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Mutants of *Dictyostelium discoideum* Defective in Spore Germination

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After activation, wild-type *Dictyostelium discoideum* spores germinate rapidly and synchronously in phosphate buffer as well as in complex medium. Mutants defective in spore germination were isolated and characterized. These mutants (called *grm*) did not germinate normally in buffer but did germinate in complex medium in the presence of bacteria. One mutant (*grm* B) swelled normally, but amoebae were not formed. Another mutant (*grm* F) swelled and germinated poorly in buffer. The members of the third group of mutants (A, C, D, and E) did not swell or give rise to amoebae in buffer.

Much attention has been paid to the morphogenetic and biochemical activities attending fruiting body construction in *Dictyostelium discoideum*, but relatively little has been paid to the process by which the products of fruit construction, the spores, germinate into amoebae. Three stages in the germination of spores are recognized: (i) activation, (ii) swelling, and (iii) emergence of amoebae (1-5). Activation of dormant spores is achieved by treatment with heat or chemicals (1-5). Experimentally, germination can proceed synchronously and quantitatively in simple buffers and does not require exogenous supplies of energy or precursors of macromolecules. Clearly the isolation and study of mutant strains defective for spore germination would be useful in describing the germination sequence with respect to the genes involved and the biochemical and morphological steps that must occur. Recently, a method of separating spores from amoebae by density gradient sedimentation was described which permitted the isolation of such defective mutants (7). The present communication describes a more convenient and direct procedure by which the mutants defective in germination can be isolated after selective killing of the germinated wild-type amoebae. The germination capacities of several such mutants are described.

MATERIALS AND METHODS

Growth conditions and organism. *D. discoideum* strain B was grown in association with *Escherichia coli* B on SM agar plates (8). After growth and fruit construction (6 days at 23 C), the spores were collected from the dish cover after the inverted plate had been forcibly struck against a counter top. The spores that fell onto the cover of the dish were collected and washed two times with 10 mM potassium phosphate buffer, pH 6.7, and were used immediately or frozen in small portions at −20 C in buffer containing 20% (vol/vol) glycerol. The frozen spores remain viable for at least 4 to 6 months.

Quantitative germination assays. Frozen spores were thawed and washed rapidly by two centrifugations or on a membrane filter (3.0-μm pore size; Millipore Corp.) with three times the volume of 10 mM phosphate buffer, pH 6.7. The spores were suspended at a density of 10^6 to 5×10^7/ml in buffer containing 20% dimethyl sulfoxide (Me_2SO) and shaken for 30 min in a New Brunswick G-76 water bath shaker at 23 C and 100 rpm to activate them (D. A. Cotter and R. W. O'Connel, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, p. 29). They were then washed to remove the Me_2SO and suspended at 3×10^7 to 5×10^7/ml in phosphate buffer. Ten-milliliter aliquots were shaken in 150-ml Erlenmeyer flasks at 23 C. (Spores activated without shaking germinate as well as shaken spores.) The germination process was followed by periodic microscopic examination at ×400 magnification.

In some experiments, heat activation was used (2-4). Washed spores in buffer were incubated at 44.5 C for 30 min. The temperature is critical because the optimum is sharp. The activated spores were then dispensed in flasks without washing and shaken as described above.

Qualitative germination assays. These were carried out on washed agar plates. The agar was washed in 20-g batches on a table-top Buchner filter with 6 liters of distilled water. Two percent agar in 10 mM phosphate buffer, pH 6.7, was autoclaved, and filter-sterilized streptomycin sulfate was added to a final concentration of 50 μg/ml before pouring. Spores from about five sorocarps from a suspected mutant clone were suspended in 0.2 ml of buffer containing 20% Me_2SO and incubated for 30 min at 23 C without shaking. A loopful was deposited on a washed agar plate and on an SM plate containing a previously

spread layer of bacteria. After a brief drying period, the plates were incubated at 23 C and inspected periodically under a microscope to determine germination.

RESULTS

Isolation of germination-defective (grm) mutants. Wild-type vegetative amoebae were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as previously described (9). Samples of 10^5 to 10^6 cells were plated on SM agar with bacteria. The plates were incubated until 2 days after mature sorocarps had appeared. The spores were collected, washed, activated with Me_2SO, washed again, and shaken in suspension for 24 h as described in above. Germination is virtually complete in 3 to 4 h, but the longer period insured that slowly germinating wild-type and leaky mutant spores would also germinate. The germinated amoebae were then selectvely lysed by incubation for 1 to 5 min with Nonidet P-40 to a final concentration of 0.2 to 0.5%. The surviving ungerminated spores were harvested, washed with buffer, and plated clonally on SM agar with bacteria. Each clone was then tested qualitatively for germination capacity on washed agar plates and on SM agar with bacteria, as described in above. Under these conditions, wild-type germination was complete in 4 to 5 h. Deficient mutants were recloned and then tested quantitatively. The characteristics of six such mutants, called *grm*, are described below.

Kinetics of germination after activation with Me_2SO. Spore samples from six mutant strains and from the wild type were activated with Me_2SO and germinated in buffer as described in Materials and Methods. In the case of the wild type (Fig. 1A), swelling of the spores started after about 30 min and was complete at about 1.5 h. Emergence of amoebae began around 1.5 h and was complete by 3 h. Under the same conditions nonactivated spores yield less than 5% germination.

The mutant strains fell into three classes as follows.

(I) In mutant *grm* B, the swelling of the spores was as rapid and complete as in the wild type, but no amoebae emerged (Fig. 1B).

(II) In mutant *grm* F, spore swelling was slower and much less complete (20 to 30% of the population). Emergence of amoebae was limited to about 5% (Fig. 1C).

(III) The spores of mutant strains *grm* A, C, D, and E neither swelled nor yielded amoebae. In contrast, sister spores, activated with Me_2SO and plated on SM agar with bacteria, germinated and yielded clones with greater than 80% plating efficiency, indicating that Me_2SO does not kill these spores.

Kinetics of germination after heat activation. Heat-activated wild-type spores of strain B swelled and yielded amoebae more slowly and less completely than Me_2SO-activated ones (Fig. 2A) (see also reference 4). The heat-activated mutant groups I and III performed precisely as they did when Me_2SO activated. However, mutant *grm* F (group II)

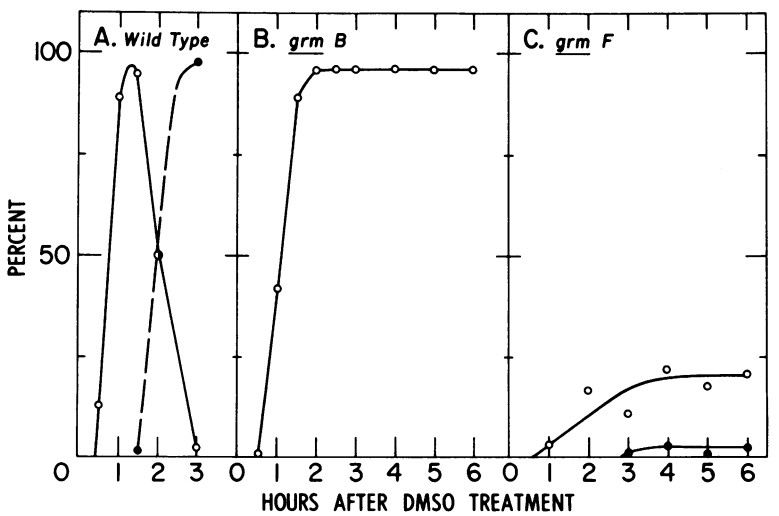

FIG. 1. *Kinetics of spore germination: Me_2SO activation. Spores of D. discoideum B wild type and mutants were activated by treatment with Me_2SO and suspended in phosphate buffer. The appearance of swollen spores* (○) *and amoebae* (●) *was monitored microscopically. (A) D. discoideum wild type; (B) mutant grm B; (C) mutant grm F.*

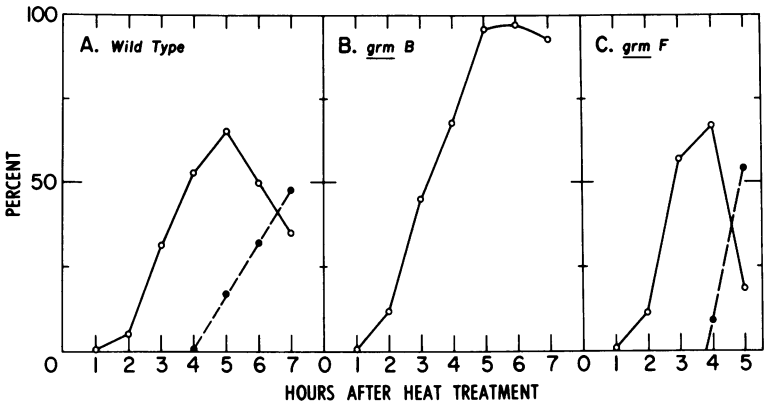

FIG. 2. *Kinetics of spore germination: heat shock. The spores were subjected to heat treatment for 30 min at 44.5 C, and germination was monitored as described in the text. The symbols are the same as in the legend to Fig. 1.*

when heat activated swelled and germinated much more rapidly and completely than when activated with Me_2SO and, in fact, was indistinguishable from the wild type.

DISCUSSION

Mutagenized spores have been incubated under conditions that permit prompt and extensive germination of the wild type and then treated with a detergent that lyses the germinated amoebae without affecting the viability of the ungerminated spores. The latter were then plated with bacteria and yielded clones. Of these about 10% turn out to be germination defective (*grm*) mutants. The procedure appears to be an efficient and convenient one for the isolation of mutants of this type.

Previous studies (1-6) have described two stages after activation in the germination process: first, a swelling of the spore and thinning of the spore coat, and second, a splitting of the coat and the emergence of a vegetative amoeba. Of the mutants described here, some produce spores that do not even initiate the swelling process under the conditions used whereas another (*grm* B) produces spores that can swell normally but from which amoebae do not emerge. Another one (*grm* F) produces spores of which only a small percentage give rise to amoebae. The spore coat is known to be multilayered, containing at least three components (6). Hence one might expect the thinning, swelling, and splitting of the coat to require the activities of a variety of enzymes.

The activation of the spores also appears to be a complex process that can be influenced by heat, exogenous amino acids, and certain chemical agents (1-5). The mechanism is unknown. The isolation of mutants unable to germinate if activated by Me_2SO but competent if heat activated attests to the complexity of the process. In addition, it should be noted that all of the *grm* mutants can be activated and can germinate rapidly and completely when spread on SM agar with bacteria. A detailed examination of specific requirements of these and other *grm* mutants for germination should help to define the steps in the activation, swelling, and emergence stages.

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