Relationship between Axenic Growth of *Dictyostelium discoideum* Strains and Their Track Morphology on Substrates Coated with Gold Particles

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ABSTRACT Amoebae of *Dictyostelium discoideum* produce tracks with two distinct morphologies on gold-coated coverslips. The wild-type strain and other strains that feed only by phagocytosis produced indistinct, fuzzy tracks, whereas mutants capable of axenic growth produced clear, sharp tracks. The sharp track morphology was found to be a recessive phenotype that segregates with axenicity and probably requires a previously unidentified axenic mutation. Axenic and nonaxenic strains also differed in their ability to pinocytose. When the two types of cells were shifted from bacterial growth plates to nutrient media, within 24 h the axenic strain established a rapid rate of pinocytosis, approximately 100-fold higher than the low rate detectable for the nonaxenic strain. However, track formation did not appear to be directly related to endocytosis. Electron microscopic examination of cells during track formation showed that both axenic and nonaxenic strains accumulated gold particles on their surfaces, but neither strain internalized the gold to any significant degree. Observation of living cells revealed that axenic strains collected all particles that they contacted, whereas wild-type strains left many particles undisturbed. The size of the gold particle clusters discarded by the cells also contributed to track morphology.

Amoebae of the cellular slime mold *Dictyostelium discoideum* carry out a variety of motile activities that include phagocytosis, pinocytosis, and cell migration. In this organism it is possible to explore the relationships among these processes by genetic as well as biochemical means. To this end, a group of temperature-sensitive motility mutants have been isolated from *D. discoideum* strain AX3. Characterization of these mutants has suggested that the motility defects in some strains affect genes involved in axenic growth (5), a finding whose implications are further explored here.

Wild-type strains of *D. discoideum* grow only by feeding on bacteria. The wild isolate best characterized genetically is strain NC4, represented in this study by the laboratory stock DdB. Mutant strains have been isolated from NC4 that can grow axenically on soluble nutrients; AX3 is such a strain. The genetic determinants of axenic growth have been partially characterized, and mutations that map to linkage groups II and III have been shown to be essential (9, 17). The gene products affected by these mutations have not been identified. Axenic strains exhibit high rates of pinocytosis when cultured in liquid media (8, 10, 11). It seems likely that rapid pinocytosis is needed for axenic growth, but this question has not been directly examined.

Characterization of the motility mutants that were derived from strain AX3 included a test of their ability to form tracks on a glass surface coated with gold particles (5). This assay method was developed for fibroblasts by Albrecht-Buehler, who termed the tracks "phagokinetic" because he found that 3T3 cells ingested the gold particles as well as collected them on their dorsal surfaces; characteristic track patterns were produced by different cell lines (1).

The *D. discoideum* motility mutants were able to clear tracks under permissive but not restrictive temperature conditions. Each strain produced one of two distinct track morphologies, which appeared to correlate with the strain's ability to grow axenically. The present study, using a variety of axenic and nonaxenic strains, confirmed this relationship, and a preliminary genetic analysis suggested that a previously unidentified axenic mutation is involved. The mechanism of track formation and the basis of the morphology difference were also examined.

MATERIALS AND METHODS

Strains and Culture Conditions: The strains of *D. discoideum* used in this study are described in Table I. They are all derivatives of the *matA1*

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TABLE I

Haploid strains of Dictyostelium discoideum

Strain	Parents	Linkage group I	II*	III*	IV	VI	VII	Unassigned	Source
DdB	NC4								‡
AX3	NC4		axeA1 axeC1 oaaA1	axeB1					§
HU217	HR7 HU166	cycA1	whiA1 tsgD12 arsB353	acrC4 axeB1					‖
HU358	TW8 HR7		tsgF6 axeA1 axeC1 oaaA1	radC35					‖
HU947	HU215 HU876	cycA1	whiA1 tsgD12 arsB353	acrC4 axeB1	bwnA1				‖
HU1116	AX3		axeA1 axeC1 oaaA1	axeB1		tsgV1826‡‡			‖
HU1376	HPS64 HU877		axeA1 axeC1 oaaA1 acrA604	tsgA1 axeB1 radC44			cobA1 or cobA353 or cobA358		¶
HU1437	HU1084 HU1136		axeA1 axeC1 oaaA1	axeB1	bwnA1			tsg-391	‖
M28		tsgE13 sprA1			bwnA1				**
MC217	M28 HU1206	tsgE13 sprA1	axeA1 axeC1 oaaA1	axeB1	bwnA1				¶

* The presence or absence of the axenic mutations in strains derived from AX3 is known from the segregation of linked markers except for MC217, where it is deduced from the strain's axenic phenotype.

‡ Kessin, R. C., personal communication.

§ Reference 6.

‖ Welker, D. L., and K. L. Williams, personal communication.

¶ This work.

** Reference 4.

‡‡ This mutation was assigned a complementation group while in proof (Welker, D. L., and K. L. Williams, personal communication).

strain NC4. Unless otherwise specified, cells were cultured on SM agar plates (14) at 21°C in association with a cobalt resistant derivative of *Klebsiella aerogenes* (18). Axenic cultures were maintained at 21°C on a rotary shaker in HL5 medium (5).

Assay for Track Formation: Cells were usually collected from the growing rim of plaques produced by spotting a cell suspension on a bacterial lawn 1–5 d before the experiment. Occasionally cells were collected from mass plates as described below for pinocytosis assays. The cells were suspended in buffer H (0.35 g KH_2PO_4, 0.35 g $Na_2HPO_4 \cdot 7H_2O$, and 50 mg dihydrostreptomycin sulfate per liter, pH 6.5) at room temperature. For some experiments, they were washed essentially free of bacteria by differential centrifugation (4 or 5 cycles of sedimentation at 100 *g* for 4 min in a bench top centrifuge, using buffer H to resuspend the cell pellets); these washes did not affect the results. The cell concentration was determined by hemacytometer count, and the cells were diluted with nutrient medium (HL5) for incubation on the gold-coated coverslips. The dilution was chosen to provide an average density of 0.2–4 cells/mm^2, depending on the length of incubation planned. Samples were incubated at 21°C or room temperature for the time period indicated in each experiment. The preparation and processing of coverslips coated with gold particles was done essentially according to Albrecht-Buehler (1) and has been previously described (5).

Pinocytosis Assay: Cells were collected from plates inoculated 36–48 h earlier with 1×10^4 to 2×10^5 cells or spores. When harvested, the cells had partially cleared the bacterial lawn, but there was no visible sign that development had begun. The cells were collected and washed at either room temperature or 4°C in buffer H as described for the track formation assay. The temperature used for these steps did not significantly affect the results. The use of buffer H was important, however, since if HL5 was substituted, the bacteria formed clumps that were not effectively removed by differential centrifugation. After the final wash, the cell pellets were suspended in HL5 at a concentration of 3–8 $\times 10^6$ cells/ml and incubated at 21°C on a rotary shaker at 175 rpm; this was the point from which "time in nutrient medium" was measured. At intervals, aliquots of this culture were shifted to smaller flasks incubated under the same conditions and, 10–30 min after the shift, pinocytosis assays were initiated by the addition of dextran labeled with fluorescein isothiocyanate (FITC-dextran, average molecular weight 70,000, from Sigma Chemical Co., St. Louis, MO). The use of this marker for pinocytosis in *D. discoideum* was described by Vogel et al. (12). Assays were run for 60 min, with samples usually taken at 10-min intervals. For each time point, duplicate 1-ml samples were withdrawn, diluted into 5 ml of cold buffer H, and kept on ice. The length of time on ice before washing did not affect uptake values. The samples were washed at 4°C by centrifugation (480 *g*, 2 min), four times in buffer H and once in 5 mM glycine (pH 9.3). The final pellets were suspended in 2 ml of 50 mM glycine (pH 9.3) containing 0.3% Triton X-100, and fluorescence was measured in a Perkins-Elmer 650-10S fluorescence spectrophotometer (Perkins-Elmer Corp., Physical Electronics Div., Eden Prairie, MN).

Uptake was calculated by comparison with standard dilutions of the FITC-dextran stock solution; this stock was filtered before use to obtain uniform low blank values. The rates were calculated as the slopes of least squares regression fits to the data points. The assays were essentially linear with time for at least 60 min (correlation coefficients routinely >0.95), although a slight sigmoid curvature was usually seen. They were also linear over a wide range of concentrations tested: cell concentrations from 5×10^5 to 1×10^7 cells/ml; FITC-dextran concentrations from 0.5 to 10 mg/ml.

Electron Microscopy: Cells were collected from bacterial growth plates, washed essentially free of bacteria as described above (Track Formation), and incubated in HL5 at 21°C on a gold-coated surface. Glass coverslips were used as the substrate except for cells to be sectioned in situ, which were placed

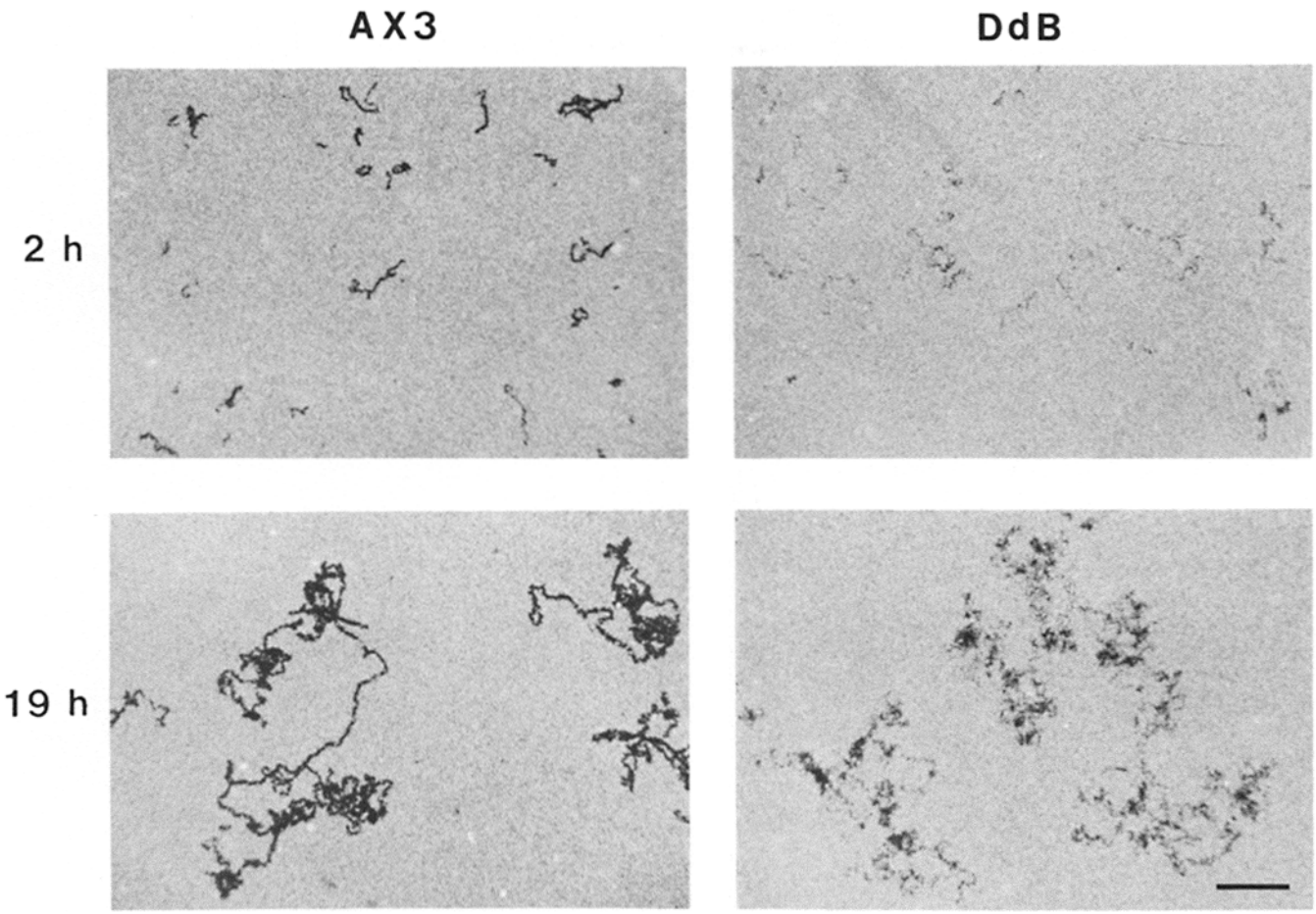

FIGURE 1 Track morphology of wild-type and axenic strains. *D. discoideum* amoebae grown in association with bacteria were incubated on gold-coated coverslips for 2 or 19 h, as indicated; the coverslips were then mounted and examined by dark-field microscopy. Wild-type (*DdB*) cells incubated in nutrient medium produced fuzzy tracks, while axenic (*AX3*) cells produced sharp tracks. Bar, 0.5 mm. × 21.

in plastic culture dishes (Falcon 1008, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) that had been coated with gold. Similar tracks were formed on either glass or plastic substrates. The cell concentration was adjusted according to the experiment planned: 2 cells/mm^2 for scanning electron microscopy (SEM)[1], 40 cells/mm^2 for in situ sections, and 2,000 cells/mm^2 for pellets. At the end of the incubation period, the HL5 was replaced with buffer H. For samples destined for SEM or in situ sectioning, the buffer was immediately withdrawn and replaced with 2 ml of 2% glutaraldehyde in 25 mM cacodylate buffer, pH 7.1. 1 ml of 1% OsO_4 in the same buffer was then added. After 20 min, the samples were washed twice with cacodylate, postfixed in OsO_4, and washed again. The SEM samples were then dehydrated through a graded ethanol series, critical point dried, and sputter coated. The samples to be sectioned were dehydrated through an ethanol series, rinsed with propylene oxide, and embedded in Epon. Sections were cut on an ultramicrotome (LKB Industries, Inc., St. Charles, MO) and stained with uranyl acetate and lead citrate. The samples that were sectioned as pellets were similarly prepared except that the cells were collected from the gold-coated coverslips by gentle pipetting, then pelleted by centrifugation before fixation. After fixation as described above, they were pre-embedded in 3% gelatin. Final embedding and sectioning were performed as described above. All procedures were carried out at room temperature unless otherwise specified.

The SEM samples were examined in a JEOL SM25 scanning electron microsope at 15 kV with a 15° tilt angle. The transmission microscopy was done with a JEOL 100S electron microscope at 60 kV.

Genetic Analysis: Standard methods were used to construct and segregate diploids and to score genetic markers (14). In some cases, axenic segregants were obtained from nonaxenic diploids by selecting for growth in HL5, with or without prior passage at high density on plates containing ben late to induce haploidization (16).

[1] *Abbreviation used in this paper:* SEM, scanning electron microscopy.

RESULTS

Track Morphology is Strain Specific

Cells of wild-type (DdB) and axenic (AX3) strains of *D. discoideum* were collected from bacterial growth plates and incubated on gold-coated coverslips as described in Materials and Methods. The characteristic track morphologies produced by the two strains were visualized by darkfield illumination (Fig. 1). When incubated in nutrient media, the wild-type and other nonaxenic strains produced indistinct, fuzzy tracks that still contained gold particles (Fig. 1, DdB). In contrast, axenic strains produced clear, broad, sharp-edged tracks (Fig. 1, AX3). The terms "fuzzy" and "sharp" are used here to denote these two track morphologies. The width of an AX3 track averaged 15 μm, or about two cell diameters. The track morphology differences were detectable within 30 min after plating and became more striking at longer incubation times; time points of 2 and 19 h are shown in Fig. 1. Characteristic tracks, although narrower and sometimes shorter, were also formed by cells incubated in buffer.

There was some variability among the cells of a given strain in the degree of sharpness or fuzziness of their tracks. An occasional AX3 cell even produced an indistinct track. The frequency of such cells was lower and the sharp appearance of the average track was more pronounced in cultures that had been passaged axenically and were shifted back to bacterial growth plates shortly before the assay. Note that the

occurrence of these occasional fuzzy tracks intermingled with the sharp tracks made it clear that track morphology is a property of the individual cell and not of the environment. However, small differences among gold preparations did affect track appearance, particularly for nonaxenic strains. On some coverslips, or even regions of a single coverslip, the tracks of nonaxenic strains became so indistinct that they were almost undetectable.

For strain AX3, cells taken directly from axenic culture were examined as well as those from bacterial plates. Cells from axenic culture produced sharp tracks, but these were substantially shorter than those of bacterially grown cells (Fig. 2). This diminished motility was also evident by direct observation. Phase-contrast micrographs taken at 1-min intervals of cells on uncoated glass slides indicated that AX3 cells from axenic culture translocated about one-fifth as rapidly as cells grown on bacteria (Sternfeld, J. M., R. Birchman, and M. Clarke, manuscript in preparation). In these experiments, the cells tested were taken from bacterial suspension cultures rather than plates, so the significant variable is not growth on a surface vs. growth in suspension, but appears to be growth on soluble nutrients vs. growth on bacteria. This suggests that the commonly observed difference in motility between axenically grown AX3 and bacterially grown NC4 cells is not strain difference, but rather a consequence of growth conditions. When both strains were grown on bacteria, they proved to be equally motile (not shown).

Genetic Basis of Track Morphology

A preliminary genetic analysis of the track morphology difference between AX3 and DdB was carried out. Diploids were constructed that contained both mutant and wild-type alleles or only mutant alleles of the axenic genes. The track morphology of such diploids and of various haploids was examined, and their ability to grow axenically was determined. Representative results are presented in Table II. Diploids homozygous for the axenic genotype produced sharp tracks, while heterozygous diploids had fuzzy tracks. Thus sharp track morphology behaved as a recessive trait exhibited by axenic mutants. It correlated with axenicity in haploid segregants selected for axenic growth or identified by screening for axenic growth from a number of different diploids heterozygous for the axenic genes. Analysis of several strains with partial axenic genotypes indicated that the *axeA1* mutation is not required for the sharp track phenotype (for example, see HU217) and that the *axeB1* mutation is not sufficient for it (e.g., HU947). These results suggest that a mutation unlinked to *axeA* and *axeB* is involved. Data from earlier studies support and extend this conclusion (see Discussion).

Endocytosis and Track Formation

Several approaches were used to examine the mechanism by which the different track morphologies were produced. Albrecht-Buehler reported that 3T3 cells phagocytose gold particles during track formation (1), so it seemed likely that *D. discoideum* cells would do the same. Furthermore, the probable relationship between axenic growth and pinocytosis suggested that wild-type and axenic strains might differ in their ability to endocytose gold particles. Therefore DdB and AX3 cells were examined for internalized gold by transmission electron microscopy.

TABLE II

Genetic Basis of Track Morphology

Strain	Axenic genotype*	Axenic growth	Track morphology
Haploids			
HU1116	*axeA axeB*	+	S‖
HU1376‡	*axeA axeB*	+	S
HU1437‡	*axeA axeB*	+	S
MC217‡	*axeA axeB*	+	S
M28	$axeA^+$ $axeB^+$	−	F
HU358	*axeA* $axeB^+$	−	F
HU217	$axeA^+$ *axeB*	+/−§	S
HU947	$axeA^+$ *axeB*	−	F
Diploids			
DMC45 (HU1116 × HU1437)	*axeA axeB*/*axeA axeB*	+	S
DMC53 (HU1116 × M28)	*axeA axeB*/$axeA^+$ $axeB^+$	−	F

* Linkage group II has been characterized as containing at least two mutations that contribute to axenic growth (9). For simplicity, we use *axeA* throughout this paper to refer to any and all genetic information on linkage group II that is required for the axenic phenotype.

‡ These axenic haploid strains were segregated from diploids heterozygous for the axenic genotype.

§ Slow growth relative to strains designated "+."

‖ *S*, sharp; *F*, fuzzy.

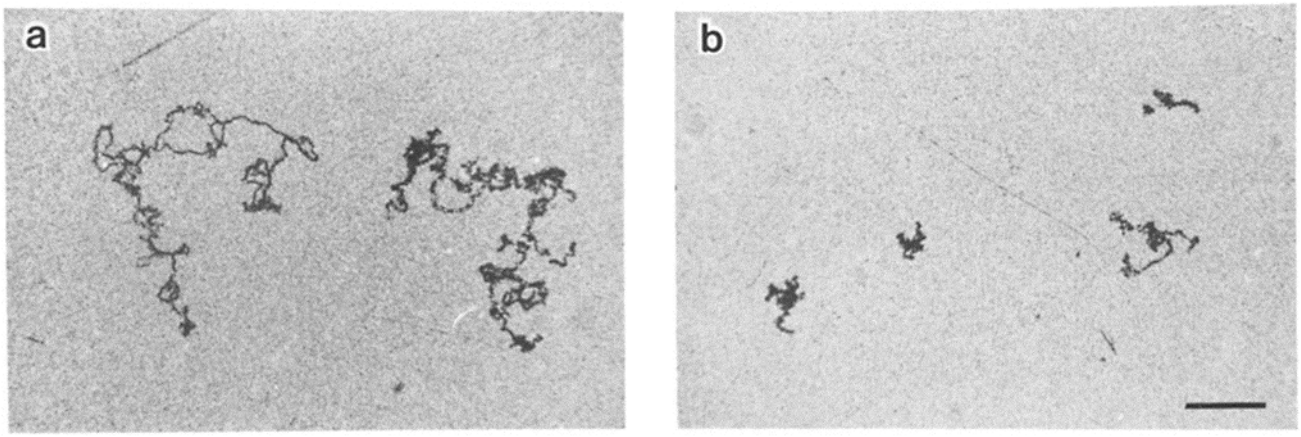

FIGURE 2 Tracks of AX3 cells grown under axenic or nonaxenic conditions. Longer tracks were produced by cells taken from bacterial growth plates (a) than from axenic culture (*b*). In both cases the cells were incubated in nutrient medium on gold-coated coverslips for 15 h, and the slips were mounted and examined by dark-field microscopy. Bar, 0.5 mm. × 21.

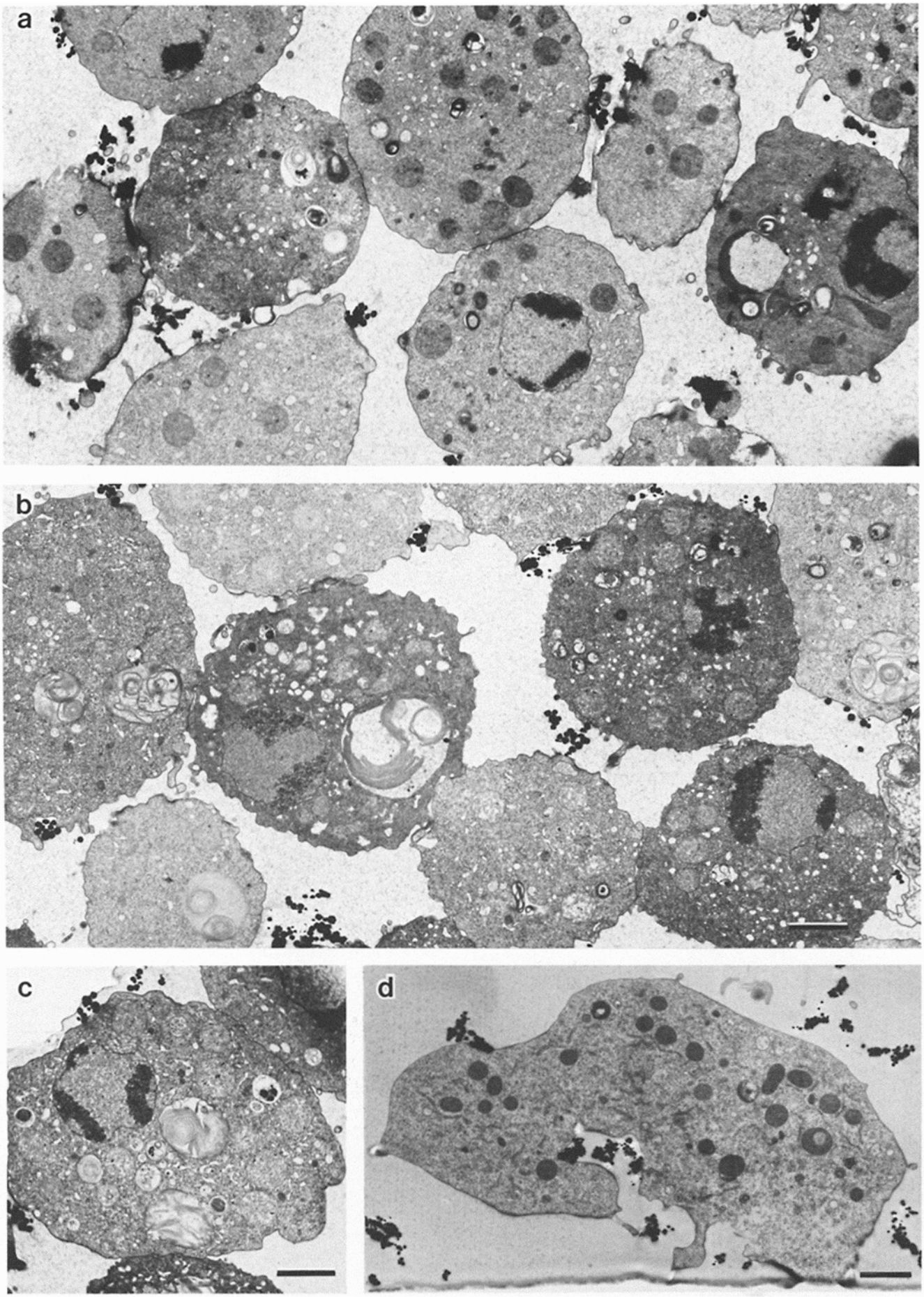

FIGURE 3 Thin sections of cells collected during track formation. DdB cells (*a*) were incubated on gold-coated coverslips in HL5 for 20 min, then collected at 4°C and fixed as a pellet. In *d*, the DdB cells were fixed in situ after a 120-min incubation in HL5. The AX3 cells shown here (*b* and *c*) were incubated and fixed as pellets, as described for DdB. Bar, 2 μm. *a* and *b*, × 5,600; *c*, × 5,500; *d*, × 4,800.

Thin sections were prepared from AX3 and DdB cells fixed during and immediately following track formation (Fig. 3). Cells were washed from gold-coated substrates either at 4°C or room temperature, and fixed as pellets (Fig. 3, *a* for DdB and *b* and *c* for AX3); other samples of both strains were fixed in situ (Fig. 3*d* shows a DdB cell). Both short and long incubation periods on gold were tested. All conditions gave the same result. Virtually all of the gold particles were external to the cells, many of them in close contact with the cell surface. Only rare sections of cells of either strain showed gold particles that appeared to be internal (Fig. 3*c*). In these cases, the gold particles were commonly found near the cell periphery, suggesting that these images represented oblique sections through particles lying in indentations of the cell surface. Serial sections sometimes confirmed this interpretation, but were usually not available because of specimen damage resulting from resistance of the gold particles to cutting. The cells shown were from bacterial growth plates; similar results were obtained with AX3 cells from axenic culture. The data made it clear that gold particles are not accumulated intracellularly whether or not they are occasionally internalized, and that the two strains do not differ in this regard.

Pinocytosis in Wild-Type and Axenic Strains

Although neither AX3 nor DdB cells accumulate gold particles intracellularly, a transient involvement of endocytosis would be difficult to detect cytologically. Pinocytosis by the two strains was measured to see whether differences in endocytic behavior were evident under conditions of the track formation assay (Fig. 4). The details of these experiments are provided in Materials and Methods. In brief, amoebae were collected from bacterial growth plates, washed free of bacteria, and resuspended in the axenic growth medium HL5. During the first two hours after being shifted to HL5, both strains exhibited very low pinocytosis rates (0.01–0.02 μl/10^7 cells/h). As illustrated in Fig. 1, the track morphology typical of each strain was already evident at this time, indicating that the two strains could make their distinctive tracks at a time when their rates of pinocytosis were very similar.

These experiments also produced the interesting observation that rapid pinocytosis is induced in AX3 cells as they adapt to axenic conditions. The pinocytosis rate remained low for both strains when the cells were incubated in buffer (not shown). Within 24 h after transfer into HL5, the AX3 cells increased their rate of pinocytosis more than 100-fold to 2.5 ± 0.3 μl/10^7 cells/h, a level similar to that of established axenic cultures in this laboratory (2.8 ± 1.2 μl/10^7 cells/h). In contrast, pinocytosis by DdB cells displayed only a transient two- or threefold increase (illustrated on a 10-fold expanded scale in Fig. 4*b*) and then dropped back to initial levels. There was no change in track morphology associated with the great increase in pinocytosis rate of AX3 cells. These results, in conjuction with the thin-section study, indicate that endocytosis is unlikely to be the determinant of track morphology, or even a significant factor in track formation.

Visualization of Track Formation

Cells and the tracks they had formed were examined by SEM, using samples that had been incubated for 1 h before fixation and processing. Viewed by SEM, the AX3 tracks (Fig. 6) were clearly visible, but the DdB tracks (Fig. 5) were usually less distinct and sometimes undetectable. Nonetheless, it was

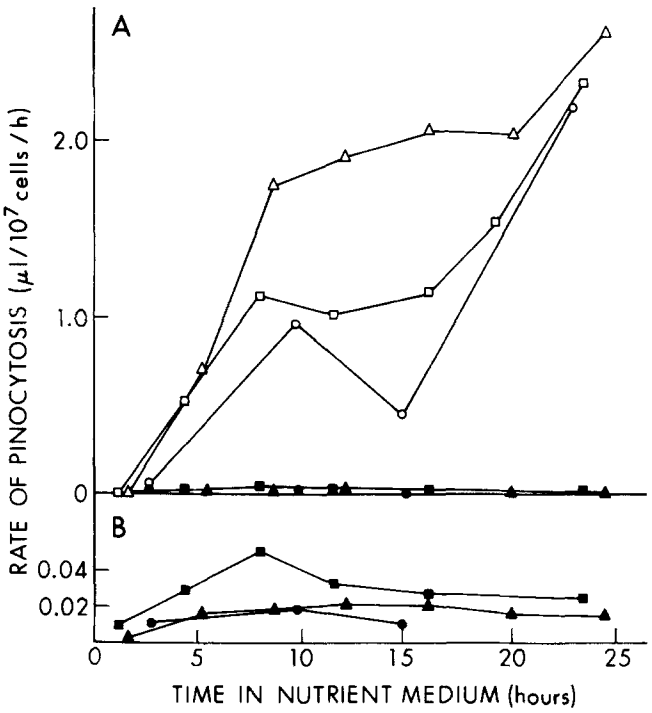

FIGURE 4 Pinocytosis by wild-type and axenic strains. Vegetative cells were shifted from bacterial growth plates to HL5, and the rate of pinocytosis was measured at intervals after the shift. The results of three separate experiments, each represented by a different symbol, are presented; in each experiment the open symbols represent AX3 and the filled symbols, DdB. For two experiments (△ and □) the cells were collected and washed at room temperature; for the third (○), the cells were processed at 4°C. The DdB data are shown on the same scale as AX3 in *A*; they are replotted on a 10-fold expanded scale in *B*.

evident that most cells of both strains had accumulated large quantities of gold on their dorsal surfaces. The particles were usually massed toward the rear of the cell, at least in cases where the cell's direction of movement could be inferred from its relationship to the track. Occasional cells of both strains were seen devoid of gold particles, although they had cleared a track (Fig. 5*a*). This implied that such cells had sloughed their collected gold, which was later confirmed by observation of living cells. The SEM images failed to reveal the basis of the distinctive track morphologies.

Substantial differences between axenic and wild-type strains became evident when the interaction between living cells and gold particles was visualized by phase contrast microscopy. Axenic cells extended multiple broad, flat pseudopodia that collected virtually all of the gold particles they contacted. These particles were lifted above the focal plane of the other gold particles, onto the dorsal surface of the cell. Here they could be seen moving about on the pseudopodia and cell body, rapidly accumulating into clumps near the central (perinuclear) region of the cell. The clumps merged into a single mass that increased in size until it covered and extended beyond the posterior half of the cell. Then all or most of the gold mass broke loose and was left behind, and the cell proceeded to form another.

An average axenic cell dropped a gold mass roughly every 15 min, having traversed and cleared during that period an area broader than the cell and about two to four cell diameters long. Although the gold masses were prominent features of

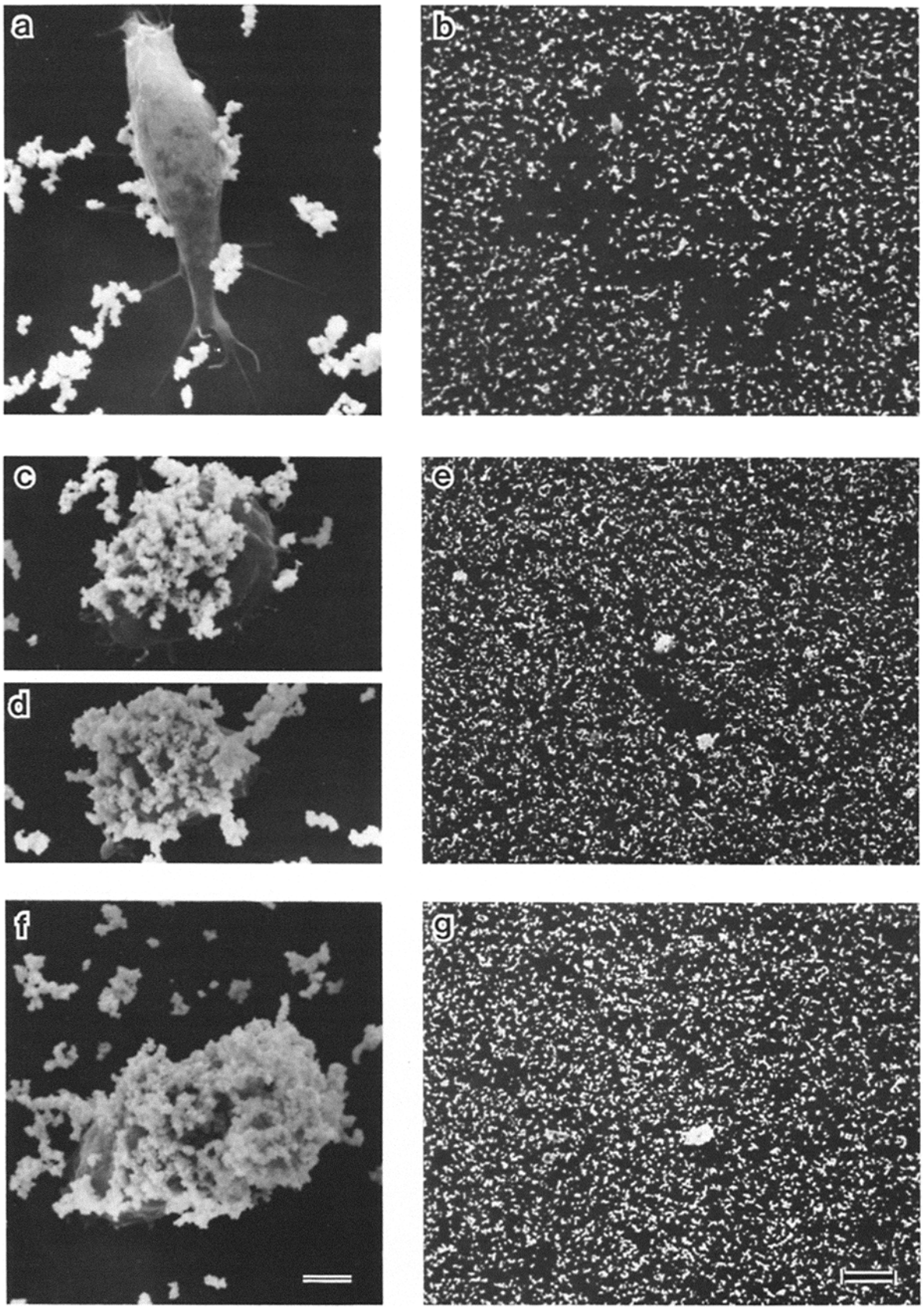

FIGURE 5 SEM visualization of DdB cells during track formation. The DdB cells were incubated for 60 min on gold-coated coverslips in HL5, then fixed in situ and examined by SEM. The range of track morphologies observed for DdB cells is illustrated. Individual cells are shown at high magnification next to the track they have produced. Note that even in the case where the track is undetectable, the DdB cell has collected a substantial amount of gold. Bars *a*, *c*, *d*, and *f*, 2 μm; *b*, e, and *g*, 20 μm. *a*, *c*, *d*, and *f*, × 4,000; *b*, e, and *g*, × 400.

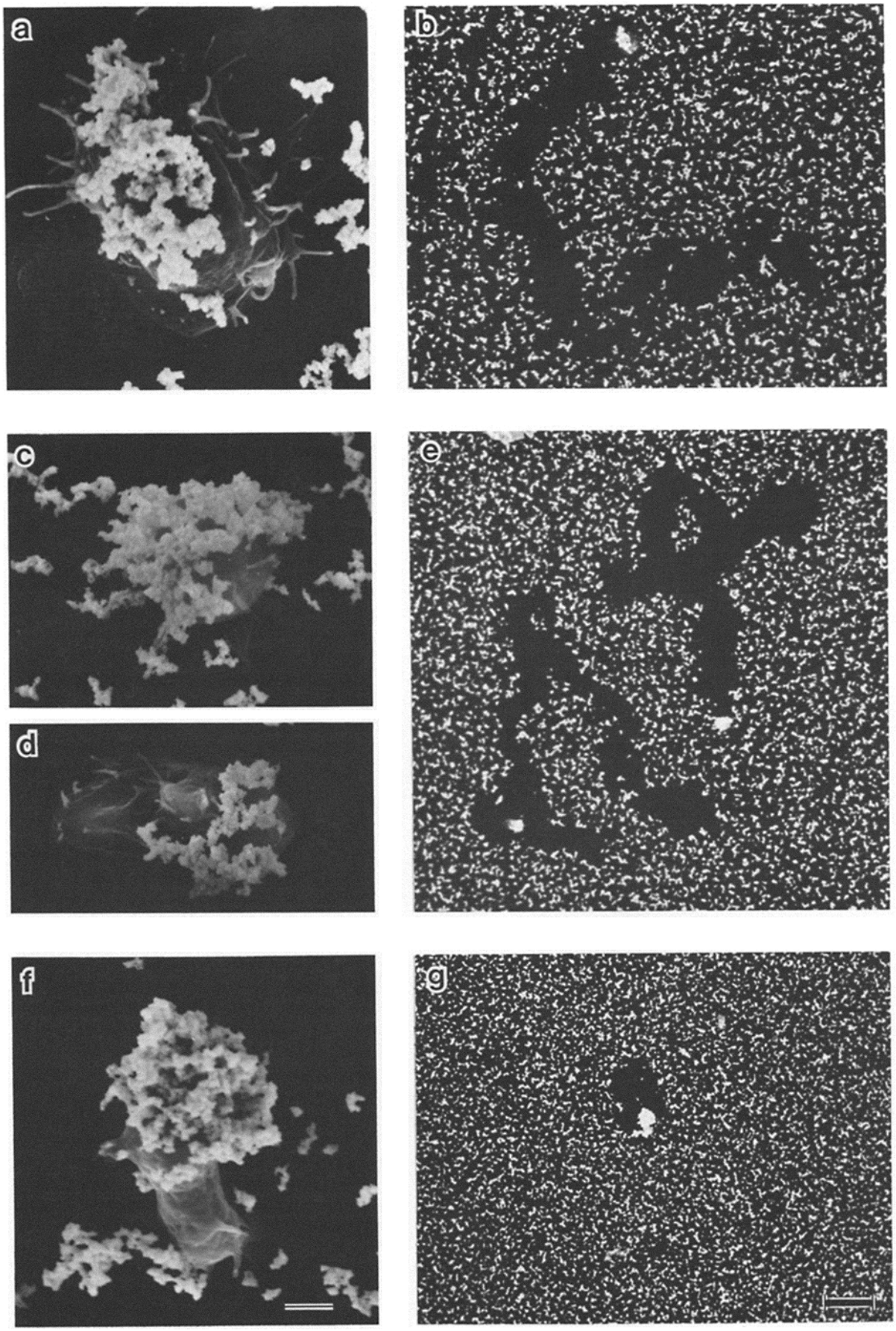

FIGURE 6 SEM visualization of AX3 cells during track formation. The AX3 cells were processed as described in Fig. 5. The sharp tracks produced by AX3 cells are clearly visible by SEM; a range of lengths is shown. Magnifications as described for Fig. 5.

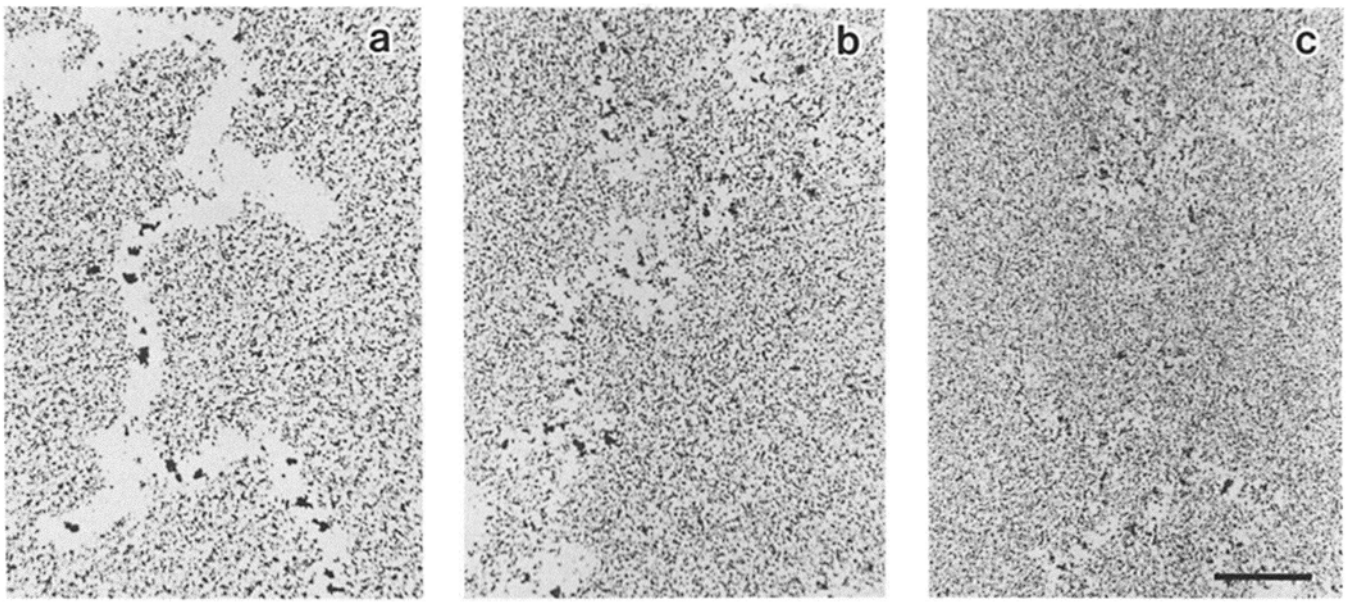

FIGURE 7 Bright-field illumination of tracks. Discarded gold clusters were best visualized by light microscopy using bright-field illumination. The tracks shown were produced by cells taken from bacterial growth plates and incubated for 22 h on gold-coated coverslips in HL5. Portions of AX3 (a) and DdB (*b* and *c*) tracks are shown. Bar, 0.1 mm. × 125.

tracks when living cells were observed, the masses were easily lost during handling of the slide and were rare in samples that had been fixed or mounted. Fig. 7*a* shows a portion of an axenic track in which several of these masses have been retained.

The behavior of wild-type cells was quite different. Most of them were more elongate than axenic cells, and their pseudopodia were not as broad or flattened. Although quite motile, these cells passed over the gold particles with a minimum of disturbance. In contrast to axenic strains, they rarely collected particles with their leading edge. Commonly the anterior portion of a cell would move across the gold particles without visibly disturbing them, although some jostling and clustering of gold particles could be seen at the rear of the cell. Most of these particles were moved only a short distance before being left behind. For many, but not all, wild-type cells, some of the particles were also accumulated into a mass on the rear dorsal surface. These masses formed more slowly and were smaller when dropped than those of axenic cells. They could usually be visualized in wild-type tracks by bright-field illumination (Fig. 7*b*), but merged in appearance with the background lawn of gold in the least distinct tracks (Fig. 7*c*). Thus, because the wild-type cells collected fewer particles and dropped them in smaller clusters, they left less evidence of their passage than did axenic cells.

DISCUSSION

This report demonstrates that wild-type and axenic strains of *D. discoideum* have distinctive track morphologies on substrates coated with gold particles. This difference has a genetic basis. Sharp track morphology was found to be a recessive phenotype in complementation studies. Its correlation with axenicity in segregants from heterozygous diploids suggests that a mutation needed for axenic growth, or a linked mutation, is responsible for sharp track morphology. Earlier work with temperature-sensitive motility mutants isolated in AX3 suggests that it is actually an axenic mutation that is involved. In that study, several spontaneous as well as induced mutations caused coordinate loss or gain of sharp track morphology and axenicity (5). As reported here, examination of available haploid strains showed that the known axenic mutations, *axeA* and *axeB*, are not adequate to account for track morphology: *axeA* is not required for sharp track morphology, and *axeB* is not sufficient. These data imply that an additional axenic mutation, unlinked to *axeA* or *axeB*, is necessary to produce sharp tracks. The existence of a previously unidentified recessive axenic mutation was also suggested by complex complementation data (not presented here) concerning axenic growth of various diploids and by the observation that some but not all *axeA*$^+$ *axeB* strains are capable of slow axenic growth. The segregation studies needed to test this hypothesis are underway.

Factors that might contribute to the track morphology difference have been examined. These studies have shown that endocytosis is not responsible for track formation. Neither strain of *D. discoideum* internalized gold to any significant degree. This was surprising in view of the report that fibroblasts make tracks on gold-coated surfaces by phagocytosis (1). It would be interesting to evaluate more carefully the relative contributions of surface collection and endocytosis in these cells as well.

Although both axenic and wild-type cells of *D. discoideum* accumulated gold particles, they differed in the extent and method of collection. Axenic cells extended broad, flat pseudopodia that picked up particles at the anterior end of the cell; the particles moved over the dorsal surface and formed a large mass that was eventually dropped. The resulting track had sharp boundaries and was devoid of gold particles, especially since the large masses were usually lost when the coverslip was handled. Wild-type cells tended to assume a thicker and more elongate shape than axenic cells. In general, the anterior portion of wild-type cells passed over gold particles without disturbing them. At the posterior end, some particles were moved or collected into masses, which were smaller than those formed by axenic cells. Thus wild-type cells removed fewer particles from the track and dropped the collected

particles in small clusters that resembled the background lawn of gold. These factors account for the fuzzy appearance of wild-type tracks.

Measurements of pinocytosis by wild-type and axenic cells shifted from bacterial plates to axenic culture have shown that the wild-type strain does not exhibit the high rate of pinocytosis observed for axenic strains. Furthermore, rapid pinocytosis is not a constitutive property of axenic strains, but is induced by replacing the normal food source (bacteria) with liquid nutrients. As is evident from Fig. 4, this induction is biphasic. This could reflect a relationship between pinocytosis and the cell cycle, since the plateau region corresponds approximately to the time cell number begins to increase after the shift to axenic conditions. However, other complex changes also occur in response to this shift. These include alterations in cell ultrastructure (7) and in the physical properties of lysosomes (15). There are also changes in enzyme levels, which parallel the onset of the developmental program (3). In particular, a subset of lysosomal enzymes is induced, including α-mannosidase-1, which is secreted by axenically growing cells (2, 3). Intracellular and extracellular levels of α-mannosidase-1 were measured at intervals following shift to HL5. Preliminary results (not shown) indicate that the first phase of the increase in pinocytosis parallels the onset of α-mannosidase-1 synthesis, and the second phase correlates with the onset of significant secretion of the enzyme. This suggests that the biphasic curve may reflect distinct states in the process of adaptation to axenic growth, and that the changes in lysosomal enzymes may be functionally related to this adaptation.

The axenic genotype has profound effects on a cell, many of which are related to motility. Some of the effects are induced only by growth under axenic conditions, like the alterations in ultrastructure, lysosomes, and secretion patterns discussed above, and the changes in pinocytosis and rate of migration described in this report. In addition, these studies have demonstrated constitutive differences in cell-substrate interactions for axenic and wild-type cells, the most striking being the ability of axenic cells to make sharp, clear tracks on gold-coated surfaces. Although the factors responsible have not yet been identified, it is evident that sharp and fuzzy tracks are manifestations of differential interactions between the cell surface and gold particles in axenic and nonaxenic strains. Several possible modifications could produce this effect. The binding of gold particles might be stronger in axenic cells because of the expression of surface components lacking or inaccessible in wild-type cells. Alternatively, binding might induce coupling of particle-receptor complexes to submembranous cytoskeletal elements in one case but not the other, or membrane cycling patterns might differ in the two cell types. Any of these alterations could also contribute to the observed differences in cell morphology during migration. (See Weatherbee [13] for a review of the postulated roles of membranes and cytoskeletal proteins in cell movement and capping.)

The relationships among these diverse consequences of the axenic mutations are not clear. Because the axenic genotype involves more than one mutation, it is possible that some of these effects are attributable to separate mutations. However, some or all of these phenotypes may represent consequences of a change in a single underlying process. These questions can be explored by biochemical, physiological, and ultrastructural characterization of strains differing in their complement of axenic mutations. The segregation analysis of axenic growth and sharp track morphology currently in progress will provide the necessary strains. Such studies should help to reveal the nature of the axenic mutations and their relationship to motile activities and how the cells undergo their remarkable adaptation to axenic conditions.

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