Isolated Fibrils Rescue Cohesion and Development in the Dsp Mutant of *Myxococcus xanthus*

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Extracellular fibrils are involved in cell cohesion and cell development in *Myxococcus xanthus*. One group of social motility mutants, Dsp, is unable to produce extracellular fibrils; these mutants also lose the abilities to cohere and to develop. Extracellular fibrils isolated from vegetative wild-type cells and added to Dsp cells fully restored the abilities of these cells to cohere and to undergo normal morphological development. The fibrils thus mimic the ability of intact, wild-type cells to carry out the same rescue. Optimal cohesion rescue by fibrils required calcium and magnesium ions, did not require protein synthesis, but was energy dependent, i.e., sodium azide and sodium cyanide blocked rescue. Cohesion rescue was also blocked by the diazo dye Congo red. Cohesion rescue is genus specific, i.e., isolated fibrils did not cause the cohesion of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus mirabilis*, *Escherichia coli*, or the related myxobacterium *Stigmatella aurantiaca*. Developmental rescue of Dsp by isolated fibrils included aggregation, fruiting body formation, and myxospore morphogenesis. Developmental gene expression in the Dsp mutant was only partially rescued by the isolated fibrils.

Myxococcus xanthus is a gram-negative soil bacterium. It goes through a complex life cycle and is uniquely characterized by social behavior that underlies its growth, gliding motility, and development. Cell-cell interactions have been demonstrated to play a role in each of these activities (11).

The two strategies for cell-to-cell communication are the exchange of soluble, extracellular signals and contact-mediated interactions. There have already been shown to be at least four signals that are required for normal development (15, 19). In addition, two extracellular appendages, pili and fibrils, have been shown to play a role in contact-mediated cell-cell interactions (10). The pili of *M. xanthus* are 6 to 8 nm in diameter, extend from one or both poles of the cell, and are required for the social motility of *M. xanthus* (18, 27). Fibrils in *M. xanthus* are approximately 50 nm in diameter, can be 10 times as long as the cell, and are arrayed peritrichously (4).

Fibrils were first described by Fluegel (13), who demonstrated their presence by staining them with India ink and referred to them as myxonemata. Subsequently, Arnold and Shimkets (1, 2) showed that the Dsp mutant, which is defective in cohesion, development, and social motility, also lacked fibrils. Shimkets (29) showed that intact wild-type cells could rescue cohesion and development of Dsp and that the rescue depended on contact between the strains. On the basis of these observations, he suggested that the fibrils played a role in the contact-mediated social behavior. This suggestion was supported and extended by Behmlander and Dworkin (4), who have isolated and characterized the extracellular fibrils, have shown that they are composed of approximately equal amounts of protein and carbohydrate (5), and have characterized the major protein component of the fibrils (6).

In this paper, we show that isolated, purified fibrils are capable of duplicating the abilities of intact, wild-type cells to rescue cohesion and development and, in addition, to some extent to rescue developmental gene expression in the Dsp mutant of *M. xanthus*. This work thus supports and adds strength to the notion that the fibrils play an integral role in contact-mediated cell-cell interactions in *M. xanthus*.

MATERIALS AND METHODS

Strains and cultivation. Strains of *M. xanthus* used in this work are listed in Table 1. *M. xanthus* was grown at 32°C in CTT broth (18) with shaking at 300 rpm. For MD1000 and other Tn5 insertion strains, kanamycin was added to CTT broth at a final concentration of 50 µg/ml. *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* were grown at 37°C in nutrient broth. *Stigmatella aurantiaca* DW135 (14) was grown at 32°C in 1% tryptone with 8 mM MgSO₄.

Fibril isolation. *M. xanthus* MD207 (originally DK1622) cells were grown on pans (25 by 37.5 cm²) containing CTT agar. Each pan was inoculated with approximately 10¹⁰ cells. After 3 days of growth at 32°C, the cells from each pan were harvested and resuspended in 200 ml of TNE buffer (10 mM Tris-HCl [pH 7.5], 100 mM NaCl, 5 mM EDTA). The cells were declumped by being blended with a Sorval Omnimixer for 1 min, suspended in 800 ml of TNE buffer with sodium dodecyl sulfate (SDS; final concentration, 0.1%), and stirred until the suspension was clear yellow. Fibrils were sedimented by centrifugation at 6,780 × g for 15 min and then washed several times with TNE buffer containing 0.1% SDS until the associated yellow color had disappeared. To remove SDS, the fibrils were washed at least three times with TNE buffer. In order to remove EDTA on the fibrils, the fibrils were washed twice with 10 mM MOPS (morpholinepropanesulfonic acid; Sigma), pH 6.8, and twice with cohesion buffer (10 mM MOPS buffer [pH 6.8], 1 mM CaCl₂, 1 mM MgCl₂). The fibrils were stored in cohesion buffer at –80°C.

Fibrils were quantified on the basis of amounts of fibril carbohydrate. Carbohydrate was determined by the phenol-

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TABLE 1. *M. xanthus* strains

Strain	Genotype	Reference
MD207	Wild type	30
LS489	Tn5 <i>lac</i> Ω DK4406	19
LS701	Tn5 <i>lac</i> Ω DK4400	19
LS713	Tn5 <i>lac</i> Ω DK4500	19
LS715	Tn5 <i>lac</i> Ω LS234	19
LS719	Tn5 <i>lac</i> Ω LS237	19
LS727	Tn5 <i>lac</i> Ω DK4435	19
MD1000	<i>dsp-1680</i> Tn5 Ω DK1407	26
LS247	<i>dsp-1693</i> Tn5 <i>lac</i> Ω DK4500	19
LS252	<i>dsp-1693</i> Tn5 <i>lac</i> Ω DK4406	19
LS700	<i>dsp-1693</i> Tn5 <i>lac</i> Ω DK4400	19
LS718	<i>dsp-1693</i> Tn5 <i>lac</i> Ω LS237	19
LS722	<i>dsp-1693</i> Tn5 <i>lac</i> Ω LS234	19
LS726	<i>dsp-1693</i> Tn5 <i>lac</i> Ω DK4435	19

sulfuric acid assay (17) with glucose as the standard. The amount of carbohydrate was thus equivalent to micrograms of glucose. In the text that follows, amounts of fibrils added will be reported in amounts of fibril carbohydrate per cell.

Cell cohesion assay. Cells were grown to a density of 1×10^8 to 5×10^8 cells per ml and were harvested by centrifugation at $6,780 \times g$ for 10 min. The cell pellets were washed once with 10 mM MOPS and then resuspended in cohesion buffer. Isolated fibrils were added to the cell suspension. The final cell density was 5×10^8 cells per ml, and the final fibril concentration was 80 or 160 mg of fibril carbohydrate per ml. Before the fibrils were added to the cell suspension, they were dispersed by sonication at 80 W for 1 min on ice. For inhibition experiments, Congo red (5 μ g/ml), sodium azide (10 mM), sodium cyanide (5 mM), ethanol (9.5%), chloramphenicol (15 μ g/ml), or rifampin (10 μ g/ml) was added at the beginning of the assay. For the assay, the cell-fibril mixture was incubated at 32°C without shaking. At each time point, 300 μ l of the mixture was centrifuged for 90 s at $250 \times g$. The optical density at 540 nm (OD_{540}) of the supernatant solution was then measured. The blank for the cells was cohesion buffer, and the blank for samples with fibrils was the supernatant of the suspension of fibrils in cohesion buffer after centrifugation. The percentage of cohesion was calculated as follows: percentage of cohesion = $(OD_t/OD_0) \times 100$, where OD_t = OD at time t and OD_0 = OD at time zero.

Developmental rescue. *M. xanthus* MD1000 cells were washed once with 10 mM MOPS and then resuspended in cohesion buffer at a final cell density of 2.5×10^9 cells per ml. Isolated fibrils were then added to the cell suspension at a final concentration of 6.4×10^{-7} μ g of fibril carbohydrate per cell. Fifty microliters of the cell mixture was spotted on TPM agar (10 mM Tris-HCl, 1 mM phosphate buffer, 8 mM $MgSO_4$ [pH 7.6]). After the spot had dried, the plates were incubated at 32°C. For counting sonication-resistant myxospores, the cells were scraped from the agar after 48 h of development and resuspended in 200 μ l of water. After sonication at 80 W for 30 s, the spores were directly counted with a Petroff-Hausser counter.

Developmental gene expression. The procedure for measuring developmental gene expression was similar to that of Kroos et al. (20). The times of expression for the various developmental gene fusions are as follows: 9 h (Ω DK4400), 10 h (Ω LS234 and Ω LS237), 14 h (Ω DK4473), 17 h (Ω DK4406), and 22 h (Ω DK4435) after initiation of development. Cells were washed and treated as described above under Developmental rescue. To break the cells, the samples were sonicated

FIG. 1. Cohesion rescue of MD1000. Solid squares, MD1000; open circles, MD1000 with 1.6×10^{-7} μ g of fibril carbohydrate per cell; solid circles, MD 1000 with 4×10^{-8} μ g of fibril carbohydrate per cell; open squares, MD207.

with a microsonication tip at 80 W for 5 min on ice with ethanol, in the presence of 200- μ m-diameter acid-washed glass beads. Protein was measured by the Lowry protein assay (24) with bovine serum albumin as the standard. The assay was performed in the presence of 1% SDS to solubilize the membrane proteins. β -Galactosidase activity was calculated as follows: Specific activity (nanomoles of *o*-nitrophenyl per milligram of protein per minute) = $213 \times OD_{420}$ /milligrams of protein $\cdot$ minutes.

RESULTS

Rescue of cohesion of Dsp mutants by isolated fibrils. Wild-type strains of *M. xanthus* cohere strongly when in the presence of the divalent cations Mg^{2+} and Ca^{2+} (28). Under these conditions, the nonfibrillated Dsp mutant fails to undergo cohesion (1). We examined the abilities of isolated fibrils to induce cohesion in the Dsp mutant MD1000. The fibrils had been washed several times to remove SDS and were stored in cohesion buffer which contains the divalent cations Ca^{2+} and Mg^{2+} to saturate any possible remaining EDTA. For the cohesion rescue assay, divalent cations were added. Immediately after the addition of the fibrils, microscopic examination revealed that the cells were already beginning to clump (data not presented) and the turbidity of the suspension had begun to decrease before the first reading could be taken (Fig. 1). By 30 min, 90% of the Dsp cells bound to the fibrils and formed a tight complex of cells and fibrils. The binding activity was nearly saturated after 30 min. To rescue the cohesion of Dsp cells to the wild-type level, 1.6×10^{-7} μ g of fibril carbohydrate per cell was required. The ratio of the amount of isolated fibrils required to rescue Dsp cells is 17 times greater than the amount of fibrils on the intact wild-type cells that is required to rescue one Dsp cell.

Figure 2 is a dose-response curve for fibril rescue of cohesion and indicates that amounts of fibrils greater than $1.6 \times$

FIG. 2. Dose response of cohesion rescue and fibril concentration. MD1000 cells were incubated with isolated fibrils for 90 min in MOPS buffer containing 1 mM $MgCl_2$ and 1 mM $CaCl_2$.

10^{-7} µg of fibril carbohydrate per cell did not increase the extent of cohesion rescue.

Cohesion rescue requires divalent cations. As a prelude to examining the requirement for divalent cations, fibrils were isolated without washing with cohesion buffer and were resuspended in MOPS instead of cohesion buffer. During the assay, 1 mM $CaCl_2$ and 1 mM $MgCl_2$ were added individually and jointly. In the absence of divalent cations, the isolated fibrils failed to rescue cohesion of Dsp cells (data not shown). With 1 mM $CaCl_2$, rescue was partially effective, i.e., 65% at 30 min. In contrast, after 30 min of incubation in the presence of both $CaCl_2$ and $MgCl_2$, the rescue was 90% of the wild-type level. In the presence of 1 mM $MgCl_2$, the rescue approached that with both divalent cations but was delayed by 30 min. The data suggest that optimum cohesion rescue required both Ca^{2+} and Mg^{2+} divalent cations. In all further experiments 1 mM $CaCl_2$ and 1 mM $MgCl_2$ were used.

Inhibition of cohesion rescue. Shimkets (28) had previously shown that energy poisons such as sodium azide and sodium cyanide inhibited cohesion of intact wild-type cells. Cohesion rescue of the Dsp mutant by isolated fibrils was likewise inhibited by similar concentrations of sodium azide (10 mM) or sodium cyanide (5 mM) (Table 2). As with intact cells, inhibition of cohesion rescue by the energy poisons was reversible. Removal of both energy poisons by washing allowed cohesion rescue to proceed (data not shown).

Cell cohesion of wild-type cells has been shown to be inhibited by Congo red (1). Congo red also inhibited the cohesion rescue of Dsp mutants by isolated fibrils. The addition of 5 µg of Congo red per ml at the beginning of the cohesion assay resulted in a 72% inhibition of cohesion rescue (Table 2). This concentration of Congo red had no effect on cell growth in CTT broth. Unlike a previous study (1), we found that concentrations of Congo red higher than 5 µg/ml resulted in cell lysis.

Shimkets (28) reported that ethanol inhibited cohesion of intact cells. However, 9.5% ethanol had little effect on cohesion rescue by isolated fibrils.

New protein biosynthesis and cohesion rescue. New protein

TABLE 2. Inhibition of cohesion

Inhibitor ^a (concn)	% Inhibition	
	Dsp cell cohesion rescue ^b	Wild-type cell cohesion ^c
Sodium azide (10 mM)	45 ± 11	59 ± 8
Sodium cyanide (5 mM)	68 ± 9	85 ± 11
Congo red (5 µg/ml)	72 ± 6	93 ± 4
Chloramphenicol (15 µg/ml)	0.2 ± 0.2	0
Rifampin (10 µg/ml)	2 ± 2	0
Ethanol (9.5%)	23 ± 9	89 ± 10

^a Each of these substances, with the exception of sodium cyanide, ethanol, and Congo red, inhibited the growth of *M. xanthus* on CTT agar.

^b Cells were incubated with 1.6×10^{-7} µg of fibril carbohydrate per cell in MOPS buffer containing 1 mM $MgCl_2$, 1 mM $CaCl_2$, and the appropriate inhibitor for 90 min.

^c Cells were incubated in MOPS buffer containing 1 mM $MgCl_2$, 1 mM $CaCl_2$, and the appropriate inhibitor for 90 min.

biosynthesis was not required for agglutination of wild-type cells (28). Likewise, cohesion rescue by isolated fibrils was not affected by the presence of 15 µg of chloramphenicol per ml or 10 µg of rifampin per ml (Table 2).

Genus specificity of cohesion rescue. Four gram-negative bacteria, *E. coli*, *P. mirabilis*, *P. aeruginosa*, and *S. aurantiaca*, and one gram-positive bacterium, *B. subtilis*, were used to determine whether the fibrils were inducing cohesion simply by virtue of a nonspecific stickiness. *S. aurantiaca* is a myxobacterium closely related to *M. xanthus*, which like *M. xanthus* undergoes specific, developmental cohesion (7). For *S. aurantiaca* DW135, the cohesion buffer contained magnesium but no calcium, as cohesion of cells of *S. aurantiaca* DW135 is induced by the presence of calcium. For the other strains, the cohesion buffer contained both magnesium and calcium ions. Figure 3 illustrates the ability of added fibrils to rescue cohesion in *M. xanthus* (MD1000); after 120 min of incubation, the isolated fibrils did not induce cell agglutination in any of the other bacteria (data not shown for *E. coli* and *P. mirabilis*).

Rescue of development in Dsp mutants. MD1000 (Dsp) not only is unable to cohere but also is unable to go through any of the visible aspects of cell development. However, when cells of the Dsp mutant were mixed with isolated fibrils immediately before being spotted on TPM agar, the cells aggregated and then formed fruiting bodies after 24 h. The fruiting body contained sonication-resistant myxospores. The extent of developmental rescue was dependent on fibril concentration. At 1.6×10^{-7} µg of fibril carbohydrate per cell, only a few irregular aggregates were formed (data not shown); however, the number of sonication-resistant spores increased approximately sevenfold (Table 3). At 3.2×10^{-7} µg of fibril carbohydrate per cell, the number of aggregates increased (Fig. 4C) and the number of sonication-resistant spores increased to the wild-type level (Table 3). At 6.4×10^{-7} µg of fibril carbohydrate per cell, the Dsp cells were able to form mound-shape fruiting bodies that were, in fact, visibly larger than those formed by the wild-type strain (Fig. 4D), and their content of sonication-resistant spores was slightly higher than that of the wild-type fruiting bodies (Table 3).

Developmental gene expression. We examined the effect of isolated fibrils on the expression of six developmental genes present in the Dsp background. Kroos et al. (20) described a number of mutants of *M. xanthus*, generated by the insertion of *Tn5-lacZ* reporter genes. Some of these genes were expressed at various times during development. Li and Shimkets (21) have transferred a number of these developmentally regulated

FIG. 3. The specificity of cohesion rescue. Cells of *M. xanthus* were incubated with 1.6×10^{-7} μg of fibril carbohydrate per cell for 120 min. (A) MD1000; (B) *S. aurantiaca* DW135; (C) *B. subtilis*; (D) *P. aeruginosa*. Bar, 20 μm .

reporter genes into the Dsp background. We selected six of these mutants whose genes were expressed at 9, 10, 14, 17, and 22 h of development and examined the effects of the isolated fibrils on development and on developmental gene expression in these strains (Table 1). In 5 of the 6 Dsp-Tn5 *lac* strains, the isolated fibrils completely restored normal development. Fibrils had no effect on LS726(Ω DK4435) in which the Tn5 *lac* is expressed normally at 22 h of development. The fibrils restored gene expression in only two of the Dsp strains. For the fusion gene Ω LS237 (normally expressed at 10 h of development) in a Dsp background, addition of isolated fibrils resulted in only one-third of the wild-type level of activity (Fig. 5). For the fusion gene Ω DK4406 (normally expressed at 17 h of

development), the gene expression in the Dsp background was rescued to the final wild-type level by the fibrils, but the increase in expression did not take place until 24 h of development had elapsed (Fig. 6).

DISCUSSION

The phenotype of the Dsp mutant has indicated that the extracellular fibrils of *M. xanthus* are causally involved in cell cohesion, S motility, and fruiting body and myxospore development (1, 2, 4, 10, 28). Dana and Shimkets (9) have shown that the introduction of suppressor mutations that increased the levels of fibril formation in mutants of *M. xanthus* restored cohesion and development to these mutants. Furthermore, it has been possible to rescue cohesion and development by mixing the Dsp mutant with intact, fibrillated cells of the parent strain (29). Unlike other examples of extracellular complementation in *M. xanthus* (15), rescue in this case required the physical proximity of the rescuer and rescuee. The goal of the present work was to demonstrate that this rescue could be duplicated by the isolated fibrils themselves, thus lending support to the notion that the fibrils play a major role in these examples of contact-mediated social behavior. We have, indeed, been able to demonstrate that isolated fibrils can rescue cohesion, fruiting body and myxospore formation, and to some extent, developmental gene expression in the Dsp mutant.

In order to restore cohesion of one Dsp mutant cell to the

TABLE 3. Sonication-resistant spores formed by wild-type, Dsp, and rescued cells

Strain	No. of spores (10^5)/cm ² (SD [%])
MD1000(Dsp).....	9 (0.6)
MD1000(Dsp) + 1.6×10^{-7} μg of fibril carbohydrate per cell.....	69 (11)
MD1000(Dsp) + 3.2×10^{-7} μg of fibril carbohydrate per cell.....	110 (10)
MD1000(Dsp) + 6.4×10^{-7} μg of fibril carbohydrate per cell.....	160 (13)
MD207 (wild type).....	120 (8)

FIG. 4. Developmental rescue of MD1000. Cells were incubated on TPM agar for 48 h. (A) MD207; (B) MD1000; (C) MD1000 plus 3.2×10^{-7} μg of fibril carbohydrate per cell; (D) MD1000 plus 6.4×10^{-7} μg of fibril carbohydrate per cell. Bar, 500 μm .

wild-type level, it was necessary to add an amount of isolated fibrils from 17 wild-type cells. Shimkets (29) reported that the ratio of intact parent to Dsp cells required to restore cohesion was 1. The amount of isolated fibrils necessary to accomplish rescue is higher but not unexpected. The isolated fibrils may have lost some of their biological activity as a result of having been repeatedly washed with SDS. In addition, their access to the cell surface receptors may be less than that of the fibrils attached to cells.

Both Mg^{2+} and Ca^{2+} divalent cations have been shown to be necessary for optimum cohesion of wild-type strains of *M. xanthus* (28). Cohesion rescue of Dsp mutants by isolated fibrils also required both calcium and magnesium. The function of divalent cations in cohesion rescue is still unclear. It has been suggested that calcium serves as an ionic bridge between cells during flocculation of *Saccharomyces cerevisiae* (16). In cohesion rescue, calcium and magnesium divalent cations may likewise play a role in ionic interactions between fibrils and the cell surface. Congo red is a diazo dye that binds to certain carbohydrates and proteins by hydrogen bonding (22). Arnold and Shimkets (1) showed that Congo red blocked cohesion, development, and social motility in wild-type cells. Their results also suggested that Congo red blocked the formation of fibrils (2). Since cohesion rescue as described here was mediated by extracellular fibrils, inhibition of cohesion rescue by the Congo red must have been due to the ability of Congo red to block the interaction of the fibrils and the cell surface. It suggests that hydrogen bonding may be one of the interaction forces in fibril-mediated cell cohesion.

Cohesion rescue required energy but was not affected by inhibitors of protein biosynthesis. Ethanol also did not substantially affect the cohesion rescue by isolated fibrils, despite an earlier report that ethanol blocked cohesion by wild-type cells (28). Since ethanol has been shown to block protein export in *E. coli* (3, 12), its effect may be on the export of fibril precursors.

The failure by the fibrils to induce cohesion in four unrelated gram-negative bacteria, one gram-positive bacterium, and the closely related myxobacterium *S. aurantiaca* has convinced us that the ability of the isolated fibrils to rescue cohesion of the Dsp mutant is not a trivial reflection of some nonspecific stickiness of the fibrils. It should be pointed out that a specific cell cohesion system has been demonstrated for *S. aurantiaca* (26).

As a paradigm of receptor-ligand complexes, the laminin-laminin receptor interaction has been shown to be both saturable and temperature sensitive (23). We found that cohesion between cells and isolated fibrils is saturable, with rescue gradually approaching a plateau, and is finally achieved at 1.6×10^7 μg of fibril carbohydrate per cell. After 30 min, the rescue was essentially saturated. In addition, the fibril-mediated cohesion rescue was temperature sensitive. At 20°C, cohesion rescue was 70% compared with 93% at 32°C, at a concentration of 1.6×10^{-7} μg of fibril carbohydrate per cell. These data are consistent with the notion that the fibril-mediated cohesion involves a receptor-ligand-type interaction. The isolation of fibril binding-minus mutants may help to resolve this issue. Technical difficulties have prevented us from

FIG. 5. Developmentally regulated Ω LS237 expression under *dsp*⁺ background (LS719; open squares), *dsp* background (LS718; open circles), and *dsp* background (LS718) plus 6.4×10^{-7} μ g of fibril carbohydrate per cell (solid circles). The cells were spotted on TPM agar and harvested at various times for assay of β -galactosidase specific activity.

determining whether isolated fibrils can rescue social motility. Thus, we are unable to comment on their role in S motility.

The isolated fibrils were able to rescue morphological development of the Dsp mutant completely. Cells thus treated formed fruiting bodies that were, in fact, slightly larger than those formed by the developmentally competent parent strain, and those fruiting bodies contained significantly greater numbers of myxospores than did the fruiting bodies of the parent strain.

Recently, Li and Shimkets (21) showed that the *dsp* muta-

tion decreased or eliminated the expression of certain developmental genes that normally were expressed between 9 and 22 h after the initiation of development. We checked the effects of fibrils on six Dsp strains containing Tn5 *lac* insertions. Isolated fibrils rescued development in five strains, whose Tn5 *lac* genes are normally expressed at 9, 10, 14, or 17 h. However, the fibrils were able to rescue gene expression in only two of those strains—those whose reporter genes were normally expressed at 10 and 17 h of development. O'Connor and Zusman (25) have shown that there are two pathways of development in *M. xanthus*. It is possible that in those mutants whose development is rescued but whose gene expression is not, the reporter gene is part of one of those pathways. Strain LS726, whose reporter gene is normally expressed at 22 h of development (21) and which does not on its own cause a developmental defect, is an especial puzzle, as neither development nor gene expression is rescued by the fibrils. In this strain, the combination of the *dsp* and the Tn5 *lac* mutations may have generated additional, as yet unknown, factors required for development.

The role of the fibrils in development is not at all clear. They have a number of possible functions, one of which may be to transmit or receive a cell-to-cell signal. The paracrine morphogen, CsgA, appears to be associated with the extracellular fibrils (31). Li and Shimkets have argued that their data (21) support the conclusion that the fibrils are not necessary for either the transmission or perception of CsgA. However, while the evidence that indicates that CsgA presentation does not require fibrils is persuasive, the argument excluding a role for fibrils in receiving or perceiving the Csg morphogen is rather indirect and less convincing. In addition, other developmental proteins have been shown to be associated with the fibrils (8). Thus, the possibility that the fibrils play some sort of a role in the exchange of paracrine signals has not yet been excluded. Another alternative possibility is that the fibrils serve as tactile antennae, functioning to sense the presence either of neighboring cells or of substrata. Finally, they may physically anchor cells to one another during social motility, developmental aggregation, or fruiting body construction.

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FIG. 6. Developmentally regulated Ω DK4406 expression under *dsp*⁺ background (LS489; open squares), *dsp* background (LS252; open circles), and *dsp* background (LS252) plus 6.4×10^{-7} μ g of fibril carbohydrate per cell (solid circles). The cells were spotted on TPM agar and harvested at various times for assay of β -galactosidase specific activity.

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