Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 3679–3683, May 1989
Developmental Biology

A glycosylation mutation affects cell fate in chimeras of *Dictyostelium discoideum*

(pattern formation/position determination/cellular slime molds)

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Communicated by J. T. Bonner, February 21, 1989 (received for review October 1, 1988)

ABSTRACT **Prestalk and prespore cells form a simple pattern in the pseudoplasmodium of the cellular slime mold *Dictyostelium discoideum*. Prestalk cells are distinguished from prespore cells by a low level of expression of a glycoantigen on their surfaces and by reduced intercellular cohesion. We examined the possible significance of these differences, using the *modB* mutation, which eliminates this glycoantigen genetically, leading to reduced intercellular cohesion. *modB* mutant cells were allowed to develop together with normal cells to form chimeric slugs. Mutant cells labeled by feeding with fluorescent bacteria were highly enriched in the prestalk cell zone at the anterior end of the slug. In contrast, normal cells, if in a minority, were concentrated in the rear part of the prespore cell zone. Immunoblot analysis and cell-by-cell double-label immunofluorescence of these mixtures showed that mutant cells underproduced several prespore cell markers. Mutant cells tended not to form spores in chimeras unless they exceeded a threshold proportion of *ca.* 30%. However, mutant cells showed no tendency to produce excess prestalk cells when allowed to develop alone. These findings are most simply explained by postulating that reduced glycoantigen expression and intercellular adhesion encourage a more anterior cell localization, which in turn causes differentiation into a prestalk cell. Since normal prestalk cells also show reduced glycoantigen expression and intercellular adhesion, this suggests that a similar mechanism may contribute to pattern formation during normal development.**

The formation of patterns of cells during development is essential to tissue morphogenesis. The cells in the pseudoplasmodium (or slug) of the cellular slime mold *Dictyostelium* form a pattern consisting of prestalk cells, which lie in the anterior 20–30% of the slug, and prespore cells, which lie in the remaining portion (1, 2). Mixed with the prespore cells is a small proportion (*ca.* 10%) of cells that most resemble prestalk cells but do not behave in the same way; these are referred to as anterior-like cells (3). Slugs form as the result of aggregation of cells after starvation and their rearrangement into a slug-shaped structure. During this phase, all cells appear to be the same biochemically and morphologically. However, evidence has accumulated that cells are determined to become prestalk or prespore cells at the time of starvation according to the phase of the cell cycle (4–6) and their glycogen content (7). These findings support the general model that cells first differentiate (8) and then rearrange themselves (3, 9–12), either by "self-assembly" (13) or with the help of a tip-localized organizer (14, 15). The mechanism of this rearrangement remains obscure, with evidence for both differential cell adhesion (16, 17) and chemotaxis (18) having been presented.

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Determination in *Dictyostelium* is reversible. For example, raising the temperature in strains carrying a temperature-sensitive (*ts*) allele of *stkA* directs prespore cells to transdifferentiate into prestalk cells (19, 20). Simple amputations cause morphallactic transdifferentiation of prestalk into prespore cells and vice versa (21). A diffusible substance (DIF) appears to act in the slug to influence prestalk-cell differentiation (22). In *Dictyostelium mucoroides*, prespore cells continuously transdifferentiate into prestalk cells as they traverse a boundary near the anterior end of the slug (23). Perhaps an initial process of deterministic differentiation and cell sorting serves to set up a provisional pattern, which is followed by a regulative phase guided by a positional component.

In this report we examine the behavior of *modB* mutant cells introduced into normal slugs and vice versa. The *modB* mutation exerts a relatively limited effect on protein glycosylation: only two of the six glycoantigens (GAgs; defined at least in part by carbohydrate) we have screened are affected, and reactivity with mannose- and GlcNAc-reactive lectins is not dramatically altered (refs. 24 and 25; data not shown). The affected GAgs (GAg-XI and GAg-XX) are normally expressed only in aggregation stage cells and later (24–26) and are highly enriched in prespore relative to prestalk cells (24, 26, 27). Though several proteins that are underglycosylated in *modB* mutants still accumulate and localize in the plasma membrane or spore coat (24, 25; unpublished data), a glycoprotein involved in homotypic intercellular adhesion (gp80) in aggregation-stage cells is not delivered properly to the plasma membrane (25), which causes intercellular adhesion to be weaker compared with that of normal cells (24, 28). In chimeras, we now find that mutant cells seek the anterior end of the slug and differentiate into prestalk cells. These observations suggest a role for cell-surface oligosaccharides in the positioning of normal cells in the pattern. Cell-surface oligosaccharides have already been suggested to be important for cell communication in this and other organisms (29, 30).

EXPERIMENTAL PROCEDURES

Cells. Strains DL117 (*modB501/+*), DL118 (*modB501/modB503*), and DL119 (*modB502/modB503*) have been described (27). These diploids were used to eliminate effects of expression of unknown recessive mutations. Strain HW10 was derived from M4-3 in the same screen used to find HW11 (renamed from HM11; ref. 31) and is phenotypically normal. WS584, classified by morphological criteria as *Dictyostelium discoideum*, is a wild-type isolate previously reported to lack the monoclonal antibody (mAb) MUD1 epitope (32) and found here also to lack the mAb 59.9 epitope. Cells were grown on SM agar in association with *Klebsiella aerogenes* or *Serratia marcescens* (Detrick) at 22°C. Pigment variants

Abbreviations: mAb, monoclonal antibody; GAg, glycoantigen (defined at least in part by carbohydrate).

of *S. marcescens* were obtained by streaking on SM agar (25) and transferring colonies with desired pigmentation to new SM agar. White variants were used to feed unlabeled cells, and variants of intermediate (not intense) pigmentation were used to feed labeled cells.

Preparation and Examination of Chimeras. For localizing marked cells, chimeras were made by the following procedure. Labeled cells were grown in association with pigmented *S. marcescens*, and unlabeled cells were grown with unpigmented *S. marcescens*. Before the bacteria were exhausted, cells were harvested, washed free of bacteria in PDF (24), resuspended in PDF at 2×10^8 cells per ml, and separately deposited at 1.5×10^6 cells per cm^2 on nonnutrient agar (24) for 6 hr. After this period, which allowed the cells to purge themselves of exchangeable pigment, cells were recovered at 2×10^8 cells per ml, mixed with partners in the desired proportions, and replated in 30-μl droplets on nonnutrient agar. Cultures were at the same morphological stage of development at the time of cell mixing. While unmixed cultures were maintained through the period of chimera development to ensure that separate strains remained isodevelopmental, trials in which one strain was slightly advanced yielded similar results. Upright fingers formed by approximately 10 hr of additional incubation were lifted with a scalpel blade, deposited on a microscope slide in a drop of PDF, and squashed under microscopic viewing (to verify the orientation of the prestalk/prespore cell axis) with a coverslip. Coverslip pressure was modulated by varying the volume of the drop to ensure collapse of the slug into a monolayer, which was essential for visualizing the distribution of pigmented cells. Fluorescence was examined by epi-illumination from a 100-W mercury arc lamp through a Nikon G-20 (rhodamine) filter set.

Other chimeras usually were formed from cells grown in association with *K. aerogenes* and in some cases were formed without a 6-hr preincubation on nonnutrient agar (this did not affect results obtained by analyzing the spore populations). For Western blot immunoanalysis, a crude membrane fraction was prepared by centrifugation (24). Gel samples were quantitated by input cell number, electrophoresed, electrophoretically transferred to nitrocellulose, immunolabeled, and detected by using an alkaline phosphatase system as described (31).

Immunofluorescence Labeling. Identification of spore strain by immunofluorescence required preextraction in 6 M urea/1% (vol/vol) 2-mercaptoethanol/10 mM NH_4HCO_3 for 5 min at 100°C. After spores were washed 3 times by centrifugation in TBS (10 mM Tris·HCl/140 mM NaCl, pH 7.5), the spore suspension was deposited onto a glass slide onto which had been freshly dried a solution (1 mg/ml) of poly(L-lysine) hydrobromide (Sigma; M_r = 59,000). Preparations were blocked with 5% (wt/vol) nonfat dry milk in TBS for 10 min. Normal spores were distinguished from mutant spores by labeling with mAb 5.1 (which recognizes the *modB*-dependent GAg-XX; ref. 24) diluted in blocking buffer for 45 min. After a rinse, the mAb was detected by incubation for 30 min with phycoerythrin-labeled goat anti-mouse IgG (Biomeda, Foster City, CA) according to the manufacturer's instructions. Spores were rinsed, overlaid with Gelmount (Biomeda), and covered with a coverslip. Fluorescence was visualized through a Nikon B-2 or G-20 filter set (G-20 allowed greater sensitivity but accelerated bleaching).

The percentage of cells that were prespore cells in slugs was determined as follows. Individual slugs were placed in a drop of 0.1% Pronase CB (Calbiochem) in freshly prepared 25 mM 2,3-dimercaptopropanol/50 mM Tris·HCl, pH 6.8 (33) on a polylysine-coated slide and were sheared through a drawn-out Pasteur pipette. After cell dissociation, the suspension was diluted 1:9 with the solution without Pronase. After 10 min slides were rinsed in TBS, dipped in methanol at room temperature for 10 min, and air-dried. Slides were blocked and labeled with mAb 83.5 and phycoerythrin-labeled goat anti-mouse IgG as for mAb 5.1 above. Antibody-negative cells are defined as those phase-visible cells that were antibody negative by viewing through the G-20 filter; >500 cells from four or more slugs were counted and averaged for each entry.

RESULTS

Fluorescent Localization of Mutant Cells in Chimeras. To observe mutant cells in chimeras, we turned to an early method in which the cell population to be marked had been fed on a diet of *S. marcescens* (21). Not only did cells that had ingested these pigmented bacteria take on a reddish hue, but also they became intensely fluorescent. Cells fed unpigmented *S. marcescens* were not fluorescent. Single chimeric slugs that formed 5–10 hr after mixing were squashed into a monolayer beneath a coverslip and viewed through fluorescence optics.

Chimeras initially were formed from strains DL118 (mutant) and DL117 (normal) in proportions of 2:8, 5:5, and 8:2. In trials in which DL118 cells were fluorescently labeled and constituted 20% of the cell number, strong fluorescence was found in some of the anterior tip cells (Fig. 1*A*). Strong anterior fluorescence was observed in each of the several examples studied in four trials. As a control, an 8:2;DL118:DL117* (the asterisk indicates labeled cells) chimera is shown (Fig. 1*B*) in which the normal DL117 cells were labeled. The fluorescent cells were more diffusely distributed but concentrated near the posterior end of the slug, rather than dispersed throughout the prespore compartment. The data suggest a tendency for mutant DL118 and normal DL117 to localize toward opposite ends of the slug, implying that lack of GAg may affect position in the slug.

In 5:5;DL118*:DL117 chimeras (in which DL118 cells were labeled), the anterior tip cells were, as expected, fluorescent. Patchy rather than dispersed fluorescence was also observed in the prespore zone (data not shown). In similar chimeras in which DL117 cells were instead labeled, a broader zone of the prespore region was fluorescent than in 8:2;DL118:DL117* chimeras (data not shown). Fluorescence tended to be patchy rather than uniformly dispersed. Corresponding results were found in DL119:DL117 and HW11:HW10 chimeras at all strain ratios (data not shown). The observations indicate that

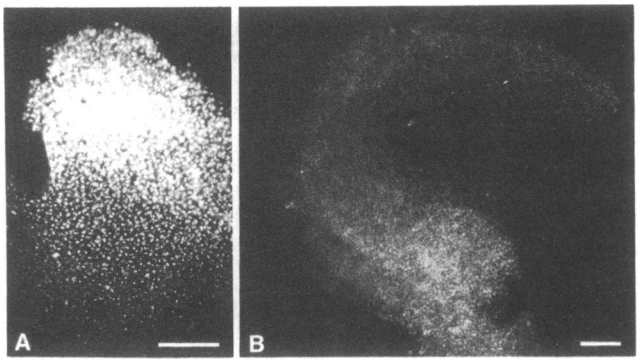

FIG. 1. Cell sorting in chimeras. Mutant or normal cells were grown in association with pigmented or unpigmented *S. marcescens* and allowed to form chimeric slugs as described. Individual slugs were picked and squashed into a monolayer beneath a coverslip, and the location of cells that had been fed pigmented bacteria was determined by fluorescence. (*A*) A 2:8;DL118*:DL117 slug (the asterisk denotes labeled cells). Only the anterior end of the slug is shown because the remainder was essentially nonfluorescent. Fluorescent (mutant) cells are concentrated in the anterior tip. (*B*) A 8:2;DL118:DL117* chimera. The fluorescent normal cells are concentrated in the posterior region of the slug. (Bar = 100 μm.)

modB mutant cells tend to localize in the anterior end of the slug, whereas normal cells tend to occupy the rear. In mixtures in which both strains occupied the middle zone, cells of the same strain still tended to cluster with one another rather than mix homogeneously or clearly sort in a polar fashion.

Expression of Cell Type Markers by Mutant Cells. The state of differentiation of the mutant cells in chimeras was examined by assaying the expression of prespore cell markers. Because the *modB* mutation reduces the apparent molecular weight of several prespore cell-specific glycoproteins (25, 31), this provided a convenient device for assaying the synthesis of prespore markers by *modB* mutant cells in the presence of normal cells.

Chimeric slugs were harvested and analyzed by NaDodSO$_4$/PAGE followed by Western blotting (Fig. 2). Fig. 2*A* shows a range of ratios of HW11, the *modB*-like mutant strain, to HW10, a normal sister strain. The 0:10;HW11:HW10 lane shows pure HW10, which expresses SP29, a prespore cell-specific glycoprotein, at the normal molecular weight position (detected using the mAb MUD1 and denoted by the arrow in the right margin). Strain HW11 produced several lower molecular weight species as shown in the 10:0 lane (31). Mixtures produced both normal and small species of SP29 (lanes designated "pre" for predevelopment mix in Fig. 2). However, lower molecular weight forms of SP29 appeared to be underproduced in the 3:7 and 1:9 HW11:HW10 lanes, in which mutant cells were in a minority. Underproduction of the mutant form of SP29 was confirmed by comparing these lanes with lanes in which pure cultures of HW11 and HW10 were mixed after developing alone (lanes depicted "post" for postdevelopment mixture in Fig. 2). For example, chimeras containing 30% HW11 cells produced the mutant form of SP29 at a level expected only if <10% of the prespore cells were mutant (compare lane 3:7 "pre" with lane 1:9 "post").

The same result was found when another prespore cell marker, WGA80B, was probed with mAb 59.9 (Fig. 2*B*). A range of ratios of a different mutant and normal strain pair, DL119 and DL117, were used. Mutant WGA80B (which was smaller) was produced in the 3:7 mutant:normal cultures at a level expected if only *ca.* 10% of the prespore cells were mutant; 5:5 cultures produced mutant WGA80B at a level expected if only *ca.* 30% of the prespore cells were mutant. Similar results were obtained when SP29 was examined in DL119:DL117 and DL118:DL117 chimeras and when WGA80B was examined in HW11:HW10 chimeras (data not shown). Repeat labeling of the blot shown in Fig. 2*B* with another mAb disclosed that two other prespore cell-specific proteins, SP75 and SP96, were produced at normal levels at all strain ratios (Fig. 2*C*). Since SP75 and SP96 are produced at the normal molecular weight in the mutant, this shows that prespore cell differentiation was normal in these chimeras.

The reduced levels of the mutant forms of SP29 and WGA80B could be the result of a failure of mutant cells to become prespore cells or of a rescue of glycosylation in the mutant cells. To determine whether rescue occurred, which might be obscured by the high background of protein produced by normal cells, chimeras were formed with a normal strain (WS584) that spontaneously lacks the epitope recognized by mAb 59.9 on WGA80B. Fig. 2*D* shows that the underproduction of WGA80B by DL118 developed with WS584 was not balanced by an accumulation of normal molecular weight forms of WGA80B, indicating that underproduction of the mutant form of WGA80B was not caused by glycosylation rescue. However, it was found in the 1:9 predevelopment mixture (i.e., chimeric) lane of Fig. 2*D* that a faint band appears at the normal WGA80B position that does not appear in the postdevelopment mixture (i.e., control) lane. This evidence for low-level glycosylation rescue

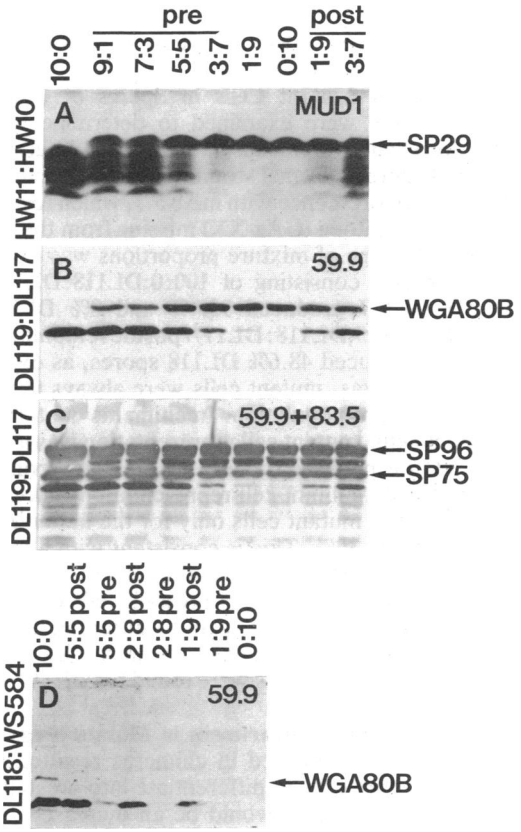

FIG. 2. Lack of expression of prespore markers by mutant cells in chimeras. Chimeras were formed as described, solubilized, electrophoresed, and analyzed by immunoblotting for the expression of prespore cell markers with either mAb MUD1 or mAb 59.9. (*A*) Strains HW10 (normal) and HW11 (mutant) were mixed in the indicated ratios and probed with mAb MUD1 to examine expression of SP29, a prespore cell-specific glycoprotein that is formed at a single molecular weight position by HW10 but as a ladder of lower molecular weight forms in HW11. The mixtures are shown under the heading "pre" to denote predevelopment mixing. To establish expected levels of marker expression, strains were developed separately and then mixed in the desired proportions (shown under heading "post" for postdevelopment mixing). (*B*) Strains DL117 (normal) and DL118 (mutant) were mixed and analyzed in identical fashion except that WGA80B was detected with mAB 59.9. DL117 produces a M_r 80,000 form and DL118 produces a M_r 68,000 form of WGA80B. (*C*) The blot shown in *B* was reprobed with mAb 83.5 to show that expression of the prespore-specific proteins detected by this antibody was similar in all lanes and, thus, that prespore cell differentiation was normal. (*D*) Strains WS584 (normal) and DL118 (mutant) were mixed and analyzed as in *B*. WS584 does not produce the epitope recognized by mAb 59.9, which allowed us to exclude the possibility that the normal strain rescued glycosylation, thereby shifting the mutant marker to the normal molecular weight position where it would be obscured by the high background level produced by normal cells.

was confirmed in other experiments (data not shown), but is not considered further here.

Underproduction of prespore markers by mutant cells might reflect a general depression of synthesis by all mutant cells or a reduced number of mutant cells that express the markers. To address this question, double-label immunofluorescence of dissociated cells in which mAb 83.5, which recognizes a prespore-specific GAg (GAg-X; refs. 24 and 34), and mAb 5.1, which recognizes normal cells, were used. Fewer than 10% of the mutant cells, and *ca.* 50% of the normal cells were marker positive in 2:8;DL118:DL117 or 2:8;DL119:DL117 chimeras (data not shown). This indicates that reduced prespore marker expression by mutant cells is due to a reduced number of expressing cells, which is

consistent with the position of mutant cells in the prestalk region of the slug, where prespore markers are not normally produced.

Representation of Mutant Cells in Spores of Chimeras. Spores of chimeras were examined to determine whether mutant cell localization was conserved as cells differentiated into stalks and spores. Normal strain spores were counted by indirect immunofluorescence with mAb 5.1, which recognizes the carbohydrate epitope (GAg-XX) missing from the mutant cells (24, 25). A range of mixture proportions was examined (Table 1). Controls consisting of 100:0;DL118:DL117 and 0:100;DL118:DL117 produced 99.4% and 0% DL118, as expected, and a 5:5;DL118:DL117 postdevelopment mixture of spores produced 48.6% DL118 spores, as expected. However, in chimeras, mutant cells were always underrepresented in the spore population. In chimeras containing up to 30% mutant cells, mutant cell representation was less than one-fourth of the predicted value. In chimeras containing >30% mutant cells, mutant cell representation reflected the input percentage of mutant cells only for the increment that exceeded the initial 30%. This is consistent with a model in which, once the proportion of mutant cells in the mixtures exceeds the normal proportion of prestalk cells (about 30%; see below), then mutant cells begin to differentiate as prespore and then spore cells in a linear fashion relative to their representation. Similar results were found for DL119:DL117 chimeras (Table 1).

Size of the Prestalk Compartment in Mutant Slugs. If the anterior localization observed in chimeras resulted from a tendency for mutant cells to differentiate into prestalk cells, it was reasoned that there would be an increased prestalk cell:prespore cell ratio in pure mutant slugs. Individual mutant or normal slugs were hand-picked, enzymatically dissociated on a microscope slide using Pronase, and processed for immunofluorescence detection of the prespore marker GAg-X (using mAb 83.5). Fluorescence-negative cells were assumed to be prestalk or anterior-like cells, and positive cells were scored as prespore cells. Slugs formed from the mutant strains DL118 or HW11 contained 68% prespore cells, and slugs composed of cells from the normal strains DL117 or HM10 contained 61% or 62%, respectively, prespore cells. Thus, there was no increase in the proportion of prestalk cells in either mutant strain relative to normal strains. This result was consistent with the results of Western blot comparisons of the levels of the prespore cell markers SP96 and SP75 (Fig. 2*C*; unpublished data), WGA80B (Fig. 2*B*), and SP29 (25), which were expressed at similar levels in total slug culture extracts on a per total protein basis. Thus, there is no mutant phenotype with respect to cell differentiation and pattern formation when mutant cells are cultured alone.

Table 1. Strain identity of spores produced by chimeric fruiting bodies

Chimeras		mAb 5.1-negative (mutant) spores, %	
		Observed	Predicted
% DL118	% DL117		
0	100	0	0
10	90	1.1	0
20	80	4.9	0
40	60	13.3	10
80	20	54.3	50
100	0	99.4	100
50	50 (post-dev. mix)	48.6	50
% DL119	% DL117		
0	100	1.1	0
10	90	0.9	0
20	80	1.3	0
40	60	7.3	10
80	20	53.5	50
100	0	100.0	100

Mutant (DL118 or DL119) and normal (DL117) cells were mixed to form chimeric fruiting bodies. Spores were labeled with mAb 5.1 as described. The controls [10:0;DL118(9):DL117, 0:10;DL118(9):DL117, and 5:5;DL118(9):DL117 mixtures] show that mAb 5.1 labels normal but not mutant spores. The middle column shows the percentage of mutant (mAb 5.1 negative) cells observed in the spore population. Since mutant cells are selectively recruited to the prestalk cell compartment, the representation of mutant cells in the spore population based on their first filling up the stalk cell compartment [assumed to be 30% of total cells (see *Results*)] was calculated and given in the right-hand (predicted) column. Approximately 300 spores were counted for each ratio in each experiment.

DISCUSSION

Five classes of glycosylation mutations in *D. discoideum* fail to block passage through any of the major phases of the life cycle, including pattern formation in the slug (24, 27, 35–37). Because pattern formation may involve relative rather than absolute values of parameters, we designed a less stringent test for the possible involvement of a protein oligosaccharide expressed on cell-surface proteins. Rather than ask whether or not mutant cells were capable of forming a normal pattern of prestalk and prespore cells, we asked whether, if provided a choice, mutant cells would prefer to contribute to one part of the pattern or the other. This test was performed by constructing chimeras consisting of a range of ratios of normal and mutant cells and scoring for the percentage of mutant cells that expressed a prestalk or prespore cell localization or phenotype. This assay discloses a tendency for *modB* mutant cells to become prestalk cells in the anterior end of the slug and stalk cells in the fruiting body that derives from the slug.

Localization of cells in the anterior region of the slug is normally tightly correlated with prestalk cell differentiation. The same is true for *modB* cells mixed with normal cells. Labeled *modB* cells were found in the anterior region by using a fluorescent cell marker. Control experiments showed that the marking method itself did not result in anterior localization and that anterior localization was not the result of faster development, which can dispose cells to become prestalk (38). Most *modB* cells appeared to be prestalk cells because they did not make two prespore cell glycoprotein markers as determined by Western blotting or a prespore cell oligosaccharide marker (GAg-X) as determined by immunofluorescence (not shown). Consistent with this interpretation, *modB* cells tended not to form spores unless they exceeded a threshold representation in the chimeras. The same results were found for all three mutant strains examined. Two of the mutants were homozygous diploid strains, to rule out a possible contribution from unknown recessive mutations that might reside in different genetic backgrounds.

The *modB* mutation exerts a relatively limited effect on protein glycosylation. Only two of the six GAgs we have screened are affected, and reactivity with mannose and GlcNAc-reactive lectins is not dramatically altered (refs. 24 and 25; data not shown). The affected GAgs (GAg-XI and GAg-XX) are normally expressed only in aggregation stage cells and later (24, 25, 39) and are highly enriched in prespore relative to prestalk cells (24, 39). Several of the proteins that are underglycosylated in *modB* mutants still accumulate and localize normally in the plasma membrane or spore coat (ref. 24; unpublished data). However, a protein involved in homotypic intercellular adhesion in aggregation-stage cells is not delivered properly to the plasma membrane (25), which explains why intercellular adhesion is weaker compared to normal cells (24, 28).

The prestalk cell differentiation of *modB* mutant cells may involve an indirect mechanism. In chimeras that contain a small proportion of normal cells, the normal cells are not found uniformly throughout the prespore cell zone but are enriched near the posterior end of the slug, suggesting that the mutation may affect cell position rather than cell type directly. An effect on cell positioning might be expected to involve a cell-surface property. Indeed, *modB* mutant cells do not express GAg-XX on their surfaces and are deficient in intercellular adhesion (24, 25, 27, 28). These very differences also distinguish anterior cells from posterior cells (17, 24, 26, 33). Differentiation of mutant cells into prestalk cells in chimeras is thus most easily explained by postulating that *modB* mutant cells sort anteriorly owing to reduced intercellular adhesion (e.g., ref. 13) and differentiate into prestalk cells according to position (1, 2). Because *modB* mutant cells seem to migrate normally during the aggregation stage (39), there is no reason to invoke altered chemotaxis (see refs. 3 and 18) to explain the sorting phenotype. That a position-dependent mechanism of differentiation might occur is in accord with findings that (*i*) the earliest expression of prespore cell markers may be position-dependent (ref. 40; but see ref. 9–11) and (*ii*) as cells traverse the prespore/prestalk boundary in *D. mucoroides*, transdifferentiation of prespore to prestalk cells occurs apparently by a positional mechanism (23).

Our model in which the mutation specifies the position of *modB* cells in slugs uniquely predicts that the *modB* mutation exerts no tendency to overproduce prestalk cells in the absence of normal cells. This is because a tendency to sort anteriorly would be meaningless without a separate population of cells against which to sort. In contrast, if the mutation directly induced cells to become prestalk cells, an elevated prestalk cell:prespore cell ratio would be predicted for mutant cells cultured alone (since cells could convert from the prespore cell pool), as has been found in other mutant strains with a sorting phenotype (15, 41) and in a phenocopy of this type of mutant (7). A higher prestalk:prespore ratio is not observed for pure cultures of *modB* cells.

In conclusion, our findings suggest that the prestalk cell differentiation of mutant cells in chimeras is an indirect result of sorting to the anterior end of the slug followed by position-dependent differentiation of these anterior cells into prestalk cells. This model does not necessarily contradict deterministic models proposed for cell differentiation. Because the level of GAg-XX expression on the surfaces of individual normal cells is known to differ at the time of pattern formation (25) and is reduced in prestalk cells (24, 26), the proposed mechanism for the anterior sorting of *modB* mutant cells in chimeras may subserve a deterministic (based on cell-cycle and/or glycogen content) mechanism of normal prestalk cell differentiation and sorting. The regulative differentiation of *modB* cells that we have observed may be related to a subsequent process of cell differentiation postulated to refine the balance of prestalk and prespore cells or to restore it in response to an environmental perturbation.

We are grateful to W. F. Loomis for supplying strains DL117, DL118, and DL119; to the late K. B. Raper for strain WS584; to J. R. Milam for *S. marcescens*; and to K. Williams for the MUD1 antibody. H. K. MacWilliams, C. Weijer, and an anonymous reviewer provided useful suggestions. This work was supported by National Institutes of Health Grant GM-37539.

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