA* strain DK5253 contains a C-dependent *lacZ* fusion at position $\Omega 4435$ that initiates expression 12 to 20 h after development begins. These strains were allowed to develop in submerged culture in the presence of C-factor at the indicated final concentrations or admixed with an equal number of wild-type cells. C-factor at 0.6, 0.8, and 1.0 units was added in divided aliquots to responder cells as described in the legend to Fig. 2 and in Materials and Methods. Strains were harvested at various times for determination of β -galactosidase specific activity as described previously (12). The maximum level of β -galactosidase expression achieved is shown here.

tor, expression of β -galactosidase in the $\Omega 4499$ *lacZ* fusion strain rose to wild-type levels, whereas no increase in β -galactosidase activity was detected in the strain containing the $\Omega 4435$ *lacZ* fusion. β -Galactosidase activity did not increase in either fusion strain when 0.6 unit of C-factor was added (Fig. 3). These observations suggest that the transcriptional response to C-factor has at least two thresholds: one for an early C-dependent transcription that normally

FIG. 4. Time course of C-factor activity extracted from developing cells. Cultures of growing wild-type cells were placed on development-inducing medium for defined periods. Cells were harvested and lysed, and C-factor was purified from each sample approximately 200-fold by methods previously published (8). Total C-factor activity was normalized to the peak C-factor levels found in cells starved for 18 h. Under these conditions, multicellular aggregates first appeared by 6 h and spores first appeared between 16 and 20 h.

begins near the onset of cell aggregation, and a higher one for a later C-dependent transcription that begins during the onset of sporulation.

Expression of the *csgA* gene increases during development and the increase is C-dependent. The existence of two C-factor thresholds suggests that changes in C-factor levels them-

selves play an important role in regulating the developmental program. It has been observed that C-factor was most abundant in extracts of wild-type cells which had been starving for 12 to 18 h on an agar surface (7) but was undetectable in cells growing in suspension. β -Galactosidase production from a *csgA-lacZ* transcriptional fusion increases approximately twofold during development in somewhat different culture conditions than employed here (4; see Materials and Methods). To monitor the production of C-factor itself, we partially purified C-factor from starved wild-type cells at different stages of fruiting body morphogenesis.

In confirmation of earlier work, little if any C-factor was found in extracts made from growing cells (Fig. 4). Low levels of C-factor were recovered in extracts made from cells 4 to 10 h after initiation of development on agar. Levels of C-factor recovered from developing cells reached their maximum after 15 h when aggregation is complete, about the time sporulation initiates. After 18 to 20 h, levels of recovered C-factor dropped sharply (Fig. 4) coincident with the onset of sporulation. Relative to levels detected at 6 to 10 h, we estimate that recoverable C-factor subsequently increased approximately fourfold.

To measure *csgA* transcription during development, we monitored β -galactosidase activity in cells containing the *csgA-lacZ* transcriptional fusion (4). This *lacZ* fusion, which was created by insertion of a properly oriented *Tn5lac* within the N-terminal half of *csgA*, prevents C-factor production (6a). In submerged culture, β -galactosidase production from the *csgA-lacZ* fusion strain increased during development at most twofold over levels expressed in vegetative cells (Fig. 5). Admixture of wild-type cells (Fig. 5, left panel) or purified C-factor (Fig. 5, right panel) allowed the *csgA-lacZ* fusion strain to produce β -galactosidase at levels approximately fourfold greater than levels in vegetative cells, and the increase was reproducible. Moreover, the fusion strain ag-

FIG. 5. Developmental β -galactosidase production from a *csgA-lacZ* transcriptional fusion. Strain LS269, a *csgA* mutant, contains *Tn5lac* inserted within the N-terminal half of the *csgA* open reading frame. LS269 left unsupplemented (○) or LS269 mixed with an equal number of wild-type strain DK1622 cells or with 1 unit of purified C-factor (■) was harvested at various times for determination of β -galactosidase specific activity. C-factor was added in a single dose 6 h after starvation initiated development. Range of experimental variation is indicated by vertical bars. Results summarize data from at least two independent experiments.

gregated and sporulated at wild-type levels when either wild-type cells or purified C-factor was added (data not shown).

The increase in β -galactosidase activity expressed from the *csgA-lacZ* fusion (Fig. 5) peaked later than the time when the increase in recoverable C-factor activity level peaked (Fig. 4). This may reflect differences in both the conditions and the type of measured activity in each assay. Nevertheless, both sets of results clearly demonstrate that the expression of *csgA* increases during development. They also suggest that *csgA* is itself a C-dependent gene, so that C-factor positively regulates transcription of *csgA*.

DISCUSSION

C-factor is a morphogenetic protein that determines both cell position (aggregation) and the level of cell differentiation (sporulation). Several observations suggest that C-factor, the product of the *csgA* gene, has at least two morphogenetic functions. Aggregation and sporulation, the two major morphogenetic events in *M. xanthus* fruiting body formation, are defective in *csgA* mutants. Solubilized purified C-factor completely restores aggregation and sporulation to *csgA* mutants. C-factor can elicit developmental sporulation without aggregation, as in the response of nonmotile cells to purified C-factor (9) (Fig. 1). C-factor can also elicit cell aggregation without subsequent sporulation, as demonstrated in the response of *csgA* Ω 4414 double mutants with their cell-autonomous sporulation defect (Fig. 1).

Although purified C-factor rescues the aggregation, gene expression, and sporulation deficiencies of *csgA* mutants, C-factor can evoke qualitatively different responses from those cells at different concentrations. At low C-factor concentrations, cells remained in undifferentiated sheets and expression of C-dependent *lacZ* fusions was not detected (Fig. 2 and 3). At an intermediate concentration of added C-factor, cells were recruited to aggregation centers and expressed an early C-dependent *lacZ* fusion but not a late one. Relatively high C-factor levels initiated aggregation, sporulation, and expression of both an early and a late C-dependent *lacZ* fusion (7) (Fig. 2 and 3). During these experiments, incremental additions of C-factor were made over 24 h. C-factor activity was found to be stable (Table 1) for over 100 h following addition to *csgA* responder cells, making it unlikely that inactivation of C-factor could account for the results. Rather, these observations are consistent with the hypothesis that C-factor has at least two activity thresholds, a lower one for early C-dependent gene expression and aggregation, and a higher one for late C-dependent gene expression and sporulation.

These results with purified C-factor suggest that *M. xanthus* cells can discern and respond to relatively small changes in C-factor concentration. The isolation of C-factor from wild-type cells at different developmental stages revealed that total C-factor concentration increases only about fourfold between the beginning of aggregation and the beginning of sporulation (Fig. 4). β -Galactosidase expression from a *csgA-lacZ* transcriptional fusion maximally increased about three- to fourfold during a similar interval (Fig. 5). It has been shown in other systems that cells may respond dramatically to small changes in the concentrations of morphogenetic substances. Retinoic acid functions as a morphogen in the chick limb bud with only a 2.5-fold posterior-to-anterior difference in concentration (29). A 10% change in concentration of bicoid protein alters the position of the boundary between head and thorax 15% in early *Drosophila*

development (2). Within a twofold range, mesoderm-inducing factor stimulates formation in *Xenopus laevis* explants of either epidermis-lined mesothelium or muscle with neural tissue (27).

The experiments reported here reveal a mechanism for increasing C-factor concentration by positive feedback. Increased transcription of *csgA* by cells after exposure to purified C-factor suggests that C-factor positively regulates its own synthesis (Fig. 5). This increase would be conditioned by the aggregation-inducing activity of C-factor (Fig. 1 and 2). Within aggregates, the local C-factor concentration would be expected to increase with cell density and with the increased efficiency of cell-to-cell C-factor transmission (9, 10).

Developmental events triggered by different threshold concentrations of the same protein could be ordered by the increasing abundance of that protein. We propose that C-factor, in addition to its role as a morphogenetic trigger, also acts as a developmental timer. Since the C-factor threshold for aggregation is lower than the C-factor threshold for sporulation (Fig. 2 and 3), rising levels of C-factor during development would be expected to first elicit aggregation, then sporulation. Unlike the essentially unicellular sporulation of bacteria such as *Bacillus subtilis*, myxobacteria have a program of multicellular aggregation and sporulation. Multicellular sporulation ensures that a sufficiently large swarm emerges from one fruiting body for efficient feeding (23) and subsequent growth on polymeric sources of amino acids.

It has been proposed that C-factor generates a relayed signal which can oscillate and is manifest as the synchronous, pulsatile, wavelike cell movements called rippling (10, 21). Support for this proposal now includes the following. (i) Rippling requires both cell motility and *csgA* function (26). (ii) Motility is required for transmission of C-factor between cells (9). (iii) C-factor induces cell movement, resulting in formation of multicellular aggregates (7, this work). (iv) As shown here, C-factor positively regulates its own net synthesis (Fig. 5). Rippling of *csgA* mutants has not yet been reconstituted with stepwise addition of purified C-factor. Rippling may require a separate signal or more focused local oscillation of C-factor than achieved by our present method of addition. In its dual aggregation and cell differentiation functions, C-factor bears similarities to cyclic AMP, a well-studied extracellular signal that coordinates fruiting body development in the cellular slime mold *Dictyostelium discoideum* (1). Whereas C-factor is normally associated tightly with cells, cyclic AMP is soluble and highly diffusible.

Intriguingly, the fruiting bodies resulting from addition of purified C-factor to *csgA* mutant cells are circularly symmetric, whereas asymmetric fruiting bodies result from wild-type cells or admixed *csgA* and wild-type cells (compare Fig. 1a and b). Exogenous C-factor may generate round fruiting bodies because of its homogeneous distribution in solution. By contrast, cell-transferred C-factor would reflect the asymmetry of the rod-shaped *Myxococcus* cells (0.5 by 5.0 μ m). It will be interesting to investigate the possibility that developmental cues provided by C-factor are not only temporally graded, but also spatially graded.

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