Biological and molecular correlates between induced dedifferentiation and spore germination in *Dictyostelium*

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Summary

When developing cultures of *Dictyostelium discoideum* are disaggregated at any time prior to cell wall formation and challenged to reinitiate development, amoebae will progress through the original sequence of morphogenetic stages, but the second time through they will do so in roughly one-tenth the original time, a process known as ‘rapid recapitulation’. However, if disaggregated cells are suspended in nutrient medium, they enter a program of dedifferentiation during which they lose the capacity to rapidly recapitulate after an 80 minute lag period in a process known as ‘erasure’. Here we show that cells that have completed the morphogenetic program and emerge from spore coats in the process of germination have also erased. In addition, the germination-specific 270 gene family is expressed during induced dedifferentiation in a unique fashion, and a germination-defective mutant exhibits a dramatic delay in erasure without concomitant defects in the program of gene regulation accompanying induced dedifferentiation. These results suggest for the first time that induced dedifferentiation and spore germination share some common processes in converting cells from a developmental to vegetative state.

Key words: *Dictyostelium discoideum*, dedifferentiation, germination, gene regulation.

Introduction

When developing cultures of the social amoeba *Dictyostelium discoideum* are disaggregated and development immediately reinitiated, cells will recapitulate the developmental stages that they had originally progressed through but, the second time around, they do so in only a fraction of the original time (Loomis and Sussman, 1966; Newell et al., 1971; Soll and Waddell, 1975). This capacity to rapidly recapitulate morphogenesis reflects the reutilization of preexisting machinery acquired during the initial, slow program of development (Soll, 1990). However, if developing cultures are disaggregated and resuspended in nutrient medium, the individual amoebae will retain the capacity to rapidly recapitulate morphogenesis for roughly 80 minutes; then, in a short time period, the entire cell population will revert to the slow developmental timing of naive vegetative cells, a process referred to as ‘erasure’ (Soll and Waddell, 1975). Disaggregation and suspension of developing cells in nutrient medium initiates a complex program of dedifferentiation which continues over a 300 minute period and includes precisely timed loss of developmentally acquired mRNAs (Finney et al., 1987; Kraft et al., 1989), deactivation of development-specific gene transcription (Chandrasekhar et al., 1990), loss of developmentally acquired proteins (Finney et al., 1987), and loss of developmentally acquired functions (Finney et al., 1979; Finney et al., 1981, 1983; Varnum and Soll, 1981). When developing cultures of the dedifferentiation mutant HI4 are disaggregated and resuspended in nutrient medium, the cells abnormally retain the capacity to reaggregate rapidly and a number of developmentally acquired mRNAs, both of which are lost at prescribed times in the wild-type dedifferentiation program (Kraft et al., 1989). HI4 does, however, normally lose chemotaxis-associated functions (Soll et al., 1984; Hedberg and Soll, 1984; Finney et al., 1983) and turns off transcription of some development-specific genes (Chandrasekhar et al., 1990) at the prescribed times, demonstrating that the dedifferentiation program involves a number of parallel pathways only one of which is defective in the HI4 mutant (Soll and Finney, 1987).

Since the discovery of the erasure process (Soll and Waddell, 1975), there has been an interest in its relationship with spore germination since both processes involve conversion of cells from a developmental to a vegetative state (Waddell and Soll, 1977). To investigate this relationship, we have tested whether freshly germinated cells have erased, whether germination-specific genes (Kelly et al., 1983; Giorda et al., 1990) are transcribed during induced dedifferentiation, and whether the germination-defective mutant HE1 (Ennis and Sussman, 1975; Shaw et al., 1986) is defective in erasure and dedifferentiation. The results
suggest that a relationship indeed exists between induced dedifferentiation and germination.

Materials and methods

Growth, development and dedifferentiation

Spores of *Dictyostelium discoideum* wild-type strain B and the germination-defective mutant HE1, obtained by nitrosoguanidine mutagenesis of strain B (Ennis and Sussman, 1975), were stored in a desiccated form on silica gel (Sussman, 1966). Spores were mixed with *Klebsiella aerogenes*, and emerged amoebae grown to mid-log phase according to the methods of Sussman (1987). Amoebae were then washed free of bacteria and development was initiated on black Eaton-Dikeman filters according to the methods of Soll (1987). To initiate dedifferentiation, cells at the loose aggregate stage were disaggregated and resuspended in either LPS plus 2% (w/v) dextrose or the axenic medium HL-5 (Cocucci and Sussman, 1970) containing penicillin (100 units per ml) and streptomycin (100 µg per ml) to discourage residual bacterial growth, according to methods previously described (Soll, 1987). Erasure was monitored by removing cells from an erasure culture, plateing on development filters, and monitoring the times to the early developmental stages (Soll, 1987). The T50 of erasure is defined as the time in erasure medium when the cell population has reverted to half of the time that it takes naive log phase cells to develop to the ripple stage.

Germination

Spores were collected, washed extensively in LPS and stored at high density in 20% glycerol (v/v in water) at −20°C. As required, spores were thawed at 37°C, washed twice in 20 mM phosphate buffer, pH 6.2 (PB), resuspended at 3-8×10^7 spores/ml in PB containing 20% dimethyl sulfoxide (v/v) and activated by rotation at 100 revs/min for 45 min at 22°C. Activated spores were washed twice in PB and resuspended at 1-3×10^7/ml in axenic medium supplemented with antibiotics. Germination was monitored microscopically under dark-field illumination (Ennis and Sussman, 1975; Shaw et al., 1986).

RNA isolation and northern blot hybridization

A sample of 5-10×10^7 amoebae were removed at each time point from an erasure culture, pelleted and frozen at −70°C. Pellets were thawed and RNA extracted by an SDS-phenol procedure (Anderson and Soll, 1984). Samples containing 12 µg of RNA per time point were loaded on a 1.25% agarose-formaldehyde gel, electrophoresed and processed for transfer according to methods previously described (Finney et al., 1987). RNAs were transferred in 10×SSC to Zetabind membranes (Cuno, Meriden, CT). RNAs were immobilized on membranes by UV irradiation, and prehybridized in a solution containing 1% BSA, 7% SDS, 0.5 M sodium phosphate buffer (pH 6.8) and 1 mM EDTA at 65°C for 1 h (Church and Gilbert, 1984). Hybridization was performed in the same buffer for 16 h at 65°C, and the filters were then washed twice in a solution containing 0.5% BSA, 5% SDS, 80 mM sodium phosphate buffer (pH 6.8), and 1 mM EDTA, and twice in a solution containing 1% SDS, 80 mM sodium phosphate buffer (pH 6.8), and 1 mM EDTA. Washes were performed for 15 min at 60°C. Membranes were then rinsed in H2O and exposed to Kodak XAR-5 film with an intensifying screen. To strip a membrane, the filter was incubated in a solution containing 2 mM Tris-HCl (pH 7.5), 2 mM EDTA and 0.1% SDS for 3 h at 80°C.

DNA probes

The plasmids containing the cDNA inserts of CP2 (Mehdy et al., 1983), gp80 (Noegel et al., 1986), D11 and D18 (Barklis and Lodish, 1983; Chisholm et al., 1984) were gifts from Dr Richard Firtel (University of California, San Diego), Dr Gunther Gerisch (Max-Planck Institute, Martinsried, Germany), Dr William Loomis (UCSD) and Dr Rex Chisholm (Northwestern University), respectively. The 270-6 cDNA was an insert in bacteriophage Lambda (Giorda et al., 1990). All probes were labelled by nick-translation (Rigby et al., 1977) without excision of inserts, purified through Sephadex G-50 columns (5 Prime-3 Prime, Boulder, CO) and used in hybridization reactions.

Results

Freshly germinated cells have erased

To characterize erasure in parental strain B (wild-type), cells at the loose aggregate stage were disaggregated, resuspended in nutrient medium, and then removed at 30 min intervals and dispersed on fresh development filters (Soll, 1987). The time for the onset of aggregation, the ripple stage, was then measured. Cells removed from the erasure culture during the first 60 min of incubation rippled within 30 min, roughly one-eighth of the time that it takes naive log phase cells plated on fresh development filters for the first time (Fig. 1A). However, cells removed from the erasure culture at 90 min rippled in 3.5 h, and cells removed at 120 min rippled in 4 hours, roughly the same time as naive log phase cells (Fig. 1A). Cells removed after 120
min continued to ripple with the same slow timing. In this example, the T50 of erasure was 73 min. The loss of the capacity to rapidly reaggregate is referred to as ‘erasure’, and in strain B, it occurs rapidly and completely between 60 and 120 min, in a fashion similar to that previously demonstrated for the axenic strain Ax3 (Soll and Waddell, 1975; Finney et al., 1979).

To test whether freshly germinated cells of strain B had retained or had lost the capacity to rapidly reaggregate, activated spores were diluted into nutrient medium to induce germination (Shaw et al., 1986). The first amoebae emerged from spore coats after 1 h. By 2 h, 5% of the spore population had germinated, by 3.5 h, 50% had germinated, and by 5 h, 80% had germinated. Amoebae were collected at 3.5 and 5 h, dispersed on development filters, and the time to the ripple stage measured. In two separate experiments, cells collected at 50% emergence began to ripple at 5.75 h and 6 h, respectively, and cells collected at 80% emergence began to ripple at 5.75 h in both experiments, roughly the same time as control (Veg) cells from mid-log phase cultures (Table 1). In both experiments, the onset of aggregation was temporally homogeneous in freshly germinated cultures, with no discernable minority of rapidly aggregating amoebae. In addition, no fruiting bodies were present at 16 h post-plating, confirming the absence of rapidly recapitulating cells in the population. To test our resolving power for identifying a minor population of rapidly aggregating amoebae in a majority of slow aggregating amoebae, mixing experiments were performed in which vegetative (slow aggregating) and loose aggregate stage (rapidly aggregating) amoebae were mixed at ratios of 50:50, 75:25, and 90:10. In all three cases, rapid and slow aggregating populations were identifiable, at roughly the proportions expected of the original mixes (data not shown). Therefore, our resolving power was good enough to identify a proportion of 10% rapidly aggregating amoebae mixed with 90% slow aggregating amoebae. Since 10% of the 5 hour sample of freshly germinated amoebae emerged during the final 24 min of incubation, their post-germination ages spanned 0 to 24 min. Since no minor population of rapidly aggregating amoebae was evident in the 5 h samples of freshly germinated amoebae in two independent experiments (Expts 1 and 2, Table 1), we conclude that erasure must have occurred sometime during sporulation, germination, or a maximum of 24 min following emergence.

However, the possibility still exists that either spores or freshly emerged amoebae secrete a factor that delays the onset of aggregation, and that the slow timing of freshly emerged amoebae therefore does not really represent erasure. To test this possibility, amoebae at the loose aggregate stage (rapid aggregating) were mixed with either ungerminated spores (no aggregation) or freshly emerged amoebae (slow aggregating) at a ratio of 50:50, and plated on fresh development filters. In the former case, the loose aggregate amoebae had rippled by 0.5 h (Table 1), demonstrating that spores in the mixture did not secrete substances that affected the capacity to rapidly reaggregate. In the latter case, the population exhibited a mixture of fast (0.5 h) and slow (6 h) aggregating cells at a ratio of roughly 50:50 (Table 1), demonstrating that the freshly emerged amoebae in the mixture did not secrete substances that affected the capacity to reaggregate rapidly.

### Table 1. The time for the onset of aggregation in populations of freshly germinated amoebae*

<table>
<thead>
<tr>
<th>Origin of cells</th>
<th>Expt. 1 (h)</th>
<th>Expt. 2 (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 100% Vegetative (Veg) amoebae from mid-log phase culture</td>
<td>5.00</td>
<td>6.25</td>
</tr>
<tr>
<td>2. 100% Loose aggregate stage (LA) amoebae</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>3. 100% Germinated (Ger) amoebae (harvested at 50% population emergence)</td>
<td>5.75</td>
<td>6.00</td>
</tr>
<tr>
<td>4. 100% Ger amoebae (harvested at 80% population emergence)</td>
<td>5.75</td>
<td>5.75</td>
</tr>
<tr>
<td>5. 50% Veg amoebae - 50% spores (Sp)</td>
<td>----</td>
<td>6.50</td>
</tr>
<tr>
<td>6. 50% LA amoebae - 50% Sp</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>7. 50% LA amoebae - 50% Ger amoebae (Ger amoebae harvested at 80% emergence)</td>
<td>----</td>
<td>0.5/6.0</td>
</tr>
</tbody>
</table>

*In experiments 1 and 2, 50% emergence had occurred at 3.5 and 4.5 h, respectively, and 80% emergence had occurred at 5.0 and 5.5 h, respectively. Experiments 1 and 2 employed different clonal isolates of strain B. In all experiments, duplicate samples were analyzed: in all cases, the duplicate samples were similar. Dashed lines denote the absence of that particular combination in experiment 1.

A germination-specific family of genes is activated and regulated in a unique fashion during dedifferentiation

The preceding results suggested that erasure may occur during germination, and that dedifferentiation induced during the forward program of development and germination may share common molecular processes. To investigate this possibility, we tested whether genes specifically expressed during germination were also expressed during induced dedifferentiation. A 2.9 kb and a 2.0 kb transcript, encoded respectively by the 270-6 and 270-11 members of the 270-gene family, are transiently expressed during germination, and that dedifferentiation induced in the mixture did not secrete substances that affected the capacity to rapidly reaggregate. In the latter case, the population exhibited a mixture of fast (0.5 h) and slow (6 h) aggregating cells at a ratio of roughly 50:50 (Table 1), demonstrating that the freshly emerged amoebae in the mixture did not secrete substances that affected the capacity to reaggregate rapidly.

As expected, vegetative and loose aggregate (0 min erasure) cells contained negligible levels of complementary transcripts (Fig. 2A). However, by 60 min in erasure medium, at least five transcripts complementary to the 270-6 member of the 270-gene family, are transiently expressed during germination, but not during vegetative growth or morphogenesis (Kelly et al., 1983; Giorda et al., 1990; Ennis et al., 1991). To test whether these germination-specific genes are also transcribed during induced dedifferentiation, strain B cells at the loose aggregate stage were disaggregated and inoculated into buffered dextrose medium. At time points, cells were removed, RNA extracted, and northern blots probed with a cDNA of the 270-6 member of the family. As expected, vegetative and loose aggregate (0 min erasure) cells contained negligible levels of complementary transcripts (Fig. 2A). However, by 60 min in erasure medium, at least five transcripts complementary to the 270-6 cDNA had accumulated to near maximum levels and, by 120 min, the transcripts had increased to maximum levels (Fig. 2A). In addition to the 2.9 kb and 2.0 kb transcripts, transcripts of 4.3 kb, 3.1 kb, and 2.3 kb were resolvable (Fig. 2A). The levels of these transcripts remained relatively constant for at least 240 subsequent minutes in erasure medium (Fig. 2A). In a repeat experiment in which loose aggregate cells were inoculated into full nutrient medium to induce erasure and samples were removed at shorter time
intervals prior to erasure, it was observed that the 270-gene transcripts accumulated at a roughly constant rate between 0 and 120 min in erasure medium (Fig. 3A). The regulation of transcription of the 270-gene family during the erasure process is different from that of all other genes, both development-specific and growth-specific, so far analyzed.

Transcripts of the germination-specific 270-gene family expressed during dedifferentiation are down-regulated by exogenous cAMP

Addition of 100 µM cAMP to an erasure culture prior to erasure or during a 30 min period following erasure, inhibits erasure in the former case, and rapidly reverses erasure in the latter case (Finney et al., 1981). The addition of cAMP also has profound effects on the levels of developmentally acquired mRNAs. In the case of the mRNA for the cohesion glycoprotein gp80, addition of cAMP prior to erasure leads to an abnormal, rapid decrease to negligible levels (Kraft et al., 1989). In the case of the mRNA for the cysteine protease CP2, addition of cAMP results in abnormal maintenance (Finney et al., 1987; Kraft et al., 1989). To test whether the accumulation of mRNAs complementary to the 270-gene family is affected by cAMP during dedifferentiation, 100 µM cAMP was added to erasure cultures at 60 min, just prior to erasure, and at 180 min, roughly 90 min after erasure. Samples were then removed at time intervals, RNA extracted and northern blots probed with the 270-6 cDNA. Within 120 min after addition of cAMP in both cases, the level of 270 complementary transcript had decreased to a negligible level (Fig. 2A), in a fashion similar to that observed for the gp80 mRNA (Kraft et al., 1989).

The germation-defective mutant HE1 is defective in erasure

To explore further the relationship between dedifferentiation and germination, the germation-defective mutant HE1 (Shaw et al., 1986) was tested for its ability to undergo erasure. When loose aggregate cells of mutant HE1 were disaggregated and development immediately reinitiated, they rapidly progressed through the ripple and loose aggregate stages in 30 and 60 min, respectively (0 min erasure, Fig. 1B), in a fashion similar to wild-type strain B cells (0 min erasure, Fig. 1A). However, in each of 6 independent experiments employing 4 clonal isolates, the time of erasure was delayed when compared to wild-type cells. In addition, in each case erasure was biphasic, and the time to ripple in log phase or erased cultures was longer than that in similar cultures of strain B (LOG, Fig. 1). In the representative erasure experiment in Fig. 1B, the time to ripple began to increase after 120 min, plateaued between 180 and 240 min, and then precipitously increased between 240 and 270 min. The T50 of erasure in this experiment was 248 min. Comparing Figs 1A and B, the delay in the onset of erasure was 60 min, and the delay in the T50 of erasure was 175 min. The average T50 of erasure computed from the data of 6 experiments with strain B was 92±22 min and the average T50 of erasure computed from the data of 5 experiments with mutant HE1 was 251±13 min.

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Fig. 2. The levels of 270 complementary transcripts during dedifferentiation, and after the addition of 100 µM cAMP at 60 min or 180 min of dedifferentiation: (A) wild-type (strain B) culture and (B) HE1 culture erased in buffered dextrose medium. Single numbers at the top of panels represent time in minutes during dedifferentiation. The first of each double number at the top of panels represents the time of cAMP addition, and the second represents the time after addition. ‘V’ represents vegetative cells. The approximate T50 of erasure for WT and HE1 cultures are noted by unfilled arrows. The numbers to the left of panel A indicate the approximate sizes, in kilobases, of the five 270 complementary transcripts.
During erasure, the 270-gene family is activated and regulated in a normal fashion in HE1

Although strain HE1 is defective in germination and exhibits a delay in erasure, the accumulation of transcripts of the 270-gene family occurred normally when HE1 cells at the loose aggregate stage were disaggregated and suspended in erasure medium (Figs 2B and 3B). Addition of 100 µM cAMP to erasure medium after 60 and 180 min of incubation resulted in rapid down-regulation of 270-complementary transcripts (Fig. 2B), in a fashion identical to that in wild-type cells (Fig. 2A).

Although erasure is delayed, HE1 cells lose development-specific transcripts at the prescribed times in the program of induced dedifferentiation

It was previously demonstrated that during the program of induced dedifferentiation in *D. discoideum* strain Ax3, the gp80 transcript continues to accumulate during the period
preceding erasure, reaching a maximum level at 90 min, roughly the time of erasure (Kraft et al., 1989). The level of gp80 transcript then plummeted to a negligible level by 150 min. The transcript of the cysteine protease gene CP2 also continues to accumulate early in the period preceding erasure, reaching a maximum level at roughly 30 min, then decreases to a negligible level by 120 min (Kraft et al., 1989). Similar results were observed during the program of dedifferentiation in D. discoideum strain B. The level of gp80 transcript remained high between 0 and 120 min in erasure medium, then decreased to a negligible level by 300 min (Fig. 4A); the level of CP2 transcript remained high between 0 and 60 min in erasure medium, then decreased to a negligible level by 180 min (Fig. 4B). In spite of the dramatic delay in the time of erasure, the time at which the level of gp80 transcript (Fig. 4A) decreased in mutant strain HE1 was roughly the same as in the parent wild-type cultures. The time at which the level of CP2 transcript (Fig. 4B) decreased in mutant strain HE1 was delayed roughly 30 min in comparison with wild type cultures, but this delay was far less than the delay in the onset and T₅₀ of erasure. These results were obtained in repeat experiments.

Immediately after loose aggregate cells of strain B were suspended in erasure medium, the transcript level of the putative prespore gene D18 (Barklis and Lodish, 1983) plummeted, reaching a negligible level by 90 min (Fig. 4C). The transcript level of the putative prestalk gene D11 (Barklis and Lodish, 1983) began to decrease after 120 min (Fig. 4D). Again, in spite of the dramatic delay in erasure in HE1, the times at which the D18 and D11 transcripts began decreasing were similar in strains B and HE1 (Fig. 4C and D, respectively). Together, these results demonstrate that although erasure timing is altered in mutant HE1, development-specific mRNAs are lost at normal times during the program of dedifferentiation.

Although erasure is delayed, HE1 responds normally to cAMP during the program of dedifferentiation

In Fig. 5A, results are presented of experiments in which 100 µM cAMP was added to an erasure culture of strain B cells at 60 min, just prior to erasure, and at 180 min, 90 min after erasure. In the former case, the capacity to rapidly reaggregate was retained so that cells removed from erasure medium at 240 min rippled in 0.6 h, while cells from parallel cultures in the absence of cAMP rippled in 4.5 h. In the latter case, the capacity to rapidly reaggregate was quickly reestablished so that cells removed from erasure medium at 300 min, 120 min after the addition of cAMP, rippled in 1 h. Similar results were obtained with strain HE1 (Fig. 5B). When cAMP was added at 60 min, cells retained the capacity to reaggregate rapidly for at least 3 subsequent hours, and when cAMP was added at 180 min, cells removed at 360 min, 3 h after cAMP addition, still rippled in roughly 1 h, compared to 6.5 h in untreated cultures. These results demonstrate that even though erasure is delayed by at least 2.5 h in HE1, the inhibition of erasure by the addition of 100 µM cAMP is intact.

When 100 µM cAMP was added to the erasure medium of WT and HE1 cultures at 60 min or at 180 min, the level of gp80 transcript in both cases decreased rapidly to minimum levels (Fig. 6A). The level of CP2 transcript in both WT and HE1 cultures remained high after the addition of cAMP at 60 min and was reestablished to developmental levels after addition at 180 min (Fig. 6B). These cAMP-mediated changes in the levels of gp80 and CP2 transcripts are similar to the cAMP-mediated changes in gp80 and CP2 transcript levels in strain Ax3 (Kraft et al., 1989). In the case of the prespore transcript D18, addition of 100 µM cAMP at 60 min reestablished the developmental level in both wild-type and HE1 cultures (Fig. 6C) and, in the case of the prestalk transcript D11, cAMP addition at 60 min maintained the pre-erasure level in both wild-type and HE1 cultures (Fig. 6D). These results demonstrate that cAMP-mediated effects on erasure and on gene expression are intact in HE1.

Discussion

The morphogenetic program in D. discoideum is quite expensive for the original cell population since it (1) occurs

![Fig. 5. The effects of the addition of 100 µM cAMP on erasure in dedifferentiating WT (A) and HE1 (B) cultures. In A and B, the kinetics of erasure in the absence of 100 µM cAMP is plotted as a solid line (○), and represents the averaged data from six independent experiments. cAMP was added at 60 min (●) or at 180 min (△), as noted by arrows. Cells were removed subsequently from treated cultures at indicated intervals, washed and deposited on a fresh development filter and the time to the ripple stage was then measured. The timing to ripple after addition of cAMP is plotted as a dashed line, and represents the averaged data from 3 independent experiments. For each cAMP-addition data point, the mean±standard deviation is shown.](image-url)
Dedifferentiation in Dictyostelium

in the absence of cell growth and results in the reduction of cell mass and protein by more than 50% (White and Sussman, 1961), (2) results in stalk formation, which involves the differentiation of approximately 20% of the cells to a nonviable terminal stalk phenotype (Whittingham and Raper, 1960), and (3) results in the differentiation of approximately 80% of the cells to wall-encapsulated spores, which serve as vehicles for dispersal and therefore result in a high proportion of cell death. It seems likely that cells enter this developmental program only when environmental conditions adverse to single cell growth provoke them to do so, and that it would be advantageous for cells that have entered the program to be able to revert en masse to the vegetative state if environmental conditions suddenly became conducive once again for growth.

Dictyostelium amoebae appear to have evolved such an escape pathway in the form of the erasure process, which can be evoked any time up to the formation of a cell wall (Soll and Waddell, 1975; Soll, 1990). However, dedifferentiation is not restricted to the erasure process since cells that have progressed through the forward program of development must emerge from the spore coats as undifferentiated cells ready to resume cell multiplication. Both induced dedifferentiation and spore germination involve the conversion of differentiated cells to the vegetative state, and therefore there may exist molecular or functional correlates between the two developmental processes (Waddell and Soll, 1977). We have therefore tested whether amoebae that have just emerged from their spore coats have undergone erasure, whether germination-specific genes are also expressed in erasing cultures, and whether a germination-defective mutant is also defective in induced dedifferentiation.

First, we have found that freshly emerged amoebae progress through morphogenesis with the slow timing of erased cells, and have, therefore, erased. Since developing amoebae can rapidly recapitulate morphogenesis right up to the time of cell wall formation, late in the developmental program (Soll and Waddell, 1975), we can conclude that cells lose the capacity to reaggregate rapidly either during spore formation, germination or the first few minutes following emergence of amoebae from spore coats.

Second, we have found that the germination-defective mutant HE1 also exhibits a defect in induced dedifferentiation. In solution, activated HE1 spores swell but do not emerge from their spore coats (Ennis and Sussman, 1975). Here we have demonstrated that the kinetics of erasure in dedifferentiating HE1 cultures are biphasic and that erasure is dramatically delayed. Wild-type strain B cultures undergo erasure in a single step between 70 and 100 min in erasure medium, while HE1 cells exhibit biphasic erasure kinetics which begin after 120 min in erasure medium. The difference in the T50 of erasure between wild-type and HE1 cells is approximately 150 min. Although there is a dramatic delay in the erasure process in HE1 cells, the times at which different developmentally acquired mRNAs are lost during the program of dedifferentiation and the effects of cAMP on gene expression are similar to those in wild-type cells. This has been demonstrated to be true for mRNAs of the gp80, D18 and D11 genes. Loss of the CP2 mRNA in HE1 is delayed by 30 min, but this delay is small compared to
the delay in erasure. The selective delay in erasure timing in HE1 represents a dissociation of erasure and the programmed loss of developmentally acquired mRNAs, and adds support to the previous suggestion (Soll and Finney, 1987) that independent pathways progress in parallel in the program of dedifferentiation, an established feature of the yeast cell cycle (Pringle and Hartwell, 1981) and the forward program of Dictyostelium development (Soll, 1979; Varnum et al., 1983).

HE1 is partially defective in both induced dedifferentiation and germination, but is deficient in neither. The defect in induced dedifferentiation represents a delay in only one parallel pathway of the program, and the defect in germination represents an incomplete block in only one aspect of the germination process, amoebal emergence in liquid medium. In HE1, spores suspended in germination solution swell normally and germination-specific genes are activated, but amoebae fail to emerge from spore coats. However, when spores are dispersed on an agar substratum in the presence of bacteria, they form colonies and must therefore emerge under these conditions. Although these observations lend weight to the argument that the defect in HE1 germination is also responsible for the defect in HE1 erasure, we have not directly ruled out the possibility that the defects are due to independent mutations.

Finally, we have found that members of one germination-specific gene family are also activated during induced dedifferentiation and are regulated in a unique fashion. Members of the 270-gene family share an internal threonine-glutamic acid-threonine-proline repeat, but are otherwise dissimilar (Ennis et al., 1991). During germination, 2 of the 4 genes in the 270-gene family are transiently expressed (Kelly et al., 1983; Giorda et al., 1990), and during induced dedifferentiation, at least five 270-complementary transcripts are synthesized. Experiments are underway, using gene-specific probes, to determine the origin of the three transcripts that are present during induced dedifferentiation.

We have therefore demonstrated that freshly germinated cells have undergone erasure, that a germination-defective mutant is also erasure-defective, and that germination-specific genes are activated during induced dedifferentiation. None of these observations alone demonstrate conclusively that germination and induced dedifferentiation are related, but together they suggest that these two processes may share common mechanisms for converting a differentiated cell back to an undifferentiated vegetative cell. Experiments are now in progress to test whether additional germination-specific and erasure-specific mutations are defective, and other germination-specific and erasure-specific genes are expressed, in the alternative process.

We thank the members of the Soll laboratory for comments on the manuscript, Steve Woodward and Kaveh Adel for preparing the figures, and Stacee Harger and Kris Wheeler for typing the manuscript. This research was supported by NIH grants GM25832 and HD18577 to D.R.S.

References


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(accepted 20 July 1992)