PROTEIN AND RNA SYNTHESIS DURING SPORE GERMINATION IN THE CELLULAR SLIME MOLD Dictyostelium discoideum

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SUMMARY

Germination of Dictyostelium discoideum is a synchronous developmental process, known to involve three distinct stages: activation, swelling and emergence of amoebae. This entire sequence is completed within 3.5 h after activation of dormant spores. Both protein and RNA synthesis appear to be required for germination. Protein synthesis, as judged from the incorporation of labeled precursors, begins during swelling. Spores do not contain polysomes, but polysomes appear during the swelling stage, coincident with the beginning of incorporation of amino acid into protein. SDS-polyacrylamide gel electrophoresis of labeled extracts shows that stage-specific changes occur in the pattern of proteins synthesized during germination. RNA synthesis also appears to start during swelling and inhibitors of RNA synthesis are shown to block germination.

INTRODUCTION

The terminal stage in the developmental cycle of Dictyostelium discoideum is the formation of the fruiting body containing spores. These spores are dormant and can germinate under the appropriate conditions (2). Synchronous germination can be induced by a variety of chemicals or heat (2-6,10), and proceeds in three well defined stages: activation, swelling and emergence.

The synthesis of macromolecules during germination has not been studied in much detail. Recently, Yagura and Iwabuchi (12) reported on the synthesis of RNA, DNA and protein during germination. Incorporation of labeled precursors into protein by germinating spores has been previously demonstrated by Bacon and Sussman (1). Feit et al, (9) have shown in the related species, D. purpureum, that the number of polysomes increased greatly during germination. In addition, cycloheximide has been shown to inhibit the emergence of amoebae

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(4,5). These observations suggested that de novo protein synthesis is required for germination, but it was not known whether simultaneous RNA synthesis is needed.

The present study is our initial investigation of spore germination in D. discoideum. We have (a) studied the incorporation of labeled precursors into protein and RNA, and detected incorporation at earlier stages than previously reported (12), (b) demonstrated that stage-specific changes occur in the proteins synthesized during germination, and (c) determined the effect of inhibitors of RNA synthesis on germination.

MATERIALS AND METHODS

Activation of spores. Spores isolated and collected as previously described (7) were activated in 10 mM potassium phosphate buffer, pH 6.7 (PB) containing 20% dimethylsulfoxide for 30 min at 23° (6). Following activation, the spores were washed on membrane filters (3.0 µm pore size; Millipore Corp.) with PB, suspended in PB at 1-2.5 x 10^7/ml and incubated in a New Brunswick gyrotory water bath shaker (at 100 rpm), at 23°. Germination was monitored microscopically.

Labeling with radioactive precursors. Labeled precursors were added immediately after activation. [^{3}H]Proline (New England Nuclear Corp., specific activity: 5 Ci/mmol) at 20 µCi/ml, and uracil-6-[^{3}H] (New England Nuclear Corp., specific activity: 21.9 Ci/mmol) at 20 µCi/ml with 0.2 µg/ml unlabeled carrier uracil, were added to follow incorporation into protein and RNA, respectively. Aliquots were collected at the indicated intervals and precipitated with an equal volume of cold 10% trichloroacetic acid (TCA). The TCA-insoluble material was collected on glass fiber filters (Reeve Angel, 934 AH 2.4 cm), dried, placed in vials containing Omnifluor (New England Nuclear) and counted in a Beckman LS-100 scintillation counter. When incorporation into protein was measured, the labeled samples were boiled for 10 min prior to filtration.

Polysome analysis. Samples, corresponding to about 5 x 10^8 spores, were removed at various times after activation and were chilled immediately by pouring over crushed ice. Cycloheximide (200 µg/ml) was then added and the cells were collected by centrifugation. The pellets were frozen immediately. Frozen cells were disrupted by grinding with dry ice. The extracts were suspended in gradient buffer (10 mM Tris-HCl, pH 7.8, 10 mM Mg-acetate and 150 mM KCl) containing 0.5% Nonidet P40 (Shell Chemical, London, England) and the unbroken cells were removed by centrifugation (5000 x g, 10 min). The extracts were applied to 15-30% sucrose gradients prepared in gradient buffer. Centrifugation was at 22,000 r.p.m. at 4° for 3.5 h in a Spinco SW 25.1 rotor. One ml fractions were collected and analyzed with the aid of an ISCO fractionator connected to a Sargent recorder.

SDS Polyacrylamide gel electrophoresis (SDS-PAGE). Activated spores (5 x 10^7 spores/ml) were labeled for 1 h periods following activation with 50-100 µCi/ml [^{35}S]methionine (Amersham/Searle Co., specific activity 200-500 mCi/mmole). Samples were collected by centrifugation and disrupted by sonication with a Biosonik III sonicator (Bronwill Scientific, Rochester, N.Y.)

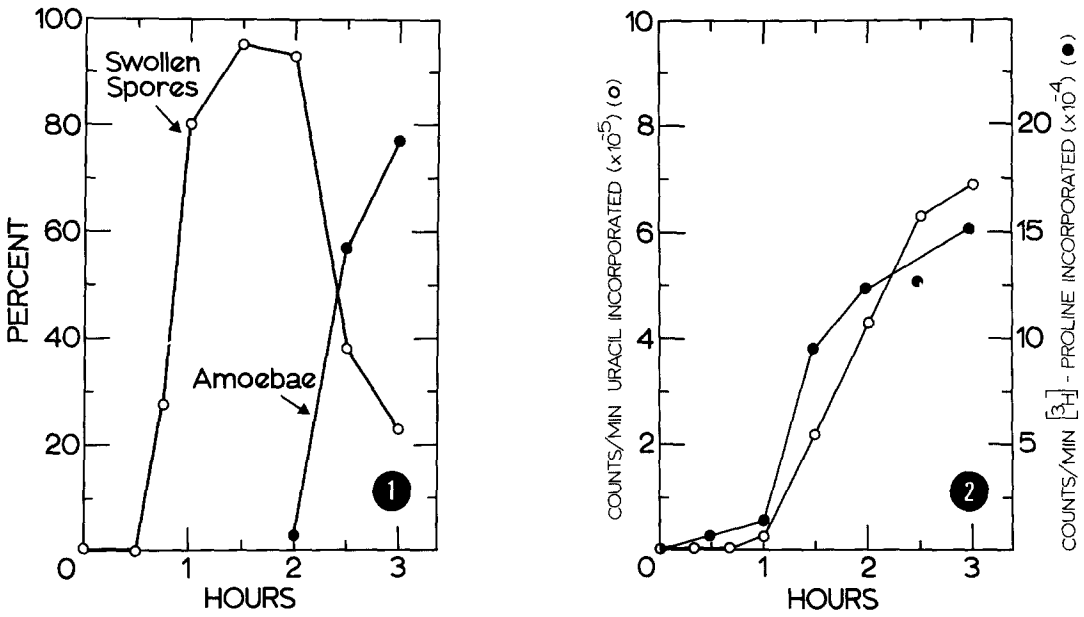

Fig. 1: Kinetics of germination of D. discoideum spores. Conditions of activation and incubation were as described in Materials and Methods. (o) percent of swollen spores; (●) percent emerged amoebae.

Fig. 2: Protein and RNA synthesis during germination. Spores were labeled immediately following activation with [^{3}H]proline or [^{3}H]uracil as described in the Materials and Methods section. One ml samples, representing 2.5 x 10^7 spores, were removed at the indicated intervals and incorporation into the TCA-insoluble fraction was determined.

until most spores appeared broken by microscopic examination. Polyacrylamide gel electrophoresis, staining and autoradiography were performed as described by Laemmli (11) and Fairbanks (8).

RESULTS

Protein synthesis during spore germination. The kinetics of germination is given in Fig. 1. As can be seen, swelling of spores occurs rapidly after activation and is complete by 2 h. Emergence of amoebae starts soon after, and the entire sequence is completed by 3.5 h.

Protein synthesis, as determined by the incorporation of labeled amino acid (Fig. 2), begins during the swelling stage, at about 0.5 h after activation, and increases rapidly from about 1 h. Since it is not known how the internal

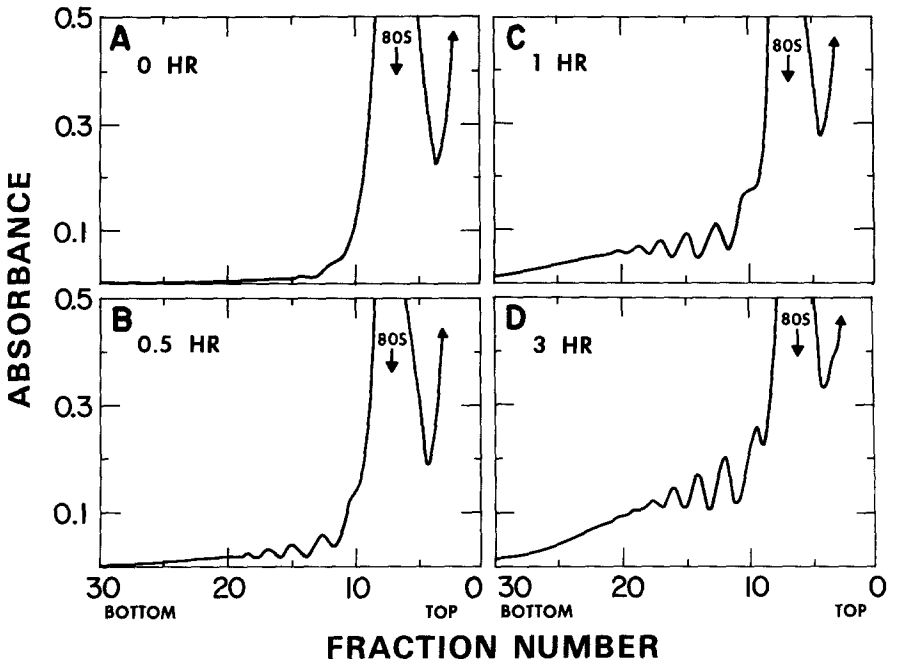

Fig. 3: Appearance of polysomes during germination. Samples were prepared as described in Materials and Methods. Extracts were obtained by grinding spores with dry ice, and 10 A_{260} units were applied to each gradient.

pool of amino acids present in spores affects the uptake and incorporation of labeled amino acids, we also monitored the appearance of polysomes as a measure of protein synthesis. As seen in Fig. 3, there is a large pool of monosomes, and no polysomes are present in spores immediately after activation. Polysomes can be detected at 0.5 h after activation, which corresponds approximately to the start of incorporation of labeled amino acids. It can also be seen from Fig. 3 that there is a large amount of monosomes in all extracts. This is not due to any appreciable breakdown of polysomes during the extraction procedure, as an extract from growing amoebae treated in the same manner showed the characteristic polysome profile of growing cells. It appears therefore that there is a large pool of 80S ribosomes in germinating spores which is not participating at any specific time in protein synthesis.

SDS-PAGE of proteins labeled with [^{35}S]methionine at hourly intervals after

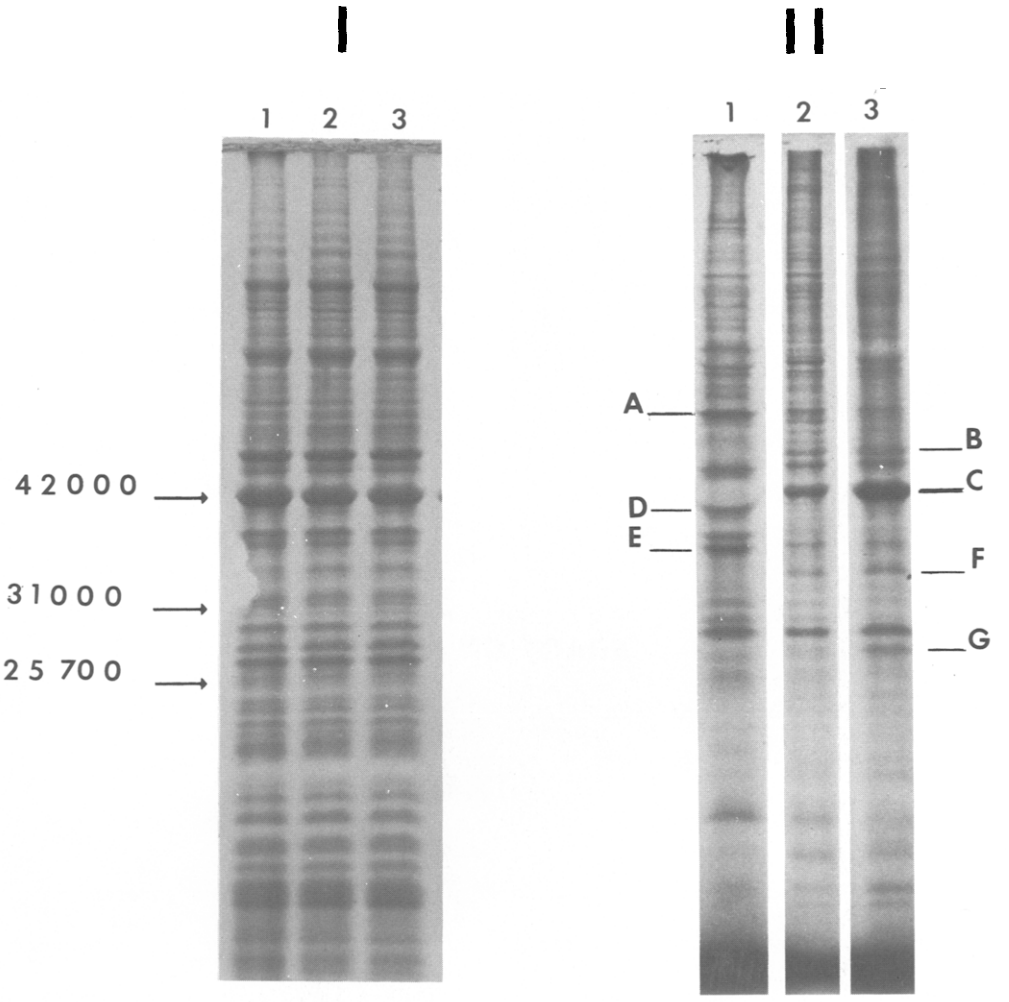

Fig. 4: SDS-PAGE analysis of proteins made during spore germinations. Samples of 5 x 10^8 activated spores were labeled for 1 h periods with [^{35}S]methionine, disrupted by sonication, and the extracts were applied to 10% polyacrylamide gels as described in the Materials and Methods. In (I) the samples were stained with Coomassie blue. Molecular weight markers used were: E. coli EF-Tu (42,000-43,000 daltons), DNase (31,000 daltons) and α-chymotripsinogen (25,700 daltons). (II) is an autoradiograph of the same labeled samples, shown in (I). 1, labeled 0-1 h after activation; 2, labeled 1-2 h; and 3, 2-3 h.

activation shows that stage-specific synthesis of proteins occurs during germination (Fig. 4II). For example, proteins A (51,000 daltons) and E (37,000 daltons) are synthesized in greater quantity during the first hour of germination than at later stages of germination. Band D (41,000 daltons)

TABLE 1

Effect of Inhibitors of RNA Synthesis on Spore Germination

Inhibitor	Concentration (μg/ml)	% Inhibition of Incorporation[a]	Effect on Germination[b] swelling	emergence
Daunomycin	250	99.6	+	-
Lomofungin	100	98.3	+	-
Thiolutin	100	97.1	-	-
Actinomycin D	12.5	39.0	+	+
	125	76.0	+	+

Activated spores (2×10^7/ml) were divided into 5 ml aliquots immediately after addition of [^{3}H]uracil and the antibiotics were added at the concentration indicated. To one sample 1% dimethylsulfoxide was added to serve as control for the lomofungin and thiolutin-treated samples as these inhibitors were dissolved in this solvent. An additional sample served as an untreated control. At intervals duplicate aliquots were removed, washed twice with cold phosphate buffer (to reduce quenching by the colored drugs), precipitated with cold 5% TCA, and counted as described in Materials and Methods. Germination was monitored microscopically.

[a]Incorporation of [^{3}H]uracil into RNA was determined at 3 h after activation.
[b](+) denotes swelling or emergence of amoebae took place in the presence of the drug; (-), no swelling or emergence was detected.

is only detected in gels labeled from 0-1 h post-activation. Other proteins are synthesized in increasing amounts as germination proceeds, as for example proteins B, F and G (47,000, 34,500 and 29,500 daltons, respectively). Protein C (which co-electrophoresed with Escherichia coli elongation factor Tu, 42-43,000 daltons) is probably slime mold actin, and its synthesis begins only during the second hour of germination.

RNA synthesis during germination. RNA synthesis, like protein synthesis, begins during the swelling stage (Fig. 2). Of the RNA synthesis inhibitors tested (Table 1), daunomycin and lomofungin effectively inhibited the

incorporation of labeled uracil and prevented emergence of amoebae. Thiolutin-treated spores (as observed under the light microscope) did not swell. Daunomycin, lomofungin and thiolutin (at the concentrations indicated in Table 1) inhibited incorporation of labeled uracil more than 90% as early as 1.5 h after activation. Actinomycin D, however, at either 12.5 μg/ml or 125 μg/ml did not inhibit germination. In the actinomycin D-treated spores greater inhibition of incorporation was observed at 3 h than at 1.5 h. For example, in the presence of 12.5 μg/ml actinomycin D [^{3}H]uracil incorporation was inhibited by 4% at 1.5 h after activation and by 39% by 3 h. This indicates that actinomycin D probably is unable to get into spores in sufficient concentration to inhibit RNA synthesis and thus germination.

DISCUSSION

Protein synthesis, as judged from the incorporation of labeled precursors and by the appearance of polysomes, starts at about 0.5 h after activation and increases rapidly at later stages (Figs. 2 and 3). Stage-specific proteins are synthesized during germination (Fig. 4). These proteins are required for germination, or maintenance of these stages in development. From a comparison between the stained portion of the gel (Fig. 4I) and the autoradiograph (Fig. 4II), it can be seen that not all of the major stained bands correspond to the main protein bands labeled during germination. As there are no detectable changes between the stained samples, the changes occurring during germination cannot be quantitatively very large. Other more subtle changes in protein synthesis, in addition to the ones seen in the autoradiograph, probably occur, but are not resolved in this gel. Two-dimensional gel analysis of the labeled proteins should resolve these minor changes.

RNA synthesis, as determined by incorporation of labeled uracil, also begins during the swelling stage (Fig. 2). Inhibition of RNA synthesis (Table I) prevents emergence (and in the case of thiolutin, swelling), indicating that

RNA synthesis is required for germination. Although there is one report to the contrary (1), it appears that actinomycin D is not a good inhibitor of RNA synthesis in germinating spores (2,12). It is possible that the spores are not permeable to actinomycin during the initial stages of germination.

In preliminary experiments (data not shown) we attempted to identify the types of RNA synthesized during germination. Results indicate that rRNA and poly A containing mRNA are synthesized in different proportions during germination and that the newly synthesized rRNA is incorporated into ribosomes. We are currently trying to quantitate the different types of RNA synthesized at each stage of germination.

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REFERENCES

1. Bacon, C.W. and A.S. Sussman. (1973) J. Gen. Microbiol., 76, 331-334.
2. Cotter, D.A. and K.B. Raper. (1966) Proc. Nat. Acad. Sci. U.S.A. 56, 880-887.
3. Cotter, D.A. and K.B. Raper. (1968) J. Bacteriol. 96, 1680-1689.
4. Cotter, D.A., L.Y. Miura-Santo, and M.R. Hohl. (1969) J. Bacteriol. 100, 1020-1026.
5. Cotter, D.A. and K.B. Raper. (1970) Develop. Biol. 22, 112-128.
6. Cotter, D.A., J.W. Morin, and R.W. O'Connell. (1976) Arch. Microbiol. 108, 93-98.
7. Ennis, H.L. and M. Sussman. (1975) J. Bacteriol. 124, 62-64.
8. Fairbanks, G., C. Levinthal, and R.H. Reeder. (1965) Biochem. Biophys. Res. Commun. 20, 393-399.
9. Feit, I.N., L.K. Chu and R.M. Iverson. (1971) Exp. Cell Res. 65, 439-444.
10. Hemes, D.E., E. Kojima-Buddenhagen, E. Hohl and M.R. Hohl. (1972) J. Ultrastruct. Res. 41, 406-417.
11. Laemmli, U.K. (1970) Nature (London), 227, 680-685.
12. Yagura, T. and M. Iwabuchi. (1976) Exp. Cell Res. 100, 79-87.