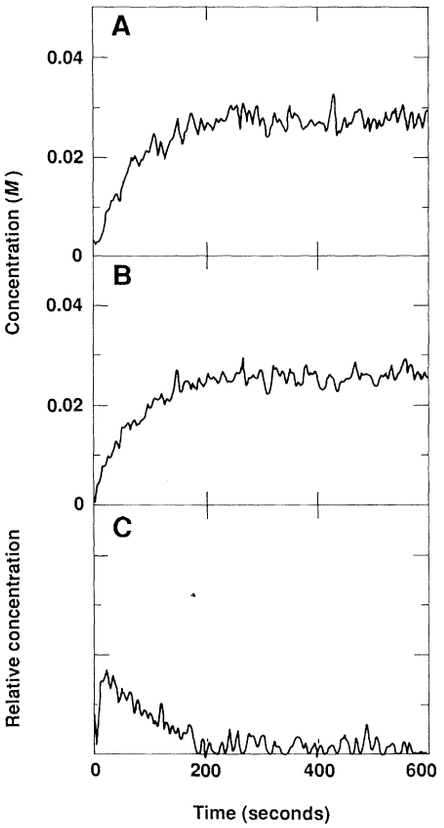

Fig. 3. Concentration as a function of time for (**A**) total H^+, (**B**) SO_4^{2-} + HSO_4^-, and (**C**) the intermediate. The initial conditions were: $[HSO_3^-] = 0.27M$; $[O_2] = 0.13M$; and $pH = 4$.

half-life of the intermediate to be about 52 seconds at 25°C and at an ionic strength μ of 0.5*M*. The decomposition rate of the intermediate appeared to be independent of the *p*H and of trace metal ions such as Fe^{2+}/Fe^{3+} and Mn^{2+} over the range studied.

From our experimental results it appears that the intermediate is formed from one oxygen molecule and two bisulfite molecules (Fig. 1) and that SO_4^{2-} or HSO_4^- or both are produced only upon decomposition of the intermediate (Fig. 3). The reaction mixture was studied by electron spin resonance (ESR) spectroscopy; no signal was observed that could be attributed to the intermediate, which indicated that it is not a free radical. The Raman spectrum of the intermediate is similar to that of dithionate ion, which is very stable and does not decay in the manner of the intermediate. From this information we propose a formula of $S_2O_7^{2-}$ for the intermediate. It could hydrolyze to form sulfate and hydrogen ions, as shown below:

$$S_2O_7^{2-} + H_2O \rightarrow 2SO_4^{2-} + 2H^+$$

The decomposition process of the intermediate, as well as the decomposition rate constant and the Raman spectrum, are very similar to those for the disulfate ion, $S_2O_7^{2-}$. The Raman spectrum of disulfate ion in solution obtained by Millen (*4*) has peaks at 325 and 1092 cm^{-1}, and possibly at 735 cm^{-1}. Two studies of the hydrolysis rate of disulfate ion (*5, 6*) agree well with one another and yield values of the hydrolysis rate constant very close to the value we obtained at 25°C. Sodium ions have been reported (*5*) to catalyze the hydrolysis somewhat. However, the effect is fairly small and does not create disagreement between our hydrolysis rate constant and the values reported for the hydrolysis of disulfate ion. With the close similarity between our results for the reaction intermediate and published data for the disulfate ion, we conclude that the intermediate is disulfate ion. The formation appears to occur by a different process than those generally used to produce disulfate, in which either gaseous SO_3 is added to sulfate solutions or a bisulfate salt is heated.

Previously Connick and co-workers (*7*) found evidence for an intermediate in the oxygen-bisulfite reaction at lower reactant concentrations (about $10^{-4}M$ in O_2 and $10^{-2}M$ in HSO_3^-). Their first-order rate constant for decomposition at 25°C of 0.0128 sec^{-1} agrees well with that reported here.

In contrast to the case for the decomposition of the intermediate, its formation appears to show a complicated dependence on reactant concentrations and trace amounts of metal ions such as Fe^{2+}/Fe^{3+} and Mn^{2+}. When the initial *p*H of the reaction mixture was increased, the relative amount of intermediate formed decreased, which suggested the presence of another reaction channel. Since the solution was unbuffered and the *p*H probe was downstream of the mixer, the initial *p*H at which this occurred is somewhat uncertain. It appears to occur at near *p*H 5. The fraction of S(IV) in the form of SO_3^{2-} increased rapidly as the *p*H increased. It may be that a second, faster oxidation reaction channel proceeds through the involvement of SO_3^{2-} rather than HSO_3^-. The observed intermediate does not seem to be involved in this process.

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Cell-Autonomous Determination of Cell-Type Choice in *Dictyostelium* Development by Cell-Cycle Phase

RICHARD H. GOMER AND RICHARD A. FIRTEL*

The developmental fate of individual cells has been examined in a system that allows *Dictyostelium discoideum* cells to differentiate in the absence of aggregation. The results show that the propensity of single amoebae to differentiate into either prespore or prestalk cells occurs by a cell-autonomous mechanism dependent on the cell's position in the cell cycle at the initiation of development. Cells that divide between ~1 1/2 hours before and ~40 minutes after the differentiation-inducing starvation become prestalk, whereas cells dividing at other times become prespore cells. These results suggest mechanisms by which an initial proportioning of the two cell types within the aggregate is achieved.

UNDERSTANDING THE MECHANISMS controlling cell-type and tissue differentiation is a central issue in developmental biology. To study the molecular basis of differentiation, we have chosen to use the eukaryote *Dictyostelium discoideum*. This organism has a relatively simple pattern of tissue differentiation and is

Department of Biology, Center for Molecular Genetics, M-034, University of California, San Diego, La Jolla, CA 92093.

*To whom correspondence should be addressed.

highly manipulatable, thus affording one a relatively simple system in which to examine cellular differentiation (*1*). Development is initiated by starvation. After establishment of an adenosine 3′,5′-monophosphate (cAMP) relay system, approximately 10^5 individual amoebae aggregate to form a multicellular organism that eventually differentiates into a fruiting body containing approximately 80% spores and 20% stalk cells. In an intermediate multicellular stage, the organism forms a migrating slug or pseudoplasmodium in which the precursors to the stalk and spore cells (prestalk and prespore cells, respectively) are spatially organized within the migrating slug. The anterior ~15 to 20% is comprised of the prestalk cells while the posterior 75 to 80% is comprised of prespore cells and a small percentage of cells denoted anterior-like cells, which have properties similar to the anterior prestalk cells (*2*). This spatial pattern is maintained in slugs ranging in size over three orders of magnitude.

A number of mechanisms have been proposed for the generation of two cell types in *Dictyostelium* including the choice of cell type being determined by a cell's position within the aggregate (*3*), or by other, nonpositional factors. The hypothesis that positional factors determine cell-type choice is supported by the observation that a dissected anterior tip will form a new, smaller slug in which prespore cells are formed by conversion of prestalk cells (*4*). Interconversion can also be seen with purified populations of prestalk and prespore cells (*5*).

Other experiments support the hypothesis that cell-type choice is, at least in part, position-independent. First, prestalk and prespore cells initially appear intermixed within the developing aggregate (*6, 7*). Second, experiments have been performed in which cells taken at a particular position in the cell cycle or grown under specific metabolic conditions are marked and mixed with other cells to examine their developmental fate. Results showed that cells taken at or immediately after the S phase (there is little or no G_1 phase in *Dictyostelium*) are preferentially localized in the anterior region whereas cells taken late in G_2 are preferentially found in the posterior (*8*). In other studies, cells grown on glucose are preferentially localized in the prespore region, whereas cells grown in the absence of glucose are preferentially localized in the prestalk region (*9*). In such studies, however, it is difficult to distinguish differentiation of the cells from sorting mechanisms based on differential binding, relative size of a cell, or other parameters.

To study prestalk and prespore gene regulation, we and others have cloned a number of genes preferentially expressed in either prestalk or prespore cells (*10*). Using the λgt11 expression vector system, we have made antibodies directed against portions of the protein products of two of these genes and have shown these antibodies to be specific markers of prestalk and prespore cells, respectively (*7*). Using these antibodies to examine tissue morphogenesis, we have observed that prestalk cells initially appear scattered through the aggregate and then presumably migrate to the tip and periphery of the later stage aggregates (*7*). Similar results have been obtained with other markers (*6*). Moreover, using conditions that allow us to examine cell type–specific gene regulation in the absence of aggregation, we have shown that both prestalk and prespore differentiation can occur in populations of starved *Dictyostelium* cells in the absence of aggregate formation (*7, 10, 11*) and that the ratio of prestalk and prespore cells is similar to that in the developing multicellular organism. One such condition involves starving cells in very low density monolayer culture in buffer medium from cells that have been allowed to develop at high density (*11*). This medium contains a developmentally regulated factor (conditioned medium factor) necessary to allow the induction of prespore and prestalk genes by cAMP. Extracellular cAMP is then added 6 hours after starvation to induce prespore and prestalk gene expression (*11*). The cAMP appears to activate the cell surface cAMP receptor which, in turn, activates a signal transduction pathway that does not involve activating adenyl cyclase (*12*). Under these conditions, prespore and prestalk gene expression is temporally regulated in a manner similar to that seen in cells developing normally. Since this phenomenon occurs with wild-type cells (*11*) rather than only with a specific mutant, we feel that regulation of gene expression and cell-type choice in the conditioned medium system is analogous to normal development.

We have taken advantage of these conditions to look for factors such as time and extent of cell-cell contact, lineage relationships, or phase of the cell cycle at the time of starvation that might influence cell-type choice during the starvation-induced differentiation. To examine this, *Dictyostelium* cells were grown on a marked slide in growth medium at very low densities in submerged monolayer culture while being videotaped through a microscope at low power (Fig. 1). After 6 to 24 hours (approximately one to three generations), the growth medium was changed to conditioned medium, which induced starvation. Cyclic AMP was added to a final concentration of 300 μ*M* 6 hours after starvation to induce prestalk- and prespore-specific genes, and the cells were fixed in situ 20 hours after starvation. Continued videotaping of the cells was done at 15 frames per minute

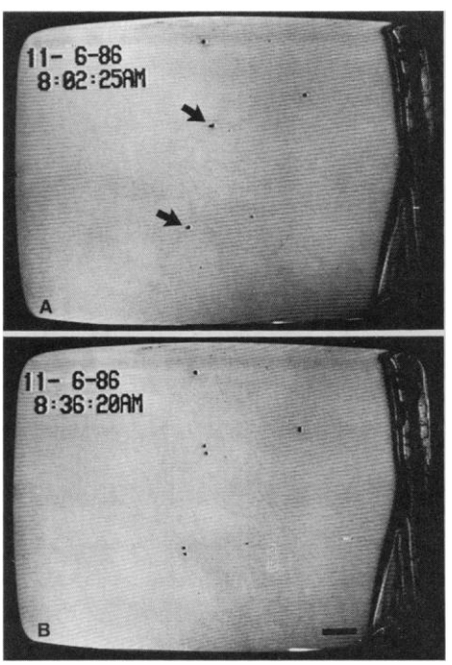

Fig. 1. Two frames of a time-lapse videotape. Axenically grown KAx-3 cells were used for all experiments. Unless otherwise stated, all operations were at room temperature. For the time-lapse studies, an "X" (partially seen at the right of the frame) and the date of the experiment were gently scribed by means of a diamond-tip scriber onto a 1.5 by 1 inch fragment of a Goldseal #3050 (Becton Dickinson) glass microscope slide. The slide was washed with Liquinox soap and very hot water, rinsed with deionized water, and dried with paper towels. The scribed upper surface of the slide was covered with polylysine (50 μg/ml) (Sigma) for 20 to 30 minutes, rinsed with deionized water, and allowed to air-dry for 5 to 15 minutes. Mid-log–phase KAx-3 cells were diluted in HL-5 growth medium containing antibiotics (*11*) to 2.5×10^3 to 4×10^3 cells per milliliter and overlaid on the slide in a tissue culture dish to a depth of 2 mm on the slide. For changing the medium or fixing the cells in 95% ethanol (see text), a dual-channel peristaltic pump was used on the microscope stage to pump in the desired material and simultaneously to pump out waste. The pump speed was ~1 ml/min for both operations. Cells were viewed through a ×4 objective on a Nikon Fx microscope with ×2 internal magnification. The area viewed and recorded was 1.2 by 0.9 mm. The light for transmitted phase-contrast illumination was from a Xenophot HLX 64-625 12-V 100-W lamp (Osram) operated at 3.3 V passed through a 540-nm interference filter (~60 nm FWHM). The videotape system consisted of a TC1005/01 (Javelin) television camera, a GYYR time-lapse recorder, and a Javelin BMW9 television monitor. Cells marked with arrows in (A) have divided in (B). Scale bar in (B) is 100 μm.

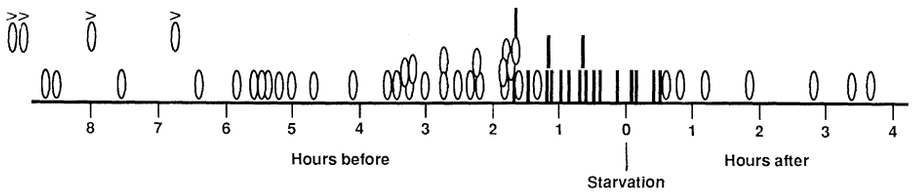

Fig. 2. Time of last division of prespore and prestalk cells. By means of the experimental conditions described in Fig. 1, cells were videotaped in growth medium and during differentiation. Twenty hours after starvation cells were fixed and stained for the prestalk marker pst-cathepsin or the prespore marker beejin by indirect immunofluorescence (*7*). Ovals, prespore cells; bars, prestalk cells; >, a division occurring an unknown time before the time shown.

throughout the entire process. The fixed slides were then stained for prestalk and prespore cells by immunofluorescence (*7, 12–14*). Using this procedure and examining the videotape, we were able to determine which cell was the sister of a given cell, what the fate of the sister cell was, which cell or cells a given cell might have touched, the motility of a cell during differentiation, and when a given cell last divided.

As expected from previous results (*7, 10, 11*), examination of 17 experiments (~570 hours of videotaping) showed that some cells differentiated into either prespore or prestalk cells without touching any other cell after their final division. Observations of vegetative cells for ~24 hours showed that all observed cells divided during this period, indicating that there are no postmitotic vegetative cells. Using double-label indirect immunofluorescence (*7, 13*) to stain cells induced to differentiate by the conditioned medium method and also normally developing cells from disaggregated slugs, we never found a cell that stained with both antibodies to prespore and to prestalk cells (anti-prespore and anti-prestalk). The fraction of cells that was positive for the prestalk or the prespore marker was variable and had a maximum of approximately 50% (*7, 12, 15*). When whole slugs were stained, we observed a nonstaining region lying between the anterior, anti-prestalk staining region at the tip and the anti-prespore staining region located in the posterior (*7*). Examination of cells from dissociated slugs (*16*) shows that 77 out of 164 = 47 ± 4% (mean ± standard deviation for a binomial distribution) of the cells stained with anti-prespore, 35 out of 293 = 12 ± 2% stained with the anti-prestalk and 41 ± 4% were null (stained by neither antibody). It is possible that another population of cells expresses a different set of markers or that some anterior prestalk cells or posterior cells do not stain with our antibodies. We do not know about the staining characteristics of the class of cells called anterior-like cells that are present, scattered within the posterior region (*2*). The number of null cells seen in our low-density experiments is roughly comparable to the situation observed from an examination of cells during normal development.

A clear pattern emerged when differentiated cells were traced backward in time until their last division. The final state of differentiation (when prestalk or prespore antigens are expressed) in relation to the time of last cell division and the time of starvation for single cells whose movements were followed from videotape are shown (Fig. 2). These cells, from the time of their last division until fixation 20 hours after starvation, either never touched another cell or touched another cell for <5 minutes. Cells that formed aggregates or touched another cell for longer periods of time were not included in this analysis. The time of last division for the cells that stain with the anti-prestalk always fell between 1 hour and 40 minutes before starvation and 40 minutes after starvation (Fig. 2). This corresponds with S and early G_2 phase (*8*). Cells that were stained by the anti-prespore last divided between >8 hours and 1 hour and 20 minutes before starvation and also between 40 minutes and 3 hours and 30 minutes after starvation. The latter group of cells were the progeny of cells that divided several hours before starvation. None of the cells that stained with the anti-prespore had their final cell division between

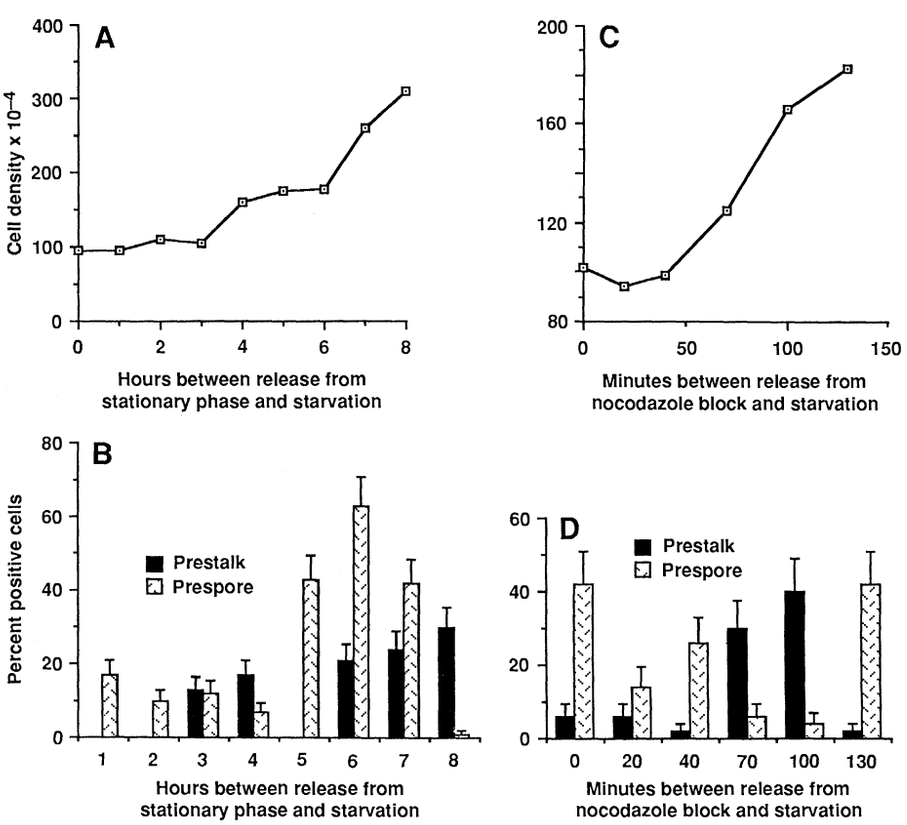

Fig. 3. Expression of prespore and prestalk antigens by synchronous populations of KAx-3 cells starved at different phases of the cell cycle. (**A** and **B**) Stationary phase cells (100 μl) from a single population of cells were added to 900-μl growth medium every hour for 8 hours. One hour after the last dilutions cell densities were determined (note that the cells approximately double between 3 and 4 hours and again between 6 and 8 hours), and the cells were pelleted by centrifugation at 1500*g* for 30 seconds, resuspended in MES-PDF (*10, 11*), washed twice again in MES-PDF, and diluted into 200 μl of conditioned medium in the well of a Miles eight-well microslide so as to give 2×10^3 cells per square centimeter when the cells settled. Six hours after starvation cAMP was added to the wells to 300 μ*M*. Twenty hours after starvation the slides were fixed and stained for prestalk and prespore cells as described previously (*7, 12, 13*). (**C** and **D**) Stationary phase KAx-3 (100 μl) were added to 900 μl of growth medium containing 10 μg per milliliter of nocodazole (*20*). Five hours after the dilution, cells were collected by centrifugation, washed twice in growth medium, and resuspended in growth medium. Starvation in conditioned medium was performed at the times indicated as described in (A) and (B). Bars in (B) and (D) indicate standard deviations.

1 hour and 20 minutes before starvation and 40 minutes after starvation, the time when most of the cells that become prestalk cells had their final division. There was no observable difference in staining pattern or intensity between the prespore cells whose last division was before starvation and those whose last division was after starvation. The prestalk cells showed some heterogeneity: those whose last division was ~1 hour or more before starvation tended to stain less intensely than those whose last division was 0 to 30 minutes before starvation. Cells that became either prespore or prestalk generally showed lower motility after starvation than cells that did not differentiate, but there was no obvious difference in the motility of the two cell types.

The fate of the sister cells of differentiated cells was also examined. In our analysis of isolated cells, only one of the two differentiated into either a prespore or a prestalk cell (only one stained with either the prespore or the prestalk antibody). There was one exception, a cell that divided 1 hour and 40 minutes before the time of starvation. Interestingly, one sister became a prestalk cell and the other became a prespore cell.

The observed pattern of regulation of choice of cell type predicts that synchronized cell populations, starved in conditioned medium and given cAMP, would differentiate into either prestalk but not prespore, or prespore but not prestalk cells depending on the cell-cycle phase at the time of starvation. We therefore examined the developmental fate of cells taken from a synchronized population at various times in the cell cycle. Synchronized cells were obtained by dilution of stationary phase cells into growth medium (*8, 17*) (Fig. 3, A and B). After a lag time (*8*), the density of the synchronized population increased, stabilized, and then increased again within an 8- to 9-hour period (Fig. 3A) as has been reported previously (*8, 17*). Cells from the various times after dilution were starved in conditioned medium to induce development (Fig. 3B). In the populations starved at the times when cells were not dividing (1, 2, and 5 hours after release from stationary phase) no prestalk cells, as determined by antibody staining, were seen. These populations did, however, give rise to prespore cells. The populations starved when cells were dividing or had just divided (3 to 4 and 7 to 8 hours after release from stationary phase) gave rise to prestalk cells and generally lower levels of prespore cells. Similar results were obtained by synchronizing cells with release from a nocodazole block (10 μg/ml) (Fig. 3, C and D). Again, we observed that cells starved while the cells were dividing and shortly after cell division gave rise to predominantly prestalk cells; cells starved at other times gave rise to predominantly prespore cells. The time after release from stationary culture that gave rise to prestalk cells corresponded to S and early G_2 (there is little or no G_1) in agreement with our observations from the videotape experiments. These data correlate with previous experiments in which cells from synchronized cell populations, when labeled by covalent addition of dye to their membrane proteins, were shown to be present in different subregions of migrating slugs (*8*).

In our experiments, the relative ratio of cells that stain with anti-prestalk or anti-prespore was approximately the same as that observed in staining of cells from whole slugs and the previously reported ratios of the two cell types. Cells taken from approximately a 2-hour period of the ~8-hour cell cycle have a propensity to differentiate into prestalk while the remainder differentiate into prespore cells. Since only one of each pair of sister cells differentiates in our system, this mechanism would predict that ~12.5% of the cells would be prestalk, a figure within the error of the observed percentage of prestalk cells in a migrating slug. Although the correlation is intriguing, it is not known if the cell-cycle mechanism alone determines prestalk cell fate.

Our results suggest that the signal determining the initial ratio of prestalk and prespore cells may depend on cell cycle–related events and that this occurs by a cell-autonomous mechanism. The videotape observations of single cells and the use of two different methods to synchronize populations of cells indicate that cells starved when in S or early G_2 phase differentiate into prestalk cells and that cells starved at other phases differentiate into prespore cells. This agrees with previous observations that cells starved in these phases sort to anterior and posterior regions of slugs, respectively (*8*). Previous results of ours and others (*6, 7, 9–11*) would then suggest that cells sort to their appropriate positions, possibly by differential cell cohesion or relative rates of mobility (*18*). In addition, it has been shown recently that cells starved in S phase have a greater propensity than cells starved in other phases to initiate aggregation centers (*17*). This corresponds to the cells that we observe to differentiate into prestalk cells, suggesting that cells destined to become prestalk cells might also initiate the cAMP-relay and -signaling system used during aggregation. Factors that have been observed to affect prespore to prestalk cell ratios such as adenosine, ammonia, differentiation-inducing factor (DIF), and growth on glucose (*9, 19*) might then either modulate the initial cell cycle–dependent choice or the subsequent maintenance within the aggregate itself.

The molecular mechanism of the cell cycle–dependent cell-type choice mechanism remains to be elucidated. The mechanism conceivably could involve a substance that is synthesized at one phase of the cell cycle. The presence or absence of the substance or a certain level of the substance would cause cells to become, for instance, prestalk. The stability of the substance could modulate cell-type ratio. Further elucidation is required of additional mechanisms, possibly involving cell-cell contact, that cause some of the cells that we observe to be null in the low-density conditioned medium experiments to differentiate during normal development.

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14. Changing the medium and fixing the cells without detaching them required, after trial and error development, the use of glass coated with polylysine as the substrate and a peristaltic pump to change the medium. Also by trial and error, we optimized the way to mark the substrate, the videotaping speed, the microscope magnification, and the initial cell density. Since cells after starvation adhere to one another, very low cell densities were required, and thus typically a final maximum total of only 15 cells per 1.2 by 0.9 mm viewed area were present in any one experiment.
15. In establishing conditions, we found that a variable fraction of the cells that divide between >8 hours before and >3 hours after starvation have neither sister cell differentiate. We have optimized conditions to obtain as many pairs of sister cells as possible to contain one differentiated cell (>80%). First, cells should be grown in monolayer culture in HL-5 growth medium for as short a time as possible. Second, the efficacy of the conditioned medium is greatly improved by using MES-PDF [8.2 m*M* 2(*N*-morpholino)ethanesulfonic acid, 20 m*M* KCl,

and 0.6 m*M* $MgCl_2$], *p*H ~6.0, to develop for the conditioned medium. Acidifying conditioned medium to *p*H 6.0 after conditioning has no effect. We also found no effect or a slightly deleterious effect from using medium conditioned by a threefold greater density of cells than described (*11*), changing the conditioned medium 1, 2, 4, or 6 hours after starvation, starving initially in MES-PDF and then changing to conditioned medium 1, 2, 4, or 6 hours after starvation, adding histidine [S. G. Bradley, M. Sussman, H. L. Ennis, *J. Protozool.* **3**, 33 (1956)] or bovine serum albumin to the conditioned medium, or adding 0.1 to 3 m*M* NH_4Cl at the time of cAMP addition. Higher levels of NH_4Cl appeared to inhibit prespore and prestalk gene expression.
16. Slugs of strain KAx-3 at 24 hours migrating toward a light source were developed as described (*10*).
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21. We thank D. Forbes, M. Price, W. Loomis, and J. Newport for loan of equipment, helpful discussions, and critical reading of the manuscript. R.H.G. was supported by a USPHS postdoctoral fellowship and by an American Cancer Society (California chapter) senior fellowship. This work was supported by grant USPHS GM24279 to R.A.F.

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Clonal Gene Therapy: Transplanted Mouse Fibroblast Clones Express Human α1-Antitrypsin Gene in Vivo

ROBERT I. GARVER, JR., ANNA CHYTIL, MICHAEL COURTNEY, RONALD G. CRYSTAL

A retroviral vector was used to insert human α1-antitrypsin (α1AT) complementary DNA into the genome of mouse fibroblasts to create a clonal population of mouse fibroblasts secreting human α1AT. After demonstrating that this clone of fibroblasts produced α1AT after more than 100 population doublings in the absence of selection pressure, the clone was transplanted into the peritoneal cavities of nude mice. When the animals were evaluated 4 weeks later, human α1AT was detected in both sera and the epithelial surface of the lungs. The transplanted clone of fibroblasts could be recovered from the peritoneal cavities of those mice and demonstrated to still be producing human α1AT. Thus, even after removal of selective pressure, a single clone of retroviral vector–infected cells that expressed an exogenous gene in vitro, continued to do so in vivo, and when recovered, continued to produce the product of the exogenous gene.

THE CHARACTERIZATION OF A growing number of heritable disorders at the gene and protein level has led to the conceptualization of therapies that are directly aimed at correcting the molecular defects. One example is the hereditary form of emphysema associated with α1-antitrypsin (α1AT) deficiency. In this disorder, inheritance of "deficiency" or "null" α1AT alleles from both parents results in low α1AT serum levels (*1, 2*). When this occurs, α1AT, the major inhibitor of neutrophil elastase in the human body, is not present in sufficient amounts to protect the lower respiratory tract from the destructive potential of neutrophil elastase (*3–5*). As a result, there is unimpeded destruction of the lung parenchyma, resulting in emphysema by the third to fourth decades (*1*). Therapies for preventing or slowing the development of emphysema in this disorder are directed toward increasing the serum and lung levels of α1AT (*6, 7*).

The phenotypic abnormality (the α1AT deficiency state) could theoretically be corrected by the addition of a normal human α1AT gene to the genome of cells of deficient individuals, such that the target cells would express the α1AT gene and secrete α1AT. Current concepts of gene therapy have focused on the use of retroviral vectors, which utilize two long terminal repeat (LTR) regions for integration of nonviral genes between them, to permanently integrate the α1AT gene into the genome of the target mammalian cells (*8*). In this regard, most interest has focused on bone marrow precursors as the target cell (*9*). Genetic disorders would be corrected by the addition of a normal gene to autologous bone marrow by in vitro infection, followed by transplantation of the infected cells into the host (*10*). By means of the N2 vector, a retroviral construct derived from an incomplete Moloney murine leukemia virus (*11*), human α1AT complementary DNA (cDNA) has been successfully integrated into the genome of mouse fibroblasts (*12*). These fibroblasts secrete a glycosylated human α1AT molecule that functions as an inhibitor of human neutrophil elastase and has a half-life in serum similar to α1AT purified from normal human plasma (*12*). We hypothesized that such cells could potentially be transplanted into the peritoneum of a recipient with the result that the integrated α1AT gene in the transplanted cells would direct the synthesis of α1AT. The α1AT would be secreted into the peritoneal cavity, diffuse into the blood, and be carried to organs throughout the body, including the lung. If possible, such an approach would have several advantages over the strategy of using autologous bone marrow cells as the target for in vitro introduction of a foreign gene. First, the process of retrovirus infection results in integration of the foreign gene in random sites in the target cell genome (*8*), and thus, the population of infected bone marrow cells to be transplanted would contain a mixture of several clonal populations, in each of which native genes might have been activated or inactivated in a deleterious fashion (*13–15*). In contrast, a monoclonal population of cells containing the integrated foreign gene could be characterized extensively in vitro prior to transplantation. Second, the level of gene

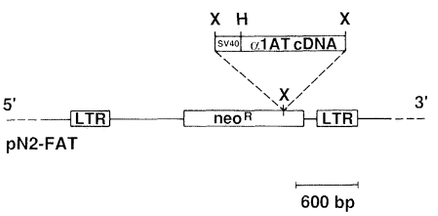

Fig. 1. Retroviral vector used to integrate the human α1AT cDNA into mouse fibroblasts. Vector pN2-FAT contained the SV40 early promoter in tandem with the forward orientation of the α1AT cDNA. The human α1AT cDNA, derived from the human α1AT-containing plasmid pTG 603 (*19*), was modified by standard techniques to create a 5′ Hind III ("H") site and 3′ Xho I ("X") site. Sequence analysis confirmed the fidelity of the α1AT protein coding region. The SV40 early promoter, with a 5′ Xho I site and 3′ Hind III site, was derived from pLSDL (provided by A. D. Miller, Fred Hutchinson Cancer Center) (*20*). The α1AT cDNA and SV40 early promoter fragments were cut from their respective plasmids and simultaneously ligated into the Xho I site of the retroviral vector N2 (provided by E. Gilboa, Memorial Sloan-Kettering Cancer Center) containing a 5′ and 3′ long terminal repeat (LTR) (*11, 12*). The orientation of the construct was established by restriction mapping. This vector also contains the gene encoding neomycin resistance, permitting infected cells to be identified by their ability to survive in medium containing the neomycin analog, G418 (*21*).

Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892 and Transgene SA, 11 Rue de Molsheim, 67000 Strasbourg, France.