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

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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 was packed 1 by 8 cm and the volumes were scaled down accordingly. Activity was measured by the incorporation of 3H-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 ribose 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
TABLE 1. Analysis of RNA polymerase inhibitors from the zoospores of Blastocladiella emersonii

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Conditions</th>
<th>Amt (nmoles) of AMP per min per mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoospore homogenate</td>
<td>Complete</td>
<td>11.2</td>
</tr>
<tr>
<td>Fraction I</td>
<td>Complete</td>
<td>56.7</td>
</tr>
<tr>
<td></td>
<td>+ Zoospore homogenate</td>
<td>58.6</td>
</tr>
<tr>
<td>Fraction II</td>
<td>Complete</td>
<td>58.1</td>
</tr>
<tr>
<td></td>
<td>+ Zoospore homogenate</td>
<td>59.2</td>
</tr>
<tr>
<td>Fraction III</td>
<td>Complete</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td>+ Zoospore homogenate</td>
<td>74.5</td>
</tr>
</tbody>
</table>

*A complete reaction mixture contained tris(hydroxymethyl)aminomethane-hydrochloride, magnesium acetate, dithiothreitol, ammonium sulfate and DNA according to Horgan and Griffin (3). Uridine triphosphate, guanosine triphosphate, cytidine triphosphate (5 µmoles per reaction mixture), 10 µliters of enzyme preparation (3 to 10 µg of protein/ml as determined by A₂₆₀/A₆₆₀), and 4.5 µmoles of adenosine triphosphate (ATP) and ³H-ATP (2.5 µCi/reaction mix; specific activity, 15.7 Ci/mmmole) 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


