Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 9360–9365, August 1998
Developmental Biology

Detection of subtle phenotypes: The case of the cell adhesion molecule csA in *Dictyostelium*

ELEONORA PONTE*, ENRICO BRACCO*, JAN FAIX†, AND SALVATORE BOZZARO*‡

*Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Ospedale S. Luigi, 10043-Orbassano, Italy; and †Max Planck Institut fuer Biochemie, D-82152 Martinsried, Germany

Communicated by J. T. Bonner, Princeton University, Princeton, NJ, June 5, 1998 (received for review December 4, 1997)

ABSTRACT ***Dictyostelium*** **amoebae aggregate into a multicellular organism by cAMP-driven chemotaxis and cell–cell adhesion. Cell adhesion is mediated by an EDTA-sensitive and an EDTA-resistant adhesion system. The latter is developmentally regulated and triggered by homophilic interactions of the membrane glycoprotein csA; on disruption of the encoding gene, EDTA-resistant contacts fail to form. Nevertheless, csA-null cells under usual laboratory conditions aggregate normally and complete development. By using experimental conditions that reproduce more closely the habitat of** ***Dictyostelium*** **amoebae, evidence is provided that csA is required for development and that its expression confers a selective advantage to populations of wild-type cells over csA-null mutants. The latter display reduced cell–cell adhesion, increased adhesiveness to the substratum, and slower motility, which lead to their sorting out from aggregating wild-type cells. It is proposed that the experimental conditions commonly used in the laboratory are not stringent enough to assess the developmental role of csA and other proteins. The assay described can be used to detect subtle phenotypes, to reexamine the developmental role of apparently nonessential genes, and to test the validity of recent models on emergence and maintenance of apparent genetic redundancy.**

Gene disruption as a method for assessing the function of a gene is a powerful and widely used approach in *Dictyostelium,* and, since its first use by De Lozanne and Spudich (1), several genes involved in motility, signal transduction, and adhesion or developmental genes of unknown function have been inactivated (2–4). In many cases, no evident or only very mild phenotypic changes were found, leading to the suggestion that many proteins are redundant, or at least nonessential for development. Among these proteins, the cell adhesion molecule csA (5) is unique. This glycoprotein starts accumulating in the preaggregation phase, is maximally expressed during aggregation, and disappears after aggregate compaction (6, 7). The csA glycoprotein mediates a homophilic type of EDTA-resistant adhesion (8, 9), and deletion of its gene generates mutants that fail to form EDTA-stable contacts during aggregation (10). Conversely, csA constitutive expression induces formation of EDTA-resistant aggregates during growth phase (11).

Despite the stringent temporal regulation of the csA gene, which points to a specific role for the glycoprotein during the aggregation phase, and despite the loss of EDTA-stable adhesion after csA gene disruption, which indicates that no other compensatory proteins mediate this form of adhesion during aggregation, no differences in aggregation timing and developmental phenotype are observed between wild-type and csA-null cells under usual laboratory conditions (10).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Commonly used solid substrata for studying *Dictyostelium* cell development include agar, filter paper, glass coverslips, or polystyrene dishes. Considering the size of *Dictyostelium* amoebae (10–12 μm in diameter) and the physicochemical properties of these materials, aggregation and multicellular development on these substrata occur on a relatively smooth, homogenous surface. In the natural environment—namely, forest detritus—cells are exposed to a rough, uneven surface, to "mountains and valleys" made of soil, decaying leaves and other debris with varying physical and chemical properties (12). Aggregation on agar or glass results in a two-dimensional streaming pattern, but aggregation is three-dimensional in soil, and chemotaxis under these conditions is very likely more difficult than on agar. Thus, defects affecting cell motility, cell–cell or cell–substratum adhesion may result in more dramatic effects on soil than under usual laboratory conditions. Indirect evidence for this can be inferred from a recent, elegant movie illustrating the difficulties of slugs in bridging the chasms between soil particles (13).

To assess the role of csA-mediated intercellular adhesion on development under conditions closer to the natural ones, we have devised a simple, reproducible method for preparing an alternative substratum. By using this assay, we show that csA is essential for development and that its expression confers a selective advantage to cells over csA-null mutants. We discuss the application of the assay to detect subtle phenotypic changes of other apparently nonessential genes and the relevance of these results in the context of recent genetic models on evolutionary stability of redundant genes.

MATERIALS AND METHODS

Cell Cultures. The following *Dictyostelium discoideum* strains were used throughout: wild type AX2 (clone 214), AX2/β-gal (see below), csA deletion mutant T10 (10), and csA-constitutive overexpressor TWT (11). Cells were grown in axenic medium (14) at 23°C and 150 rpm in a climatic cabinet equipped with gyratory shakers (Kühner, Bielefeld, Switzerland). They were harvested at a density of no more than 4×10^6 cells/ml. Transfected cells were kept under constant selection by adding 20 μg/ml G418 to the medium.

For development in suspension, cells were washed twice, were adjusted to 1×10^7 cells/ml in 17 mM Sörensen Na/K phosphate buffer (pH 6.0), and were shaken as above. Under these conditions, cells become competent to aggregate in 4–5 hours (15, 16). For development on nonnutrient agar, cells were plated at a concentration of 1×10^8/90-mm in diameter plate.

Development on Soil Plates and Colony Blots. Commercially available garden soil (type universal, neutral pH) was sieved to obtain particles of homogenous size (<0.4 cm in diameter) and was autoclaved. Aliquots of 20 g were distributed homogeneously on 90-mm in diameter Petri dishes and were moistened

‡To whom reprint requests should be addressed. e-mail: sbozzaro@polito.it.

with 0, 5, or 10 ml of sterile water to obtain three soil plates, respectively A, B, and C, with different degrees of moisture. Cells were washed free of axenic medium, were concentrated to 1×10^8/ml in Sörensen phosphate buffer, and aliquots of 0.25 ml were pipetted onto soil plates to cover an area of ≈2 cm^2. Four samples can be accommodated easily on the same Petri dish, with enough space left in between to avoid any interference among colonies. The plates were covered with a lid and were incubated at 23°C. The autoclaved soil can be stored for several weeks at room temperature. Plates were prepared fresh before starting the experiment, and, under these conditions, sequence and timing of development were reproducible. Development and fruiting body formation on soil plates was monitored with a Wild M3Z stereomicroscope (Heerbrugg, Switzerland) equipped with overhead optical fibers (Intralux 6000) (Volpi Heerbrugg, Switzerland).

To determine the efficiency of spore formation on soil, starving cells were plated on agar or soil plates A–C as above, and, after 48 h, the agar piece or the soil particles of each whole colony were transferred in 50-ml test tubes containing 10-ml Sörensen phosphate buffer. After repeated vortexing, the larger soil or agar particles were let to sediment, and 5 ml of the suspension were immediately transferred to 15-ml test tubes and treated with 0.5% SDS for 5 min (17). A 1-ml aliquot of the suspension containing spores then was transferred to a new 15-ml test tube and was centrifuged at $1,000 \times g$ for 5 min. The pellet was resuspended in 10-ml Sörensen phosphate buffer, and samples containing spores were diluted 1,000- or 10,000-fold, respectively, and were plated on nutrient agar with *Escherichia coli B/2*. Under these conditions, in two duplicate experiments, the number of plaques per plate formed by spores of AX2, T10, or TWT originally collected from agar varied between 80–95 (1,000-fold dilution) or 19–29 (10,000 fold dilution), allowing a good estimate of the spore yield.

For co-development experiments, starving cells of T10 and AX2 were mixed in a proportion of 1:1 and were pipetted on plate B. After 40 h of incubation, sorocarps were picked up randomly with a loop, were transferred in Sörensen phosphate buffer, and were treated with 0.5% SDS; spores were washed free of SDS by dilution and centrifugation, were mixed with *E. coli B/2* and plated on nutrient agar in a proportion of 20 spores per plate. When colonies were about 1 cm in diameter, they were blotted onto a nitrocellulose filter as described (16), and the blot was incubated with the mAb 448, which is specific for the csA polypeptide (18). Immunodetection was performed using the Amersham ECL Immunoblotting System procedure.

Sorting out Experiments. Binary combinations of AX2 with T10 or TWT were used for co-aggregation and sorting out experiments. Starving cells of the strains to be tested were washed once in cold Sörensen phosphate buffer, were resuspended at 1×10^7 cells/ml, were mixed in a proportion of 1:1, and were shaken at 150 rpm for 30 min. In a first series of experiments, aggregates were fixed as described (19) and were treated with mAb 448 and FITC-labeled anti-mouse IgG. In a second series, AX2 transfected with *lac-Z* reporter gene under the control of an actin-15 promoter was used (named "AX2/β-gal"), and the aggregates formed were fixed in suspension with 1% glutaraldehyde in sodium phosphate buffer (pH 7.2) for 4 min. After washing by dilution with sodium phosphate buffer (pH 7.2), the aggregates were stained with β-gal staining solution (20). No qualitative, and only slight quantitative, differences in the results were found between both procedures, but using AX2/β-gal allowed us to perform sorting out experiments also with growth-phase cells that do not express csA.

Cell–Substrate Adhesion Measurement. Cells at the desired stage were resuspended at 2.5×10^6cells/ml in cold phosphate buffer and 20-μl aliquots were spotted in double at the periphery of 60-mm bacteriological polystyrene dishes. After 10 min incubation at 23°C, 10 ml of Sörensen phosphate buffer was pipetted gently in the center of each dish, and the dishes were rotated for 1 min on a gyratory shaker at a speed varying between 80 and 160 rpm. The buffer was removed totally, and the film of buffer covering the spots of adhering cells was dried in the air. To determine the extent of cell binding in each spot, the absorbance was measured in an Eppendorf photometer 1101M equipped with a 400- to 600-nm pass-band filter, and a 1×4 mm screen slit, by placing the dish between the light source and the photomultiplier. A standard curve was obtained by spotting 20-μl cell suspensions at concentrations varying between 0.2 and 5×10^6/ml and drying after 10 min incubation. In this range, the extinction curve increased linearly from 0.05 to 0.55.

Cell Motility Measurement. A total of 5×10^6 cells at 0.5 or 4 h of starvation were plated on a 60-mm in diameter bacteriological polystyrene dish (Falcon 1016 or 1007). Random motility at the beginning of development or after 4 h of starvation was tested in buffer. Chemotactic cell motility was tested by stimulating 4h-starved cells with cAMP diffusing from a microcapillary filled with 1 μM solution. Serial images were time-lapse recorded for 1 h with a Zeiss ccd camera (ZVS-47DE) mounted on a Zeiss Axiovert 35 microscope connected to a Panasonic video-recorder AG-6010 (Panasonic, Secaucus, NJ). Paths of individual cells moving toward the capillary were projected on a monitor, were traced with a pen onto transparency sheets, and were analyzed in a Digicad graphic tablet (Digicad, Irvine, CA) with the Videoplan Image processing system (Zeiss). In each experiment, 50 to 70 cells were followed for 30 to 45 min. The length of migration per unit time per cell was used to determine the average speed.

RESULTS

Development of Wild-Type, csA-Null, or Overexpressing Cells on Soil vs. Agar Plates. Starving wild-type AX2, csA-deletion mutant T10, or constitutively expressing TWT cells were incubated on agar or soil plates A, B, and C. Under these conditions, AX2 cells underwent development on soil with a timing that is a function of the relative moisture of the plates. Compact aggregates were formed after 7 h from starvation both on agar and on soil plate A and after 9 h on soil plates B and C (Fig. 1). The time elapsed for fruiting body formation, after aggregation, was comparable on all plates. In contrast to agar, where most tip aggregates exposed to diffused light underwent culmination, on soil plates they first formed migrating slugs. Slug migration accounts for the short delay in culmination observed on all soil plates (Fig. 1). Size and shape of fruiting bodies were similar both on agar and soil, but, compared with agar (100%), their number on soil plates A, B, and C was reduced gradually from ≈85% to 60 and 45%, respectively, suggesting that developmental conditions become more stringent with increasing soil wetting. The sequence and timing of development were highly reproducible, provided that soil plates were prepared fresh.

csA-null T10 cells incubated on soil formed compact aggregates with a delay of 8 (plates A and B) to 10 h (plate C), compared with AX2 cells (Fig. 1). Fruiting body formation was inhibited strongly on all soil plates; on plate C, T10 cells formed <15% of the fruiting bodies compared with wild-type internal control. This corresponds to ≈1% of the fruiting bodies normally formed by T10 or wild-type cells on agar (Fig. 2 and 3). On agar or filter, no differences in developmental timing or fruiting body number were found between csA-null and wild-type cells (Figs. 1 and 3). It is possible that T10 cells form very small slugs and fruiting bodies on soil that do not manage to reach the surface of the soil and thus escape scoring. In this case, the csA-null cells would appear to be inefficient at fruiting, though their spore yield could be similar to the wild type. We therefore determined the number of viable spores in the whole colonies, in addition to counting the fruiting bodies, by collecting the soil particles of the entire colony area, lysing

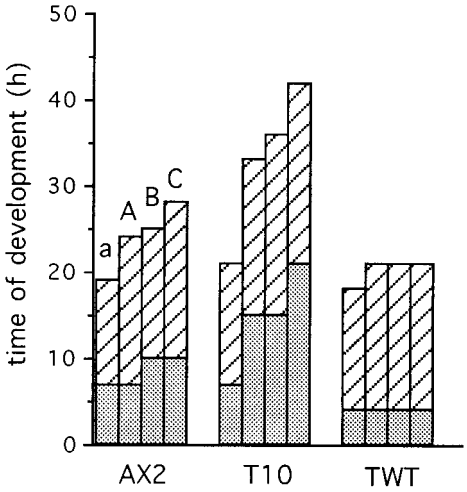

FIG. 1. Timing of development of AX2, T10, and TWT cells on agar or soil plates. At the beginning of starvation, cells of the indicated strain were resuspended at 1×10^8/ml in Sörensen phosphate buffer, were plated on agar or soil plates, and were incubated at 23°C. Development was monitored at regular intervals with a stereomicroscope. Time elapsed from beginning of starvation for appearance in the colonies of the first compact aggregates (dotted columns) or fruiting bodies (dashed columns) is indicated in the ordinate. a, agar; A, B, and C, soil plates with increasing degree of moisture, prepared as described in *Material and Methods*. Average times of three (a) and eight (A, B, and C) experiments are shown. The SDs did not exceed ±1 h for both compact aggregate and fruiting body formation in all cases.

entrapped amoebae with SDS, and plating spores on bacterial lawns. As shown in Fig. 3, the percentage of viable spores arising from T10 cells was reduced strongly on all soil plates, compared with wild-type cells, to an extent similar or even higher than the fruiting body number. Thus, under conditions closer to the natural ones, csA gene disruption dramatically affects the ability of cells to aggregate and form fruiting bodies and viable spores, which is not evident on standard laboratory substrata, namely agar or filter paper.

Constitutive expression of csA, obtained by placing the csA gene under the control of an actin promoter (11), resulted in the opposite effects, leading to early aggregation and fruiting body formation on all plates (Fig. 1). Fruiting body and viable spore numbers were similar to or higher than the wild-type (Figs. 2 and 3), though normal-size fruiting bodies were mixed with several smaller ones.

Differential Fitness of Wild-Type vs. csA-Null Mutant. To determine whether csA expression confers a selective advantage to cells, starving AX2 and T10 cells were mixed on soil plate B in a proportion of 1:1 to form fruiting bodies. After fruiting body formation, spores were collected randomly and were cloned on a bacterial lawn. Colonies were blotted on nitrocellulose filter and were labeled with mAb 448, which is specific for csA (18), to distinguish colonies derived from wild-type or mutant spores. In six different experiments, the number of colonies arising from T10 spores averaged ≈18% (Table 1), a value in the same range as that obtained when T10 cells are cultured alone under similar conditions (Fig. 3). This observation suggests that csA-null cells either fail to co-aggregate with wild-type cells or, if co-aggregating, are directed preferentially toward the prestalk pathway. csA gene disruption seems, therefore, to affect not only the aggregation efficiency of mutant isolates but also their capacity to be rescued by wild-type cells.

These results prompted us to investigate whether csA deletion results in sorting out of mutant from wild-type cells during aggregation. Previously, we found that csA-null T10 cells co-aggregating on agar or glass with wild-type AX2 cells

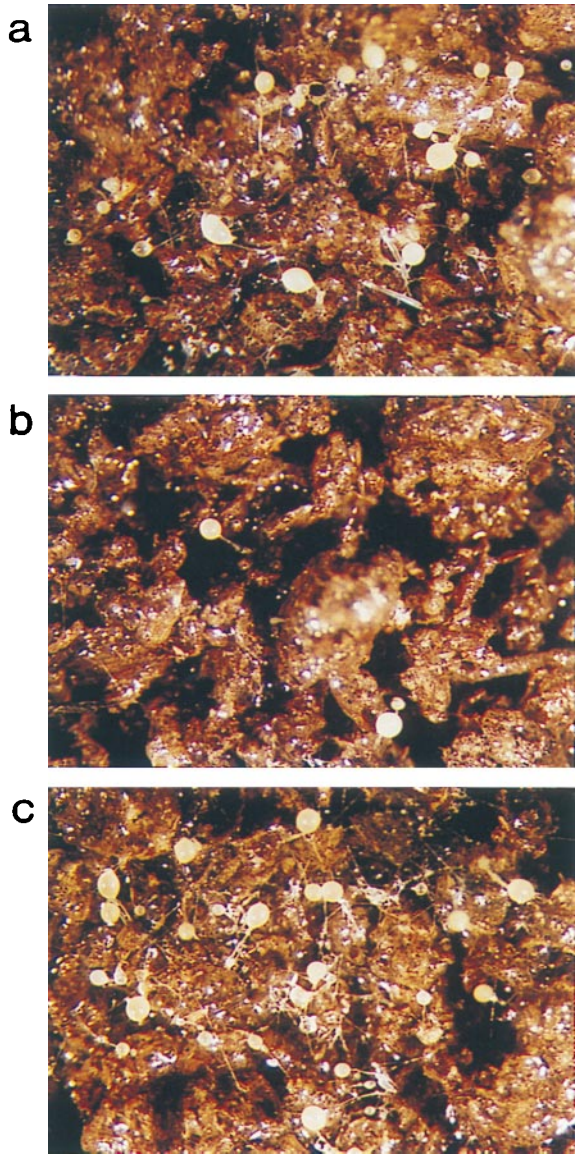

FIG. 2. Fruiting bodies formed on soil plate C by AX2, T10, and TWT cells. (*a*) Wild-type AX2, (*b*) csA-deletion mutant T10, and (*c*) csA constitutive expresser TWT. See Fig. 1 for experimental details.

formed mixed aggregating streams and mounds (data not shown). The data reported above indicate, however, that aggregation on these substrata is not a stringent enough condition to assess the potential ability of csA to mediate cell sorting. Co-aggregation experiments, therefore, were performed under shaking, where shear forces interfere with cell–cell adhesion. Under these conditions, aggregation-competent wild-type and csA-null cells sorted out in strain-specific aggregates (Table 2). Growth-phase cells of both strains, as expected, formed mixed aggregates; in contrast, growth-phase TWT cells, which express csA constitutively, underwent sorting when mixed with growth-phase AX2 cells (Table 2). Examples of the different types of aggregates that could be obtained are shown in Fig. 4. Thus, differential csA expression is sufficient to induce sorting of two cell populations, at least under conditions that require stronger cell–cell cohesion.

Effects of csA Expression on Cell–Substratum Adhesion and Cell Motility. In cells co-aggregating on a solid surface, cell sorting is likely to depend on relative differences between cell–cell and cell–substratum adhesion, which affect cell motility. csA-null and wild-type cells differ in their cohesiveness

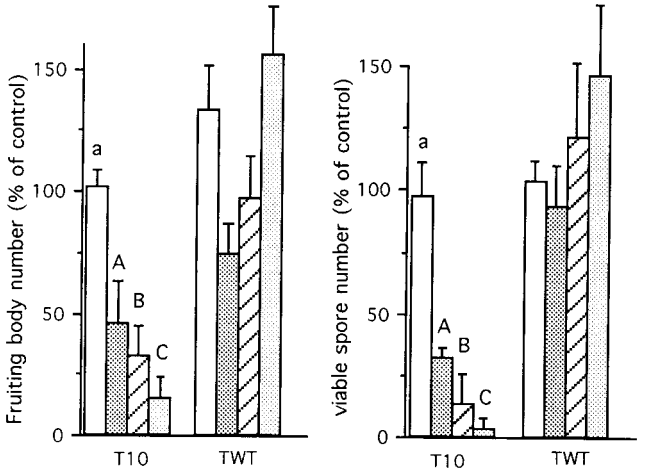

FIG. 3. Efficiency of T10 and TWT cells in fruiting body and spore formation on soil. At the beginning of starvation, AX2, T10, or TWT cells were resuspended at 1×10^8/ml in Sörensen phosphate buffer, and a total of 2.5×10^7 cells per colony were plated on agar (a) or soil plates (A, B, and C) and were incubated at 23°C for 48 h. (*Left*) The number of fruiting bodies per colony formed by T10 or TWT was scored and expressed as percentage of AX2 fruiting bodies formed on each plate. (*Right*) The number of viable spores in each colony was determined after treating the soil particles with SDS as described in *Material and Methods* and was expressed as a percentage of the internal AX2 control. Mean values of three (a) and eight (A, B, and C) experiments (*Left)* or two duplicate experiments *(Right)* are shown. Vertical bars, SDs.

during the aggregation stage, as shown by their differential response to EDTA (6, 10) and by the fact that vortexing easily dissociates csA-null but not wild-type aggregates (S.B., unpublished observations). To determine whether csA expression also interferes with cell–substratum adhesion, cell binding to bacteriological polystyrene dishes was measured. Previous observations in our laboratory have indicated that *Dictyostelium* cell adhesiveness is higher to bacteriological polystyrene than tissue-culture polystyrene dishes, glass coverslips, or agar (in that order). Thus, strain differences in cell–substratum adhesion are more evident on this substratum. The results of a representative experiment are summarized in Fig. 5: csA-null T10 cells, both during growth and at aggregation stage, adhered to polystyrene to a similar extent as AX2 growth-phase cells whereas aggregation-competent AX2 and growth-phase TWT cells were less adhesive to the substratum.

Table 1. Efficiency of spore formation by T10 cells co-cultured with AX2 cells on soil

T10 cells at beginning of experiment, %	Number of colonies in colony blots		T10 spores in mixed fruiting bodies, %
	AX2	T10	
50	16	3	15.8
50	13	6	31.6
50	18	2	10.0
50	62	11	15.0
50	46	11	19.3
50	67	17	20.2
Totals	222	50*	18.4

AX2 and T10 at beginning of starvation were mixed in a proportion of 1:1 and were incubated for development on soil plate B. After fruiting body formation, spores were collected randomly and were plated on *E. Coli B/2* at a density of 20 spores per agar plate. When colonies were ≈1 cm in diameter, they were blotted onto nitrocellulose, and the blots were incubated with mAb 448, which recognizes a peptide epitope of csA. The bound antibody was detected by chemiluminescence, and the colonies also were stained with red Ponceau (16). The number of colonies positive (AX2) or negative (T10) for csA in six experiments was counted, and the percentage of T10 spores in mixed fruiting bodies was extrapolated.
*$P < 0.001$ (χ^2 test), relative to total number of colonies.

Table 2. Strain-specific sorting out of cells co-aggregating under shaking

Starvation time, h	Strain combination	Aggregate sorting out		
		% strain-specific	Partially mixed	Mixed aggregates
0.5	AX2 + T10	0	0	100
0.5	AX2 + TWT	99 ± 1.9	0.8 ± 1.7	0
5	AX2 + T10	69 ± 2.9	31 ± 2.9	0

AX2/βgal, T10, and TWT cells were starved separately at a density of 1×10^7 cells/ml. At the indicated time, cells were mixed 1:1 and were shaken at 150 rpm for 30 min. The aggregates were fixed, washed, and labeled with β-gal staining. Stained (AX2) and unstained (T10 or TWT) aggregates could be found ("strain-specific"), as could aggregates with distinct strain-specific areas ("partially mixed") or in which cells of two strains were mixed randomly ("mixed aggregates"). The number of aggregates in the three categories in each experiment was determined and expressed as % of total. Mean values ± SD of three experiments are shown. A total of 138 (AX2 + T10 at 0.5 h), 122 (AX2 + TWT at 0.5 h), and 174 (AX2 + T10 at 5 h) aggregates were counted.

Similarly, aggregation-competent wild-type cells showed more random and chemotactic motility on bacteriological polystyrene dishes than csA-null cells. The average speed was 10.5 and 6 μm/min for, respectively, AX2 and T10 when cells were stimulated with cAMP (Table 3). No difference in random motility was found in cells at the beginning of starvation, strongly suggesting that the observed changes during development are linked to csA expression in wild-type cells. These differences in cell–substratum and cell motility were not sufficient to induce strain-specific sorting of cells co-aggregating on polystyrene dishes (data not shown), but they are indicative of pleiotropic effects resulting from csA expression that very likely play a role for optimal aggregation in the natural environment. The same differences also can explain the observation that T10 cells form larger streams on glass or polystyrene than AX2 cells (10).

DISCUSSION

Homophilic interactions between csA molecules on opposing surfaces generate EDTA-resistant intercellular adhesion during the aggregation stage of *Dictyostelium* cells. We have shown here that deletion of csA results in increased cell–substratum adhesiveness and reduced cell motility. These concomitant effects can explain the phenotype of csA null-mutants on soil: In the absence of the csA glycoprotein, cell–cell cohesion is weakened, cell–substratum adhesion increases, and cell motility is reduced, resulting in delayed aggregation and in many cells failing to enter aggregates, thus, in a dramatically lower number of fruiting bodies and viable spores formed. csA expression confers a selective advantage to wild-type cells, which aggregate faster and sort out into homotypic aggregates, apparently leaving behind csA-null cells. This offers a teleonomical explanation of why csA, whose expression is strictly regulated during the aggregation stage, has been retained over millions of years, despite its apparent functional redundancy under laboratory conditions; wild-type and spontaneously arising mutant amoebae coexist in the same environment, possibly also with amoebae of other species. Homophilic cell–cell adhesion during aggregation ensures species–specific formation of aggregates (6, 19) and, as shown here, negative selection of mutant cells that do not aggregate as efficiently.

Gene deletion is the method of choice to assess the developmental role of a given gene. In *Dictyostelium*, this approach

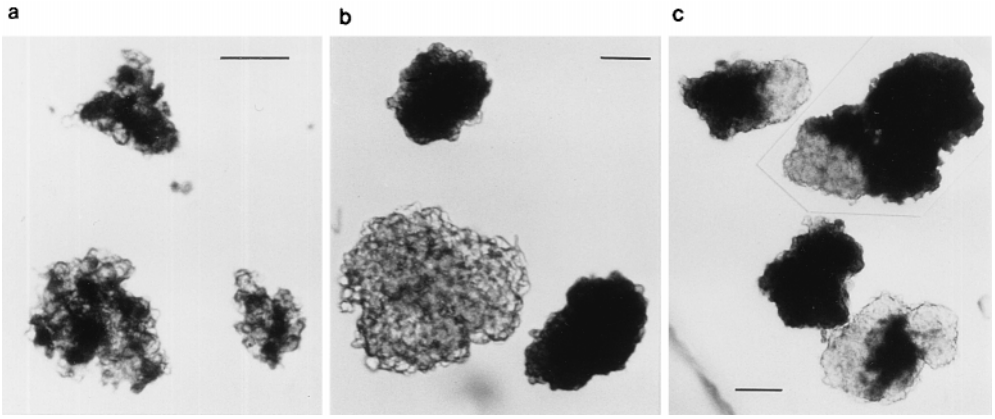

FIG. 4. Types of cell aggregates formed in co-aggregation experiments. AX2 cells, expressing the *lac-Z* reporter gene (AX2/β-gal), were mixed in binary combinations with T10 or TWT cells in a proportion of 1:1 at a final concentration of 1×10^7 cells/ml and were shaken for 30 min at 150 rpm on a gyratory shaker. The aggregates were fixed and stained to detect AX2 cells in the aggregates (black areas). (*a*) "Mixed" aggregates formed when AX2/β-gal cells were incubated with T10 cells at the beginning of starvation; (*b*) "strain-specific" and (*c*) "partially mixed" aggregates formed when AX2/β-gal cells were incubated with TWT cells at the beginning of starvation as well as with T10 cells at aggregation stage. See Table 2 for quantitative data. (Bars = 0.2 mm.)

is favored by the organism's haploid genome, but it has often led to very mild or no evident phenotypic changes, favoring the notion that many proteins are redundant or nonessential for development. We suggest that the apparent redundancy results in many cases from the standard artificial substrata used to support development of *Dictyostelium* cells not being as selective as the natural substratum. The experimental approach described in this paper for csA mutants has been extended in our laboratory to deletion mutants in actin binding proteins; 7 of 10 mutants, which develop normally on agar, are at selective disadvantage when incubated on soil (E.P., A. Noegel, and S.B., unpublished observations).

We propose that the developmental role of other genes whose deletion has not led to clear phenotypic changes should be reexamined by using the approach described in this paper. The soil plates also can be used systematically as a selection test, complementing development on agar, for detecting new developmental mutants generated by restriction enzyme mediated insertion (21).

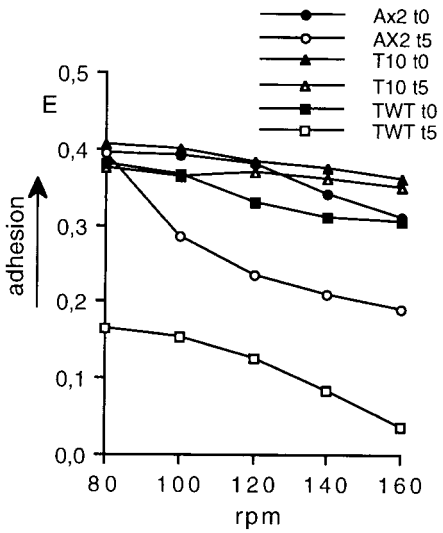

FIG. 5. Cell adhesion to polystyrene dishes. Growth-phase (t_0) or aggregation-competent (t_5) cells of AX2, T10, or TWT were washed once and were resuspended at 2.5×10^6 cells/ml in ice-cold Sörensen phosphate buffer. Aliquots of 20-μl cell suspension were spotted at the periphery of 60-mm in diameter polystyrene dishes. After a 10-min incubation at 23°C, 10 ml of Sörensen phosphate buffer were pipetted gently at the center of each dish, and dishes were rotated for 1 min at the speed indicated in the abscissa. Unbound cells were aspirated with the buffer, and bound cells were determined as described under *Material and Methods*.

The finding that, under certain experimental conditions, some genes are not required for development offers an opportunity to examine the question of genetic redundancy. Redundancy seems to be widespread in genomes of several other organisms (refs. 22–27; see also ref. 28 for selected examples of redundant genes). At a cellular and organismal level, redundant genes performing in part or *in toto* a similar function introduce flexibility, permitting a process to continue as normal under a wider range of environmental conditions, and possibly also leading to the emergence of new properties (28). Because for a protein to be redundant other proteins with compensatory or overlapping functions must exist, the question arises of how a redundant gene can be protected against accumulation of deleterious mutations and thus whether redundancy can be stable during evolution. Thomas (28) has proposed several mechanisms by which partially or fully redundant genes may optimize a function or increase the fidelity of a process that selection could act on, thus fixing genetic redundancy. Nowak *et al.* (29) have developed genetic models and computer simulations to explain emergence and maintenance of genetic redundancy. According to these simulations, two genes that perform the same function with the same efficiency will be maintained stably in a population if the mutation rates in both genes are the same. Two genes performing the same function with different efficiencies also would be maintained in a stable equilibrium if the gene performing the function with lower efficiency also has a lower mutation rate than the other gene. The same holds true for two genes that perform distinct functions but with partial overlap.

Other authors have, instead, questioned the existence of redundant genes as contradictory with Darwinian theory,

Table 3. Random and chemotactic cell motility of AX2 and T10

Strain	Starvation time, h	Speed, μm/min	
		Buffer	cAMP
AX2	0.5	1.8 ± 0.2	n.t.
	4	7.2 ± 0.3	10.1 ± 1.0
T10	0.5	1.9 ± 0.2	n.t.
	4	5.1 ± 0.4	6.1 ± 0.6

AX2 and T10 cells at the indicated time of development were assayed for random or cAMP-stimulated cell motility as described in *Materials and Methods*. Values of two experiments are given as means ±SD; n.t., not tested.

stressing the need for new assays to be developed that detect subtle phenotypic changes (30). The present results favor the notion that gene redundancy is relative to a given environment and that, at least in many cases, it results from standard experimental assays being unable to detect graded phenotypic defects.

Nevertheless, the experimental conditions described in this paper may help to test models of genetic redundancy. Deletion of the csA gene results in complete loss of EDTA-stable adhesion during aggregation; thus, no other protein substitutes for csA in mediating the EDTA-stable form of adhesion during the aggregation stage. During the same stage, a second class of adhesion molecules, termed csB, is responsible for an EDTA-sensitive form of adhesion (9). Thus, csA and csB behave as two loci that perform a similar function, i.e., cell–cell adhesion, albeit with different biochemical mechanisms and different efficiencies. Both csA and csB may act synergistically or cumulatively, thus promoting adhesion with higher fidelity, as suggested by the Thomas' model (28). As shown in this paper, neither csA nor csB are truly redundant under natural or semi-natural conditions, but, under the less stringent standard laboratory conditions, csA-dependent adhesion is dispensable, and its loss has no detectable phenotypic changes on the efficiency of fruiting body and viable spore formation.

Because *Dictyostelium* cells can be grown to high density with a generation time of a few hours and sensitive assays for measuring adhesion (16) and probes for DNA and protein analysis (7, 11, 18) are available, it should be possible to test whether a functional csA gene is maintained stably over generations with the same efficiency in cells developing on agar as on soil. This analysis could be extended to actin-binding proteins, thus offering an experimental framework for testing theoretical models of gene redundancy, such as those of Thomas (28) and Nowak *et al.* (29).

We thank Dr. A. Noegel for providing T10 cells and for critical reading of a first draft of the manuscript, Dr. A. Ceccarelli for the actin-15/β gal plasmid, and Dr. G. Gerisch for helpful suggestions. This work was supported by funding of the Italian Ministero dell'Universitá e della Ricerca Scientifica e Tecnologica and Consiglio Nazionale delle Ricerche.

1. De Lozanne, A. & Spudich, J. A. (1987) *Science* **236,** 1086–1091.
2. Loomis, W. (1996) *Microbiol. Rev.* **60,** 135–150.
3. Gerisch, G., Noegel, A. A. & Schleicher, M. (1991) *Annu. Rev. Physiol.* **53,** 607–628.
4. Witke, W., Schleicher, M. & Noegel, A. A. (1992) *Cell* **68,** 53–62.
5. Müller, K. & Gerisch, G. (1978) *Nature (London)* **274,** 445–449.
6. Bozzaro, S. & Ponte, E. (1995) *Experientia* **51,** 1175–1188.
7. Noegel, A., Gerisch, G., Stadler, J. & Westphal, M. (1986) *EMBO J.* **5,** 1473–1476.
8. Siu, C., Cho, A. & Choi, H. C. (1987) *J. Cell Biol.* **105,** 2523–2533.
9. Beug, H., Katz, F. E. & Gerisch, G. (1973) *J. Cell Biol.* **56,** 647–658.
10. Harloff, C., Gerisch, G. & Noegel, A. (1989) *Genes Dev.* **3,** 2011–2019.
11. Faix, H., Gerisch G. & Noegel A. (1990) *EMBO J.* **9,** 2709–2716.
12. Bonner J. T. (1982) in *The Development of* Dictyostelium discoideum, ed. Loomis, W. F. (Academic, New York), pp.1–33.
13. Higuchi, G. & Yamada, T. (1992) *Behavior and Differentiation of Cellular Slime Mold: A Clarified Mode of Life on Soil* (Cine Document, Tokyo, Japan), 16-mm color, sound film, 21 min.
14. Ashworth, J. M. & Watts, D. J. (1970) *Biochem. J.* **119,** 175–182.
15. Gerisch, G. (1959) *Naturwissenschaften* **46,** 654–656.
16. Bozzaro S., Merkl R. & Gerisch, G. (1987) *Methods Cell Biol.* **28,** 359–385.
17. Bozzaro S, Hagmann J., Noegel, A., Wetsphal, M., Calautti, E. & Bogliolo, E. (1987) *Dev. Biol.* **123,** 540–548.
18. Bozzaro S. & Merkl, R. (1985) *Cell Differ.* **17,** 83–94.
19. Bozzaro, S. & Gerisch, G. (1978) *J. Mol. Biol.* **120,** 265–279.
20. Dingermann, T., Reindl, N., Werner, H., Hildebrandt, M., Nellen, W., Harwood, A., Williams, J. & Nerke, K. (1989) *Gene* **85,** 353–362.
21. Kuspa, A. & Loomis, W. F. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8803–8807.
22. Brookfield, J. F. Y. (1997) *Adv. Genet.* **36,** 137–155.
23. Rudnicki, M. A., Braun, T., Hinuma, S. & Jaenisch, R. (1992) *Cell* **71,** 383–390.
24. Saga, Y., Yagi, T., Ikawa, Y., Sakakura, T. & Aizawa, S. (1992) *Genes Dev.* **6,** 1821–1831.
25. Laney, J. D. & Biggin, D. M. D. (1996) *Development* **122,** 2303–2311.
26. Schorle H., Holtschke T., Hunig, T., Schimpl, A. & Horak, I. (1991) *Nature (London)* **352,** 621–624.
27. Pekny, M., Leveen, P., Pekna, M., Eliasson, C., Berthold, C. H., Westermark, B. & Betsholtz (1995) *EMBO J.* **14,** 1590–1598.
28. Thomas, J. H. (1993) *Trends Genet.* **9,** 395–399.
29. Nowak, M. A., Boerlijst, M. C., Cooke, J. & Maynard Smith, J. (1997) *Nature (London)* **388,** 167–171.
30. Hochgeschwender, U. & Brennan, M. B. (1994) *Nat. Genet.* **8,** 219–220.