

Cytokinesis Is Defective in *Dictyostelium* Mutants with Altered Phagocytic Recognition, Adhesion, and Vegetative Cell Cohesion Properties

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Abstract. Mutants that have been selected for defects in phagocytic recognition, adhesion, and vegetative cell-cell cohesion were found to be larger and more highly multinucleate than their parent strain. This defect is associated with the complex mutant phenotype of these mutants since revertants of the mutants coordinately acquire the wild-type phenotype for all of the defects. The larger size and multinuclearity were due to a high frequency of failure of cytokinesis in cells of wild-type size. This was shown by purifying the small cells in mutant populations and observing their growth and cell division.

The mutant phenotype is more penetrant during axenic growth. Most of the mutants are not multinucleate when grown on bacteria. Recently, new mutants have been isolated that are also multinucleate when grown on bacteria by a strong selection procedure for non-adhesion to tissue culture dishes. The pleiotropic mutant phenotype and the greater penetrance of the mutant phenotype in axenic culture can be explained by hypothesizing a deficiency in a membrane component of the actomyosin motor that is involved in all of the processes defective in the mutants.

To understand the basic cell mechanisms involved in phagocytosis, cell adhesion, and cell-cell cohesion, we have been studying mutants of the cellular slime mold, *Dictyostelium discoideum*, that exhibit a pleiotropic defect in all of these processes (Vogel et al., 1980; Vogel, 1983; Duffy, 1986). Revertants of these mutants, which have been selected by their adhesive properties, also re-acquire wild-type phagocytic and cell-cell cohesion properties, suggesting that all are due to a single underlying mechanism that is altered by these mutations (Vogel, 1983; Duffy, 1986). Genetic analysis has shown that recessive mutations at two loci, *phgA* and *phgB*, lead to the mutant phenotype (Duffy and Vogel, 1984). Revertants are due to dominant suppressor mutations at two additional loci (Duffy, 1986).

Here we report that these mutations also lead to a defect in cytokinesis. Mutant strains exhibit a much broader size distribution that is often bimodal: one peak corresponds to cells of wild-type size and a second peak to cells approximately twice this size. The mutants are multinucleate, the majority having two to three nuclei. Small cells, purified from mutant populations by velocity centrifugation, failed to divide at the time when a parallel culture obtained from wild-type cultures underwent a semi-synchronous division, indicating a cytokinesis defect.

We propose that a common element of the actomyosin motor responsible for cytokinesis, phagocytosis, cell-substrate adhesion, and cell-cell cohesion is deficient or defective in these mutants. Competition for this element by these differ-

ent processes may provide an explanation for the pleiotropic phenotype of these mutants and the inhibition of adhesive and phagocytic activities during mitosis (McDonald and Durston, 1984; Berlin et al., 1978).

Materials and Methods

Growth of Amebae

Sporulating strains were stored on dried, sterile silica gels at 5°C (Reinhard, 1966). Stock cultures were started from silica gels every 2–3 mo by sprinkling a few granules on lawns of *Escherichia coli* B/r growing on lactose-peptone (LP)¹ agar (0.2% bacteriological peptone [Oxoid Ltd., Basingstock, England], 0.2% lactose, 2 mM KH₂PO₄, 2 mM Na₂PO₄, 1.5% Bactoagar [Difco Laboratories Inc., Detroit, MI]).

For most experiments, *Dictyostelium discoideum* strain AX-2 and mutants derived from it were grown in AX-2 medium (Watts and Ashworth, 1970) supplemented with 1.8% maltose instead of glucose (1.43% bacteriological peptone [Oxoid Ltd.], 0.715% yeast extract [Oxoid Ltd.], 1.8% maltose, 3.5 mM KH₂PO₄, 3.6 mM Na₂PO₄). Liquid suspension cultures were shaken on rotary shakers (110 rpm) at 21°C.

For bacterial suspension cultures, *E. coli* B/r bacteria were grown on a nutrient broth (1% bactotryptone [Difco Laboratories Inc.], 0.5% yeast extract [Oxoid Ltd.], 0.5% NaCl, 1% glucose). The bacteria were harvested at late exponential phase and washed five times with phosphate buffer (PB) (14.67 mM KH₂PO₄, 2.83 mM Na₂PO₄, pH 6.1). Bacterial suspensions ($A_{420} = 5$) were inoculated with amebae or heat-shocked spores (30 min, 45°C) and shaken as above for axenic cultures.

1. Abbreviations used in this paper: LP, lactose-peptone; PB, phosphate buffer.

Cell Density and Cell Volume Determination

For cell counts and cell volume determinations 0.5-ml aliquots of axenic or bacterial suspension cultures were incubated on ice for 15 min and vortexed until a single cell suspension was obtained. Cell counts and cell volume were determined using an electronic particle counter (model C₈₁) equipped with a Channelyzer (model C256) from Coulter Electronics, Harpenden, UK. The samples were diluted so that the amebal density did not exceed 2×10^4 per ml to minimize coincidence counting. Volume distribution data were transmitted to a computer (model IIe; Apple Computer Inc., Cupertino, CA). A computer program was written in Pascal to store the data on diskettes, plot it on a matrix printer, and perform routine statistics. Interested parties may obtain a copy of the program by sending a blank diskette to D. R. Waddell.

Mutagenesis

Approximately 10^9 amebae harvested from axenic culture were washed twice with PB and resuspended at 2.5×10^7 cells/ml in PB. Immediately before use 0.2 g of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was dissolved in 0.5 ml DMSO and added to the cell suspension. After a 20-min incubation at 21°C, the cells were pelleted at 200 g at 0°C and washed four times with ice cold PB. Viability assayed by clonal growth was between 1 and 10%.

Selection of Non-adhesive Mutants

After mutagenesis cells were divided into two batches and suspended in axenic growth medium. The cells were allowed to recover for 4 d in suspension culture. The surviving cells were washed free of axenic medium and resuspended in PB at 5×10^6 cells per ml. 10 ml of the suspension were introduced to 600-ml tissue culture flasks (Greiner, Nuertingen, FRG) and allowed to settle and adhere (~20 min). The flasks were gently tilted and the non-adherent cells were carefully decanted using a 10-ml pipette. The selection was repeated until most cells appeared to be non-adherent when viewed with an inverted microscope. After the initial selection, the cells were grown on LP agar plates in association with *E. coli* bacteria. Before amebae had cleared the bacterial lawn, they were harvested, washed free of bacteria, and reselected for adhesion on tissue culture dishes. After ~30 rounds of selection and growth on bacteria, the amebae were cloned by serial dilution, and four clones from each batch of mutagenized cells were isolated. These strains were cloned a second time before being characterized further.

Size Separation of Cells

Velocity sedimentation was used to separate cells according to size. The drift velocity, V_d , of spheres of radius r and density ρ_c in a medium of density ρ_m and viscosity N is given by the following equation:

$$V_d = [2gr^2(\rho_c - \rho_m) / 9N],$$

where g is the centrifugal force (Berg, 1983).

The velocity is therefore proportional to the surface area of the spheres. Slime mold amebae assume a spherical shape when incubated on ice for a few minutes. Preliminary calculations based on the assumption that they were spherical indicated that good size separation was possible. We chose to use Ficoll 400 (Pharmacia, Uppsala, Sweden) solutions. Ficoll 400 is a large polymer (400,000 D) composed of sucrose monomers. The solutions are biologically inert, autoclavable, and because of Ficoll's high molecular mass, they exert a minimal osmotic pressure on cells.

The density of vegetative cells of *D. discoideum* was determined by centrifuging the cells in a 4–20% Ficoll gradient prepared in PB. The cells were centrifuged until they reached their isopycnic density. The cells were in a broad band at ~17–18% Ficoll. Density marker beads (Pharmacia) indicated that this corresponded to a density of 1.05–1.06 g/ml.

For velocity centrifugation 4–14% gradients were chosen. A gradient was necessary to prevent bulk and convective flow. Linear gradients (12 ml) were formed in 13.5-ml Quick-seal ultracentrifuge tubes (Beckman Instruments, Inc., Palo Alto, CA). A 1-ml cushion of 20% Ficoll was injected under the gradient using a long needle and syringe. Approximately 5×10^6 cells in 0.3 ml PB were carefully loaded to the top of the gradient. The cells were centrifuged through the gradients for 30 min at 160 g at 0°C. It is critical that the centrifugation be carried out in a swing-out centrifuge rotor with light buckets so that the tubes are horizontal during the centrifugation. When centrifugation was carried out in tubes at a slight angle to horizontal the cells concentrated at the edge of the tube and flowed down without

separating. The top of the unsealed Quick-seal tubes can be conveniently fitted with tubing and connected to a fraction collector. The gradients were fractionated by puncturing the bottom of the tubes with a syringe needle and pumping 25% sucrose below the 20% Ficoll cushion and collecting the cells in 0.7-ml fractions.

Determination of Nuclei Per Cell

To determine the number of nuclei per cell of amebae from axenic cultures, the cells were washed twice with cold PB and fixed by adding either 70% ethanol or methanol/acetic acid (3:1, vol/vol) while vortexing the tube. The cells were stained in a 0.1 µg/ml bisbenzimidazole solution (Hoechst dye H33258) prepared before use in Hoechst buffer saline (20 mM EDTA, 2 M NaCl, 40 mM K₂HPO₄, 10 mM KH₂PO₄, pH 7.4). The cells were washed once with Hoechst buffer saline and viewed using a fluorescent microscope. At least 500 cells were scored for each determination.

To determine the nuclei per cell in amebae from bacterial cultures, the cells were washed free of bacteria and then resuspended in axenic medium containing 500 µg/ml streptomycin sulfate. The cells were allowed to digest already ingested bacteria for 45 min and treated as above for axenic cultures.

Results

Cell Volume Distributions

Cell size distributions of wild-type and mutant cells were obtained using an electronic particle counter (see Materials and Methods). Since by this method cell volume is recorded as a voltage pulse when a particle passes through a small aperture, it is critical that the cell suspension is dissociated. Although axenically grown wild-type cells were not highly aggregated when in growth medium (vegetative cell cohesion is assayed in non-nutrient salt buffers), small clumps did exist. These clumps could be removed by incubation of the cells on ice for 15 min and vortexing vigorously for 15–20 s. Although the mutant cells were defective in vegetative cell cohesion they were treated identically. Representative cell volume distributions of mutant and wild-type strains are presented in Fig. 1.

The cell volume distribution of logarithmically grown axenic cultures of wild-type cells exhibits a median cell size of ~0.44 pl, which corresponds to a spherical cell diameter of 9.5 µm. Axenic cultures of mutant cells also exhibit a peak at this size and often a peak at 0.88 pl, which corresponds to twice the volume and to a spherical cell diameter of 11.9 µm. The mutant defective at the *phgA* locus (Duffy and Vogel, 1984) shown in Fig. 1 exhibits such a bimodal distribution. However, many cultures of mutant strains exhibit only a broad shoulder toward the larger sizes. The size distribution of a mutant in the *phgB* locus shown in Fig. 1 is representative of these cultures. The strikingly bimodal distribution of the *phgA* mutant does not appear to be a function of mutants at this locus since we have also observed cultures of the *phgB* mutant that were also bimodal (not presented). A revertant of the *phgB* mutant obtained by selection for adherence to plastic surfaces (Vogel, 1983; Duffy, 1986) exhibits the wild-type cell volume distribution. Therefore, the larger size of the mutants belongs to a complex mutant phenotype that includes defective phagocytosis, cell-substratum adhesion, and vegetative cell-cell cohesion.

Growth in Axenic Medium and on Bacteria

In axenic medium the doubling time of the genetically characterized phagocytic mutants is very similar to that of wild-type strains (Table I). This suggests that the larger size

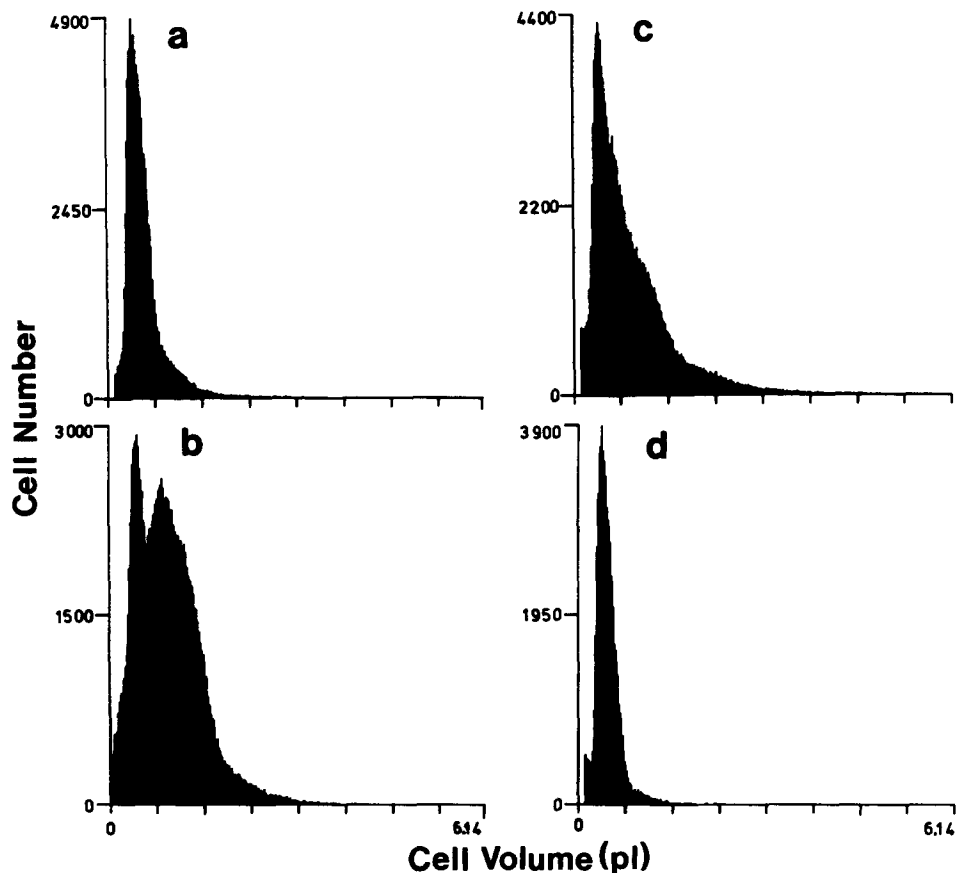


Figure 1. Cell volume distributions of genetically characterized strains (Duffy and Vogel, 1984; Duffy, 1986). Cells were obtained from logarithmically grown axenic culture at cell densities less than 2×10^6 per ml. Cell volume was determined using a particle counter. Volume scale was calibrated with latex particles of known size. (a) Wild-type (AX-2); (b) HV32 (*phgA1356*); (c) HV29 (*phgB 1351*); (d) HV155 (*phgB 1351, su[phgB 1351]*), a revertant of HV29.

is not due to a delay in the cell cycle. However, the doubling time of axenically growing cells (9–12 h at 21°C) is longer than that of bacterially growing cells (3–4 h) so that a defect that delayed the cell cycle would be more likely to affect cells growing at the minimum doubling time. The doubling times of the genetically characterized mutants as well as more recently isolated mutants were 23–77% greater than that of wild-type or revertant strains when growing on bacteria (Table I).

Unexpectedly, we found that the cell volume distribution of mutant cells growing on bacteria were very similar to those of wild-type or revertant strains (not shown). Since axenic cultures of mutants contain a large percentage of normal-sized cells, we hypothesized that the mutants were only partially defective in an important if not vital cell process. If this were so then perhaps more “penetrant” mutants could be obtained which might also show the defect in bacterially grown cultures. The original mutants were obtained

Table I. Growth Rates of Phagocytosis and Non-Adhesive Mutants

Strain	Bacteria (<i>E. coli</i> B/r)*		Axenic medium	
	Doubling time [‡]	% Difference to AX-2	Doubling time [‡]	% Difference to AX-2
	<i>h</i>		<i>h</i>	
HV32(<i>phgA1356</i>)	4.3 (3.0)	+43	11.8 (11.3)	+4
	4.6 (2.6)	+77	12.5 (11.3)	+8.8
HV29(<i>phgB 1351</i>)	3.7 (3.0)	+23	11.4 (11.3)	+0.9
	4.1 (2.6)	+57	11.8 (11.3)	+4
HV155(<i>phgB 1351, su[phgB 1351]</i>)	2.8 (2.7)	+ 3	11.4 (11.3)	+0.9
HV197	5.2 (3.9)	+33	10.3 (9.8)	+5
	5.2 (4.2)	+24	9.38 (8.96)	+4.7

* Phagocytosis mutants can feed upon bacteria or other particles that contain glucose groups via a lectin-like receptor, which still functions in these mutants (Vogel et al., 1980; Vogel, 1983).

[‡] Doubling times were determined by least squares fit to an exponential curve. Wild-type controls were always counted in parallel since the growth rates vary between different batches of bacteria and axenic medium. Doubling time of control (AX-2) is presented in parentheses. Cultures were counted at least four times at cell densities between 10^4 and 2×10^6 per ml.

by selecting for their phagocytic and perhaps cohesion properties (Vogel et al., 1980; Duffy, 1986). By using the adhesive properties of cells on tissue culture dishes a strong and repeatable selection can be performed. The selection was carried out at restrictive temperature (27°C), and the cells were grown on bacterial lawns at 21°C with a minimum of growth between rounds of selection. After ~30 rounds of selection strains were obtained that also exhibit the cell size defect when grown on bacteria. All the mutant strains isolated were defective at both restrictive and permissive temperatures although some appear to be more defective at the restrictive temperature. The cell volume distribution of a bacterially grown culture of one of these strains is compared with that of wild-type in Fig. 2.

Bacterially grown cells were much smaller than comparable axenically grown cells. The median cell volume of wild-type cultures was 0.140 pl, which was approximately one-third the volume of axenically grown cells and corresponded to a spherical diameter of 6.5 μm. As with axenically grown cultures, the mutants grown on bacteria exhibited a peak at the cell volume of wild-type cells. A second, broad peak of cells approximately two to three times as large was also observed. The ability to isolate such strains indicates that the cell volume change that these mutants exhibited was not a peculiarity of axenic growth. This was important to establish since axenic growth is not the natural method of growth by cellular slime molds, and growth in the most widely used media requires at least two mutations (Watts and Ashworth, 1970; Williams et al., 1974). A hypothesis to explain why the defect is more apparent in axenic culture will be discussed below.

Mutants are Multinucleate

Although wild-type axenically grown *Dictyostelium* cultures contain a higher percentage of multinucleate cells than bacterially grown cultures, 80–90% are mononucleate (Fig. 3, *a* and *e*, and Fig. 4 *a*; McDonald and Durston, 1984). However, the majority of mutant cells in axenic cultures are multinucleate, most frequently having two nuclei (Fig. 3, *b–d*, and Fig. 4 *b*). The strongly selected mutant, HVI97, also exhibits a high frequency of multinucleate cells (29%) in bacterial culture (Fig. 3 *f*). This mutant also exhibits a high frequency of multinucleate cells (78.5%) in axenic culture. Since a high percentage of cells have more than two nuclei, a better measure of the multinuclearity is the percentage of

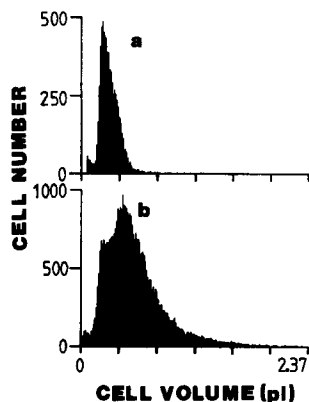


Figure 2. Volume distribution of a strongly selected non-adhesive mutant growing on bacteria. The non-adhesive mutant (HVI97) was isolated as described in Materials and Methods. Strains were grown in suspension in association with *E. coli* B/r and the cell volume distribution determined using a particle counter as described in Materials and Methods. (*a*) Wild-type (AX-2); (*b*) non-adhesive mutant (HVI97).

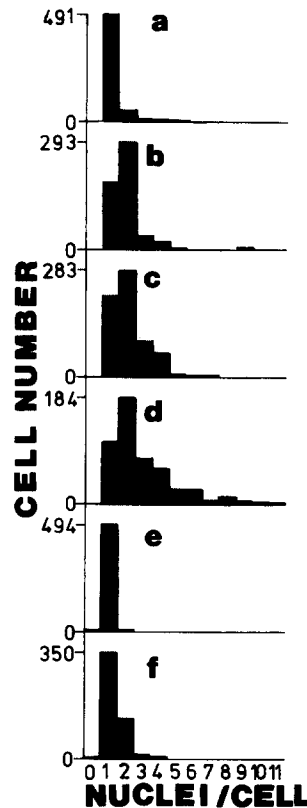


Figure 3. Nuclei per cell. Logarithmically grown cells in axenic culture were fixed and stained with bisbenzimidazole and nuclei per cell determined using a fluorescent microscope. Axenic cultures: (*a*) wild-type (AX-2); (*b*) HV32 (*phgA1356*); (*c*) HV29 (*phgB1351*) (*d*) HVI97. Bacterial cultures: (*e*) wild-type (AX-2); (*f*) HVI97.

nuclei in multinuclear cells. For the strongly selected mutant in axenic and bacterial culture these figures are 92.4 and 47.4%, respectively.

Size Increase Occurs within Two Cell Division Cycles

There are several ways in which large multinucleate cells could be formed: cells could fuse, cytokinesis could fail after nuclear division, or cytokinesis could occur but the furrow could be inaccurately placed and hence the daughter cells would receive unequal numbers of nuclei and cytoplasm.

To distinguish among these possibilities, we developed a method to purify the small, “normal-sized” cells from mutant cultures. If cell fusion were the cause then we expected larger cells to appear suddenly without a period of continuous cell growth. If cytokinesis failure were the cause then we expected the size-purified cells not to divide when they reached the size at which division occurs in wild-type cultures and thereby achieve the large size within one or two division cycles. If inaccurate placement of the division furrow were the cause, then we expected the size-purified cells to divide but produce unequal-sized daughter cells and require several generations to produce the broad size distribution observed for logarithmically grown mutant cells. In extreme cases division of cells with only two nuclei would be expected to produce anucleate cells.

We developed a velocity sedimentation method to separate cells according to size. Spheres of different sizes are known to sediment in viscous media at a rate proportional to their surface area (see Materials and Methods). Since amoebae kept on ice closely approximate spheres, it was possible to estimate the appropriate viscosity and centrifugation field to obtain a good separation of cells according to size. Ficoll

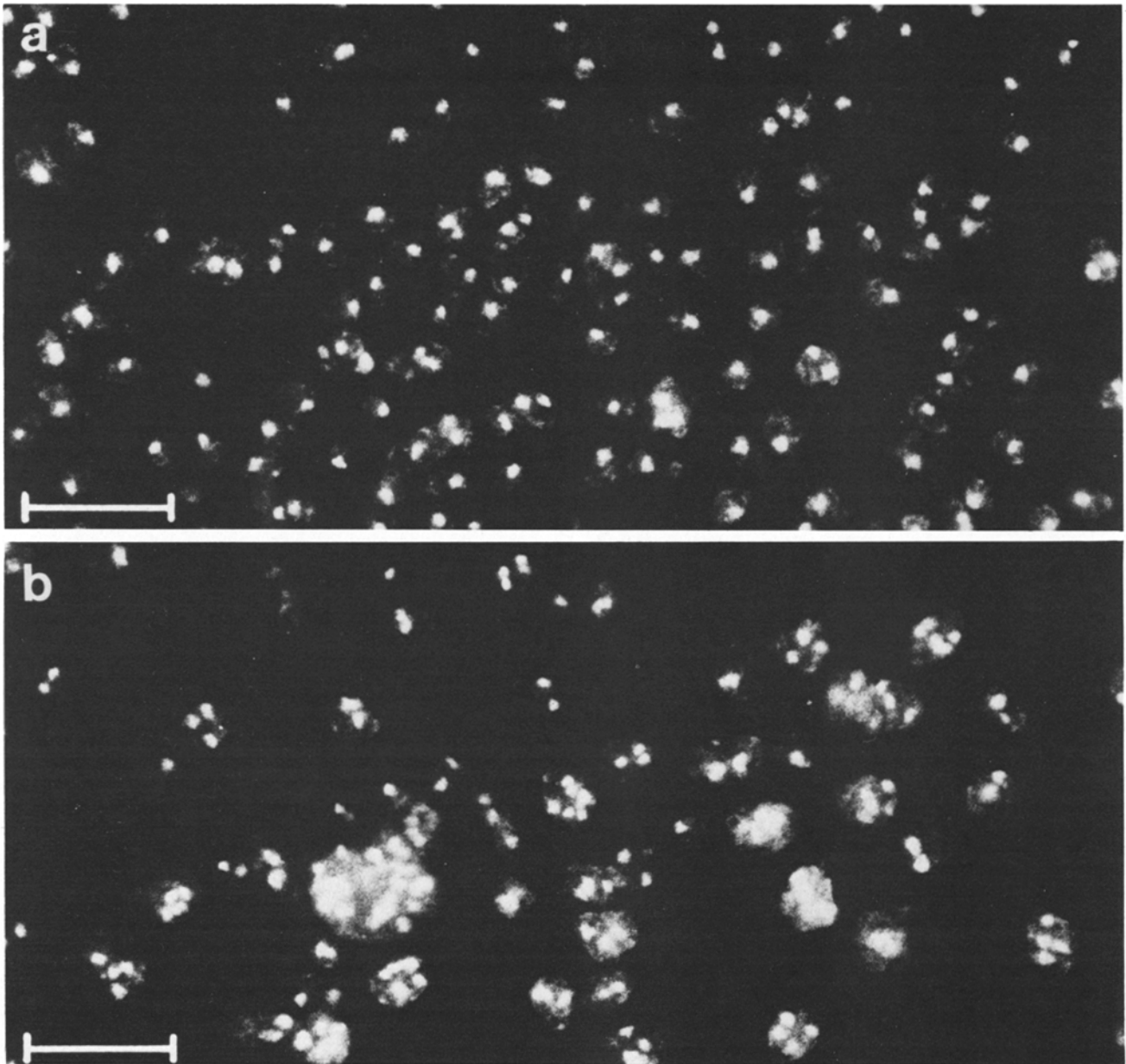


Figure 4. Micrographs of wild-type and mutant cultures. Logarithmically grown cultures were fixed in 2% formaldehyde, extracted for 5 min in acetone at -20°C , and stained with bisbenzimidazole (0.1 $\mu\text{g}/\text{ml}$). Photographs were made with Ilford HP-5 film using an Olympus BM2 microscope equipped for reflected light fluorescence. (a) Wild-type (AX2). (b) HV197. Bar, 30 μm .

gradients (4–14%) were chosen since the high molecular mass of Ficoll (400,000 D) minimizes the osmotic shock to the cells. A typical size separation of mutant cells is presented in Fig. 5. Fractions differing in mean volume over a fourfold range were obtained. The fractions at the top of the gradient containing small cells were very pure (coefficient of variation = 0.31). Fractions of larger cells near the bottom of the gradient were contaminated with small cells (coefficient of variation = 0.55).

For these experiments, logarithmically grown wild-type and mutant cell populations were centrifuged on identical gradients and the first two to three fractions containing small cells were pooled. The cells were then washed once and re-suspended in fresh nutrient medium. The results of a typical experiment are presented in Figs. 6 and 7. From initial popu-

lations of wild-type and mutant populations with mean sizes of 0.629 and 0.888 pl, respectively (not shown), cell populations with mean cell sizes of 0.518 and 0.456 pl, respectively, were obtained (Fig. 6, a and c). Nearly all wild-type (96.6%) size-purified cells were mononucleate. 88.3% of mutant size-purified cells were mononucleate and most of the remaining were binucleate (9.2%).

The wild-type cell population divided semi-synchronously between 4 and 7 h (Fig. 7 a). During the division the cell size distribution showed two distinct peaks: one of freshly divided cells and a second containing large cells that had not yet divided (Fig. 6 b), and the cell volume decreased sharply (Fig. 7 b). Over the same time period, the mutant cell population continued to increase in cell volume (Fig. 7 b) with only a slight increase in cell number (Fig. 7 a). By the time

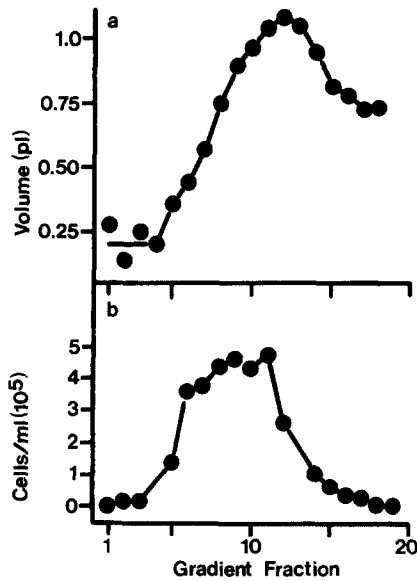


Figure 5. Size separation of a mutant strain. 3×10^6 cells of an axenically grown non-adhesive mutant (HV197) were harvested and resuspended in 0.3 ml growth medium. The cells were loaded on top of a cold 4–14% linear gradient (12 ml total vol) of Ficoll 400 prepared in PB and centrifuged at 160 g, 30 min, 0°C. 0.7-ml fractions were collected and the cell volume (a) and cell density (b) of each fraction determined using a particle counter. Fraction 1 is at the top of the gradient.

the wild-type cells began their second division the mutant cell population had almost reached the mean cell volume of the logarithmically grown parent population of 0.880 pl. Shortly after the wild-type cells divided, a peak at the size of freshly divided cells began to appear in the mutant culture but the majority of mutant cells failed to divide. In a repeat experiment after a cell doubling had occurred in the wild-type control culture, the percentage of mononucleate cells had dropped to 33%. The majority of the cells were then binucleate (61.8%). Regardless of how the binucleate cells present at the beginning of the experiment divided, we estimate that between 70 and 80% of the cell divisions failed to yield this percentage of mononucleate cells (Fig. 8).

Therefore, we believe that the increased size of the mutants is due to the failure of the majority of the cells to undergo cytokinesis after the nuclei had divided and the cells had reached the size at which the wild-type cells divided. Unequal division may be occurring, but it is not the major cause of the size increase since the parent cell size distribution was reached within two cell cycles and during this period the division rate was greatly reduced. Extreme asymmetry of division does not account for the size increase since only two out of 500 mutant cells examined lacked a nucleus after 14 h. One out of 500 wild-type cells lacked a nucleus. Fusion of cells is unlikely since cell growth occurred at a continuous rate until cells at least twice as large as wild-type were formed.

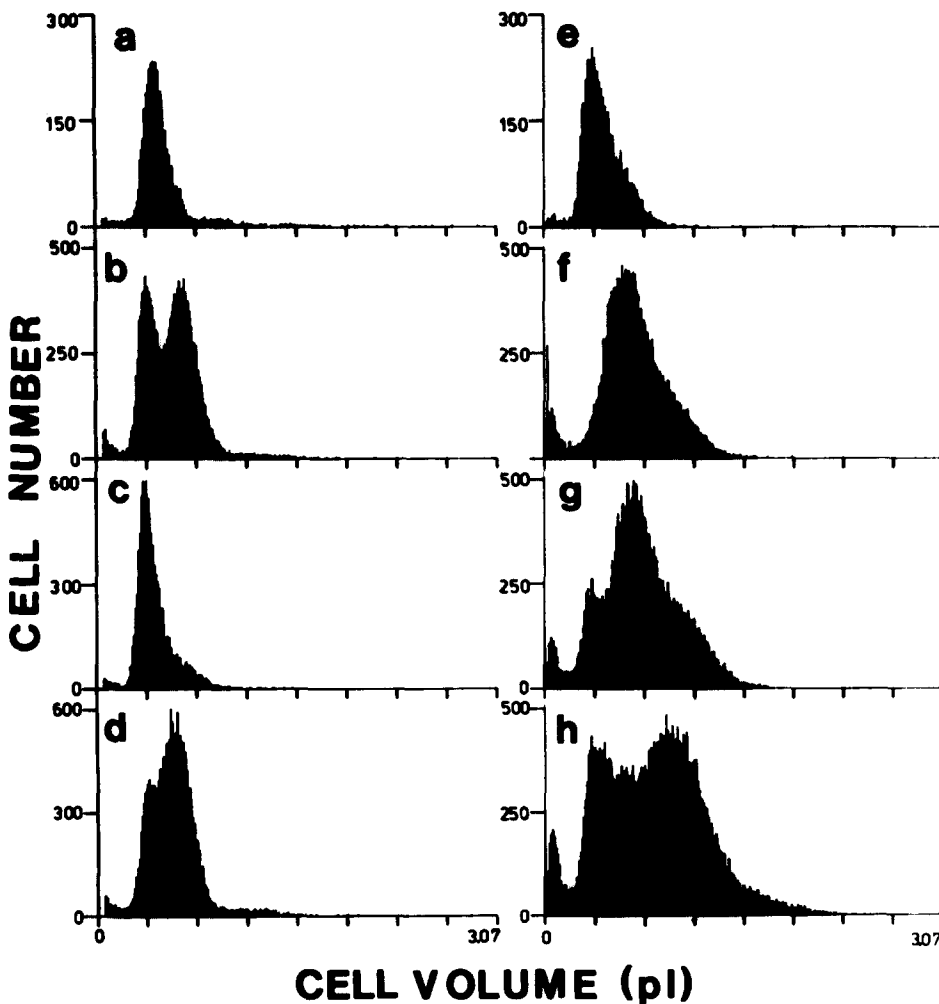


Figure 6. Volume distributions of size-purified wild-type and mutant cells at different times after reintroduction to growth medium. Small cells were purified from axenic cultures of wild-type (AX-2) and mutant (HV197) cultures by velocity sedimentation. The cells were washed once and reinoculated into fresh medium. Samples were taken every 1–2 h and the cell volume distribution determined using a particle counter. (a–d) Wild-type; (e–h) mutant (HV197); (a, e) 0 h; (b, f) 5 h; (c, g) 7 h; (d, h) 13 h.

Discussion

Phagocytic mutants of *D. discoideum* isolated on the basis of their phagocytic or adhesive properties exhibit a complex mutant phenotype that includes altered phagocytosis and defects in adhesion and cell-cell cohesion (Vogel et al., 1980; Vogel, 1983; Duffy, 1986). Here we have shown that these mutations also produce defects in cytokinesis. This suggests that a component of a mechanism common to all of these processes is defective in *phg* mutants.

Actomyosin-based motors, which are assembled on the cytoplasmic side of the cell membrane, are used by cells for phagocytosis (Reaven and Axiline, 1973; Stendahl et al., 1980; Hartwig et al., 1980; Fukui and Imamoto, 1985), cytokinesis (Schroeder, 1973, 1976; Meeusen et al., 1980), cell-substratum adhesion (Cappuccinelli and Ashworth, 1976; Isenberg et al., 1976; Heath and Dunn, 1978; Kreis and Birchmeier, 1980), and cell motility (Yumura et al., 1984). For a recent review of the actomyosin motor in *Dictyostelium*, see Fukui and Yumura (1986).

The pleiotropic mutant phenotype could be explained by hypothesizing that an element of the actomyosin motor, or an element involved in its assembly, is deficient or lacking

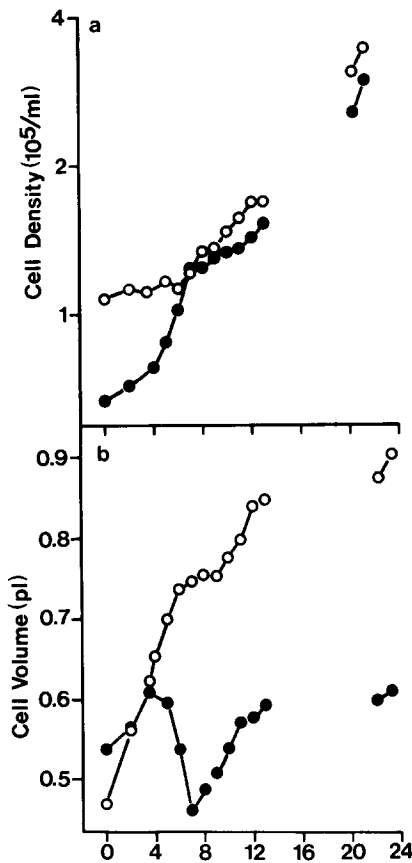


Figure 7. Cell density and cell volume of size-purified wild-type and mutant cells at different times after reintroduction to growth medium. Small cells were purified from axenic cultures of wild-type (AX-2) and mutant (HV197) cultures by velocity sedimentation. The cells were washed once and reinoculated into fresh nutrient medium. Cell density (a) and cell volume (b) were determined using a particle counter. (●) Wild-type (AX-2); (○) mutant (HV197).

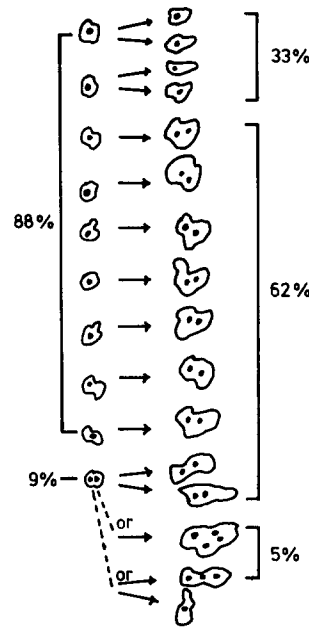


Figure 8. Schematic diagram of the division of mutant size-purified cells. At the beginning of the experiment 88.3% of the mutant cell population were mononucleate, 9.2% were binucleate, 1% had 3 nuclei, and the rest lacked nuclei. For simplicity only the mononucleate and binucleates are represented at left part of the figure. After a cell doubling of the wild-type control only 33% of the mutant cells were mononucleate. Because the multinucleate cells present at the beginning could have divided in a variety of ways, it is not possible to determine the exact percentage of failed cell divisions. However, regardless of how this portion of the cell population divided, between 70 and 80% of the cells did not divide to yield the

percentages of mononucleate and binucleate cells that were observed. Of the nine mononuclear cells presented in the schematic figure the upper two were depicted to be successful.

high enough activity in the mutants. If the synthesis rate of this component is proportional to the number of haploid nuclei then as a mutant cell becomes multinucleate enough of it may eventually become available for efficient cytokinesis. Such a relationship could explain why, at least in axenically growing cultures, the mutants can achieve division rates close to those of wild-type cultures after they are larger and multinucleate. For efficient cytokinesis in the mutants described here, the necessary number of nuclei appears to be somewhat greater than twice that required by wild-type cells.

The altered phagocytotic properties of these mutants can be most easily understood in terms of cell surface receptors. The mutants are altered in nonspecific phagocytic recognition. They can bind hydrophobic particles (latex) but not more hydrophilic particles (carboxylated- or amino-latex). However, they can phagocytose bacteria and other particles containing glucose groups via a lectin-like receptor (Vogel et al., 1980; Vogel, 1983). This would imply that a membrane receptor responsible for nonspecific phagocytic recognition, adhesion, and vegetative cell cohesion is the component of the actomyosin motor that is deficient or altered in these mutants and that this receptor also participates in cytokinesis. In contrast to most of the other actomyosin requiring processes, cytokinesis does not appear to require the transduction of signals from receptors on the outer surface but instead appears to be activated by a clock that also determines the timing of nuclear division (Conrad and Rappaport, 1981). However, concanavalin A binding proteins have been shown to cluster over the cytokinetic furrow in macrophages, suggesting that membrane receptors, although not transducing signals, could be involved in this process (Berlin et al., 1978).

The higher penetrance of the mutant phenotype in axenic culture as well as the multinuclearity of commonly used ax-

enic strains in axenic culture could also be attributed to a membrane component of the actomyosin motor. The commonly used axenic strains (AX2 and AX3), which have been derived from a single parent (NC4), contain at least two mutations that greatly improve growth in axenic media (Williams et al., 1974). Mononucleate axenic cells of strains derived from NC4 are approximately two to three times as large as their bacterially grown counterparts. Hence the same amount of the component would be distributed over a larger cell membrane area in axenically grown cells. We would predict that the multinuclearity exhibited by these strains in axenic culture is a secondary effect due to the larger size and hence a greater cell surface to nuclear ratio of axenically grown cells.

Recently myosin deficient mutants have been constructed in *Dictyostelium* strains by introduction of transcribable antisense myosin genes (Knecht and Loomis, 1987) and disruption of the myosin gene by homologous recombination with a myosin heavy chain gene containing a deletion (De Lozanne and Spudich, 1987). The most striking phenotype of these strains is the formation of large, multinucleate cells presumably because of defects in cytokinesis. Similar to our mutants, these mutants are motile. However, unlike our mutants, they do not grow well in suspension cultures, adhere to tissue culture dishes, and exhibit defects during development. Phagocytosis of bacteria is reported to occur but is not completely normal. Whether defects in phagocytic recognition of particles lacking glucose groups exist was not reported. However, the differences between the method of selection of our mutants and differences in phenotype suggest that a different aspect of the motor or its assembly is affected in *phg* mutants. Since *phg* mutants map to at least two distinct loci, at least one and possibly both are not in the myosin gene. Nevertheless this work supports the notion that defects in the actomyosin system could lead to the mutant phenotype we observe.

We thank Verena Daum and Brendan Davies for excellent criticism of earlier drafts of this manuscript.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Vo 348/1-2).

Received for publication 16 March 1987.

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