Aphidicolin Inhibition of DNA Synthesis and Germination in Spores of *Anemia phyllitidis* L. Sw.

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**ABSTRACT**

Aphidicolin inhibits DNA synthesis and nuclear division in spores of *Anemia phyllitidis*. In spite of blocked DNA replication, spores germinate under continuous dark conditions, if induced by addition of 5 x 10^-3 grams per milliliter gibberellic acid. Differentiation of aphidicolin-treated prothallia indicate the existence of a prepattern in the dry spore which is realized independent of cell division during early events of spore germination.

Spore germination in the schizaeaceous fern *Anemia phyllitidis* is controlled by phytochrome as well as antheridiogen A, a pheromone produced and secreted by female prothallia of this species (10). Gibberellins can be substituted for the natural chemical signal (22). Although dry spores contain polyadenylated poly (A)^+ -RNA (4) the activation of processes connected directly with spore germination are induced neither by a general induction of translation of this stored mRNA nor by triggering transcription, as has been suggested by Raghavan (12-15). Both processes occur in high rates already in imbibed but noninduced spores (4).

By autoradiographic methods Rutter and Raghavan (17) have proved that the nuclear cycle of ripe fern spores is blocked in G1-phase. Thus, it is possible that the induction of germination by Pfr or chemical signals may be triggered by induction of replication in the spore nucleus. We therefore investigated the effects of Aph\(^1\) on germination and DNA synthesis of *Anemia* spores. This compound, produced by the fungus *Cephalosporium aphidicola* (3), selectively inhibits nuclear DNA polymerase (9, 19, 24, 25), whereas organellar DNA synthesis is not affected by this diterpenoid (5-8, 25).

**MATERIALS AND METHODS**

**Plant Material and Culture Conditions.** Spores of *Anemia phyllitidis* L. Sw. were harvested in 1983 from plants grown in the greenhouses of the University of Ulm and have been stored until use at 4°C in the dark. Spore sterilization and standard growth conditions have been described elsewhere (4).

**Inhibition of DNA-Synthesis.** Spores preimbibed in the dark on mineral medium containing 50 µg ml^-1\) Aph were induced to germinate by addition of GA\(_3\) (final concentration 5 x 10^-3 g ml^-1\). Samples not induced to germinate as well as samples without inhibitor have been used as controls.

**Labeling and Extraction of DNA and Proteins.** Labeling of DNA happened after a 72 h period of imbibition. For this purpose to each sample (100 mg spores in 4 ml medium) 740 KBq methyl-1',2'[\(^3^H\)]thymidine (4,0 TBq mmol^-1\) have been added. For labeling of proteins 740 KBq [\(^3^S\)]methionine (54 TBq mmol^-1\) were used.

Extraction, separation, and determination of DNA and proteins followed the methods of Fechner and Schraudolf (4).

**RESULTS AND DISCUSSION**

\(^3\)H-thymidine (185 KBq ml^-1\) added after a period of dark imbibition (72 h) to noninduced spores of *A. phyllitidis* and to those spores which have been induced to germinate by the

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1 Supported by the Deutsche Forschungsgemeinschaft.
2 Abbreviation: Aph, aphidicolin.
Aphidicolin and Spore Germination in A. phyllitidis

Table I. Effects of Aph on the Incorporation of Methyl-1,2[^3]H]Thymidine (Tdr) into DNA of Imbibed and Germinating Spores of A. phyllitidis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Preimbibition (72 h)</th>
<th>Incubation (72 h)</th>
<th>Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/A350 nm</td>
<td>[3H]Tdr</td>
<td>[3H]Tdr + GA3</td>
</tr>
<tr>
<td>Imbibition</td>
<td>Medium</td>
<td>8100</td>
<td>14900</td>
</tr>
<tr>
<td>Germination</td>
<td>Medium</td>
<td>720</td>
<td>710</td>
</tr>
<tr>
<td>Imbibition + Aph</td>
<td>Medium + Aph</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germination + Aph</td>
<td>Medium + Aph</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

 simultaneouse addition of GA3 (5 × 10−5 g ml−1), labels exclusively the DNA-fraction nucleic acids, if these are isolated 72 h after the addition of nucleoside or nucleoside and GA3 (19). Continuous dark conditions; 21°C.

Fig. 2. Germinating spore of A. phyllitidis treated with aphidicolin (50 μg ml−1) induced to germinate by GA3 (5 × 10−5 g ml−1). Continuous dark conditions; 21°C.

The simultaneous addition of GA3 (5 × 10−5 g ml−1), labels exclusively the DNA-fraction nucleic acids, if these are isolated 72 h after the addition of nucleoside or nucleoside and GA3 (19). Continuous dark conditions; 21°C.

The significant labeling of DNA in noninduced and just imbibed spores and its inhibition by Aph (Fig. 1; Table I) demonstrates that at least some nuclear DNA synthesis occurs independently from the induction of spore germination. This finding is incompatible with Raghavan's (13) observations. He found with autoradiographic methods no incorporation of label into the nucleus of Anemia spores if grown in the basal medium only (13; Fig. 32). In contrast to a 12 h application of [3H]thymidine by this author, DNA extraction in our experiments has been accomplished after a 72 h imbibition period, followed by 72 h of labeling. These differences in method may be responsible for the disagreement in the observations.

In how far DNA synthesis and nuclear division are prerequisites for germination is controversially discussed. For seed germination, problems are shortly reviewed by Galli (5). For fern spores qualitatively different effects of an application of inhibitors of DNA synthesis have been observed in different species (14, 15, 17). Unexpectedly, in A. phyllitidis not only crest opening and chloronema extension turned out to be independent of DNA synthesis but also the first steps of cell wall pattern formation. Even without preceding nuclear division the wall of the outgrowing germ tube shows already a double protrusion, one corresponding to the growth axis of the chloronema, one to that of the rhizoid (Fig. 2). The intine of the germinating spore, or more probably the adjoining plasmalemma, seems to be prepatterned before a future chloronema rhizoid-pole, respectively. This information is realized independently from the division of the spore nucleus whenever germination is induced by an external signal. The stability as well as the independency from nuclear division indicate that the information for this bipolar intine differentiation may be already manifest in the dry spore and may derive from a polarization during meiosis and/or spore differentiation. Prepatterns connected with rhizoid differentiation in form of metabolophilic zones which are detectable during early germination steps of the spore are known from other fern species (1, 2, 11, 16). Although metabolophilic prepatterns are not found in spores and young prothallia of A. phyllitidis, the realization of a bipolar pattern of the intine in Aph-inhibited prothallia

Table II. Effect of Aph on Protein Synthesis in Imbibed and Germinating Spores of A. phyllitidis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Preimbibition (72 h)</th>
<th>Incubation (72 h)</th>
<th>Protein Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/mg</td>
<td>[35S]methionine</td>
<td></td>
</tr>
<tr>
<td>Imbibition</td>
<td>Medium</td>
<td>21,300</td>
<td></td>
</tr>
<tr>
<td>Germination</td>
<td>Medium</td>
<td>25,000</td>
<td></td>
</tr>
<tr>
<td>Imbibition + Aph</td>
<td>Medium + inhibitor</td>
<td>19,500</td>
<td></td>
</tr>
<tr>
<td>Germination + Aph</td>
<td>Medium + inhibitor</td>
<td>24,000</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 3. Effect of aphidicolin (50 μg ml⁻¹) on protein synthesis. Fluorogram of the protein banding of de novo synthesized proteins, isolated from imbibed and germinating spores of A. phyllitidis. SDS-PAGE (12%) separation. 1, Imbibed spores (control); 2, induced spores in presence of aphidicolin; 3, induced spores (control); 4, imbibed spores in presence of aphidicolin. The arrow denotes the 20.5 kD protein (4) (each right-hand channel of a pair represents the double amount of protein).

could be the expression of a comparable polarized state of the spore.

A study of the time course of protein synthesis in germinating spores has shown that 48 h after setting the germination stimulus a new mRNA appears which, after 72 h, gives rise to a 20,500 D protein. The meaning of this protein for the process of germination has been discussed and it has been connected with the postgermination processes than with the induction process itself (4). The observation that Aph-treated spores germinate without further cell differentiation, permits a check of this statement. Protein synthesis itself is not inhibited by Aph application (Table II). SDS electrophoresis of soluble proteins of Aph-treated prothallia shows that in spite of a significant growth of the germination tube, none of the new protein bands present in control prothallia becomes detectable (Fig. 3). As supposed in the preceding paper (4) new proteins appearing 72 h after induction of control prothallia are not directly implicated in the primary processes of germination.

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