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In Vitro Ribonucleic Acid Synthesis in the Zoospores of the Aquatic Fungus *Blastocladiella emersonii*

PAUL A. HORGEN

Department of Forest Botany and Pathology, School of Biology, Chemistry, and Ecology, State University College of Forestry, Syracuse, New York 13210

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The zoospores of *Blastocladiella emersonii* possess three ribonucleic acid polymerases. No general or specific inhibitor for the enzymes were found in the protoplasm of the spores.

The zoospores of *Blastocladiella emersonii* are motile and respire (1, 2) but were reported not to grow or to synthesize ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or protein (6). Focusing on the inability of zoospores to synthesize RNA, an examination was made of the DNA-directed RNA-synthesizing enzymes. Multiple RNA polymerases have been reported from a number of different eukaryotic organisms (3-5, 8, 9). Three forms of RNA polymerase were recently reported in the vegetative ordinary colorless (OC) thalli which are actively making RNA (3). These same multiple polymerases were isolated from the synthetically deficient zoospores. No general or specific RNA synthesis inhibitor of any of the three forms of polymerase was detected in the whole cell homogenates of spores. The results indicate that the zoospores have a functional in vitro RNA-synthesizing machinery when utilizing salmon sperm DNA as a template.

Single-generation cultures of *B. emersonii* were grown in aerated carboys at 23 C (2). OC thalli were harvested by filtration and washed with glass-distilled water. Motile spores were produced by placing washed OC thalli in aerated beakers of glass-distilled water. The spores were filtered to remove thalli and collected by centrifugation. The enzymes were isolated directly from the zoospores. RNA polymerase was partially purified by the method of Mertelsmann and Matthaei (7), except that the column used was 1 by 8 cm and the volumes were scaled down accordingly. Activity was measured by the incorporation of ^{3}H-adenosine triphosphate into trichloroacetic acid-insoluble polynucleotides as previously reported (3).

Polymerases isolated from zoospores were resolved into three species of enzyme activity (Fig. 1). The zoospore polymerases were eluted at approximately the same ammonium sulfate concentration as were the OC polymerases. They were DNA-dependent, dependent on all four riboside triphosphates, and had the same general properties as the vegetative OC polymerases (3).

The whole cell homogenate from the zoospores, as well as from the OC thalli, exhibited in vitro polynucleotide synthesis, indicating that no general RNA synthesis inhibitor was present in the protoplasm of the spores (Table 1). Mixing the whole cell homogenate from the spores with each of the three species of polymerase gave no observable effect, indicating that no specific inhibitor was present that may affect one or more

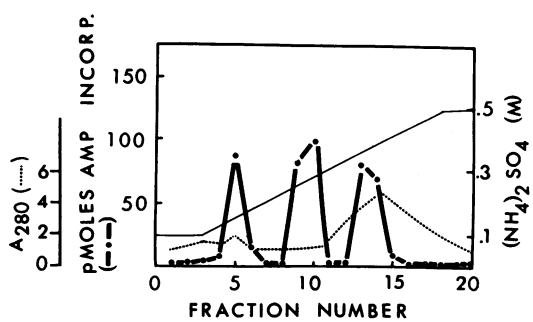

FIG. 1. *Diethylaminomethyl cellulose chromatography of Blastocladiella emersonii RNA polymerases from zoospores. A 4-ml sample of soluble polymerase isolated by the method of Mertelsmann and Matthaei (7) was chromotographed on a column (1 cm by 8 cm). The column was washed with 150 ml of buffer as described (3) and eluted with a linear gradient of buffer from 100 to 500 mM ammonium sulfate. Reaction mixtures were as in Table 1. The temperature during chromatography was maintained at 4 ± 1 C. AMP, adenosine monophosphate.*

TABLE 1. *Analysis for RNA polymerase inhibitors from the zoospores of Blastocladiella emersonii*[a]

Enzymes	Conditions	Amt (nmoles) of AMP per min per mg of protein
Zoospore homogenate	Complete	11.2
Fraction I	Complete	56.7
	+ Zoospore homogenate	58.6
Fraction II	Complete	58.1
	+ Zoospore homogenate	59.2
Fraction III	Complete	72.0
	+ Zoospore homogenate	74.5

[a] A complete reaction mixture contained tris(hydroxymethyl)aminomethane-hydrochloride, magnesium acetate, dithiothreitol, ammonium sulfate and DNA according to Horgen and Griffin (3). Uridine triphosphate, guanosine triphosphate, cytidine triphosphate (5 μmoles per reaction mixture), 10 μliters of enzyme preparation (3 to 10 mg of protein/ml as determined by A_{280}/A_{260}), and 4.5 μmoles of adenosine triphosphate (ATP) and ^{3}H-ATP (2.5 μCi/reaction mix; specific activity, 15.7 Ci/mmole) were also added. AMP, adenosine monophosphate.

of the polymerases. Thus, the spores had the same active RNA-synthesizing enzymes in vitro as the vegetative thalli.

It appears that zoospores and vegetative OC thalli of *B. emersonii* have three forms of RNA polymerase whose properties are very similar to the multiple RNA polymerases reported from other eukaryotes (3). The zoospores, which are reported to lack the ability for in vivo RNA synthesis, are not deficient in the RNA-synthesizing enzymes and therefore must be under some other kind of internal restraint.

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LITERATURE CITED

1. Cantino, E. C., and J. S. Lovett. 1964. Non-filamentous aquatic fungi: model systems for biochemical studies of morphological differentiation. Advan. Morphog. **3**:33-93.
2. Horgen, P. A., and D. H. Griffin. 1969. Cytochrome oxidase activity in *Blastocladiella emersonii*. Plant Physiol. **44**: 1590-1593.
3. Horgen, P. A., and D. H. Griffin. 1971. Specific inhibitors of the three RNA polymerases from the aquatic fungus *Blastocladiella emersonii*. Proc. Nat. Acad. Sci. U.S.A. **68**:338-341.
4. Jacob, S. T., E. M. Sajdel, and H. N. Munro. 1970. Different responses of soluble whole nuclear RNA polymerase and soluble nucleolar RNA polymerase to divalent cations and to inhibition by α-amanitin. Biochem. Biophys. Res. Commun. **38**:765-770.
5. Kedinger, C., M. Gniazdowski, J. L. Mandel, Jr., F. Gissinger, and P. Chambon. 1970. α-Amanitin: a specific inhibitor of one of two DNA-dependent RNA polymerase activities from calf thymus. Biochem. Biophys. Res. Commun. **38**:165-171.
6. Lovett, J. S. 968. Reactivation of ribonucleic acid and protein synthesis during germination of *Blastocladiella* zoospores and the role of the ribosomal nuclear cap. J. Bacteriol. **96**:962-969.
7. Mertelsmann, R. and H. Matthaei. 1968. Isolation and purification of DNA-dependent RNA polymerase from nuclei of human placenta. Biochem. Biophys. Res. Commun. **33**: 136-139.
8. Mondal, H., R. K. Mandal, and B. B. Biswas. 1970. Factors and rifampicin influencing RNA polymerase isolated from chromatin of eukaryotic cell. Biochem. Biophys. Res. Commun. **40**:1194-1200.
9. Roeder, R. G., and W. J. Rutter. 1969. Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. Nature (London) **224**:234-237.