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Short Communication

Aphidicolin Inhibition of DNA Synthesis and Germination in Spores of *Anemia phyllitidis* L. Sw.[1]

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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 5×10^{-5} 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[2] 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 5×10^{-5} 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 [^{35}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

H^3-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

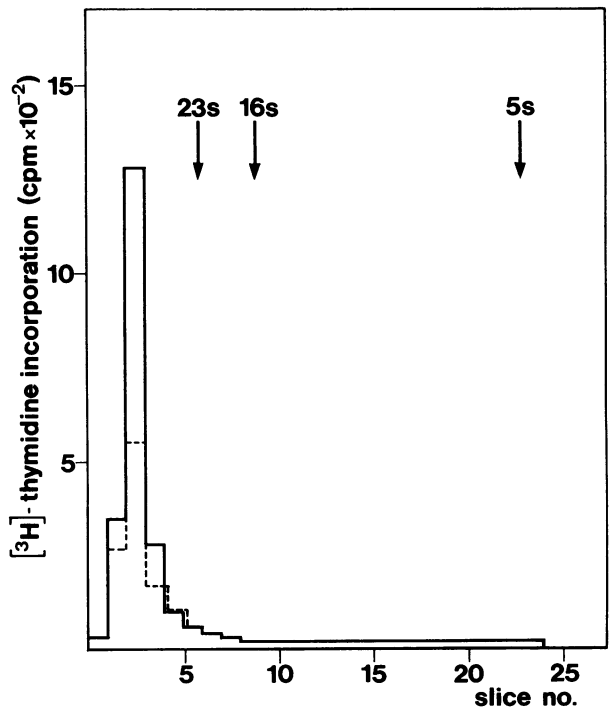

FIG. 1. Incorporation of [^{3}H]thymidine into DNA prepared from imbibed spores (---) and spores induced to germinate (———) by addition of GA_3. Labeling of spores was for 96 h. Nucleic acids were separated on a polyacrylamide-agarose gel (2% polyacrylamide, 0.5% agarose). Arrows denote migration of *Escherichia coli* RNA.

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[2] Abbreviation: Aph, aphidicolin.

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*

Continuous dark conditions; 21°C. The concentration per ml of GA_3 was 5×10^{-5} g and of Aph was 50 μg. The amount of DNA was measured by UV $A_{260\,nm}$. RNA was removed by treatment with RNase A.

	Treatment		
	Preimbibition (72 h)	Incubation (72 h)	Incorporation
			cpm/$A_{260\,nm}$
Imbibition	Medium	[^{3}H]TdR	8100
Germination	Medium	[^{3}H]TdR + GA_3	14900
Imbibition + Aph	Medium + Aph	[^{3}H]TdR	720
Germination + Aph	Medium + Aph	[^{3}H]TdR + GA_3	710

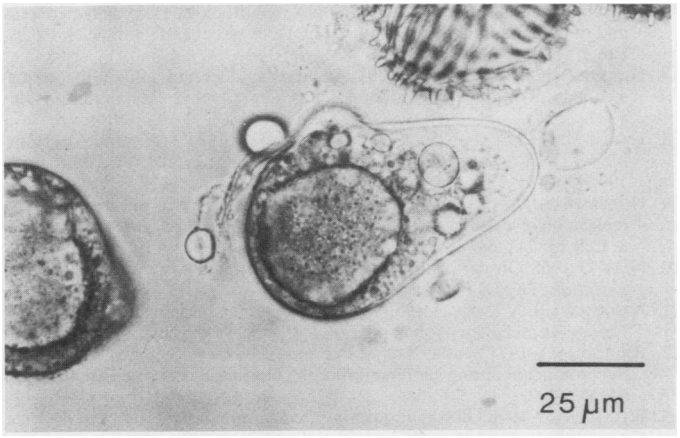

FIG. 2. Germinating spore of *A. phyllitidis* treated with aphidicolin (50 μg ml^{-1}) induced to germinate by GA_3 (5×10^{-5} g ml^{-1}). Continuous dark conditions; 21°C.

simultaneous addition of GA_3 (5×10^{-5} g ml^{-1}), labels exclusively the DNA-fraction of nucleic acids, if these are isolated 72 h after the addition of nucleoside or nucleoside and GA_3 (Fig. 1). According to observations reported by Raghavan (13) and Rutter and Raghavan (17), induction of spore germination stimulates DNA synthesis. This is confirmed by the increased specific activity of DNA isolated from induced spores (Table I). Preincubation of *Anemia* spores with Aph (50 μg ml^{-1}) inhibits thymidine-incorporation in imbibed and induced spores. Both treatments produce identical and low specific labeling of DNA. This indicates that comparable to animals and spermatophytes (18, 20, 21), Aph also inhibits nuclear DNA synthesis in archegoniates. The resistant fraction of [^{3}H]thymidine incorporation of imbibed and induced spores probably presents the DNA-synthesis of cell organelles, especially ctDNA which in *A. phyllitidis* cannot be separated from nuclear DNA by physical methods (23). Indeed, an Aph-resistant DNA-polymerase which is involved in ctDNA synthesis has been found in spinach chloroplasts (19). The autoradiographic localization of DNA synthesis in the cytoplasm immediately surrounding the nucleus of GA_3-induced spores of *A. phyllitidis* (13, 14) as well as in phytochrome controlled spores of *Pteris vittata* (15) supports this possibility.

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 [^{3}H]thymidine used 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 into 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 metalophilic zones which are detectable during early germination steps of the spore are known from other fern species (1, 2, 11, 16). Although metallophilic 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*

Continuous dark condition; 21°C. The amount of protein was measured by the biuret method.

	Treatment		
	Preimbibition (72 h)	Incubation (72 h)	Protein Synthesis
			cpm/mg
Imbibition	Medium	[^{35}S]methionine	21,300
Germination	Medium	[^{35}S]methionine + GA_3	25,000
Imbibition + Aph	Medium + inhibitor	[^{35}S]methionine	19,500
Germination + Aph	Medium + inhibitor	[^{35}S]methionine + GA_3	24,000

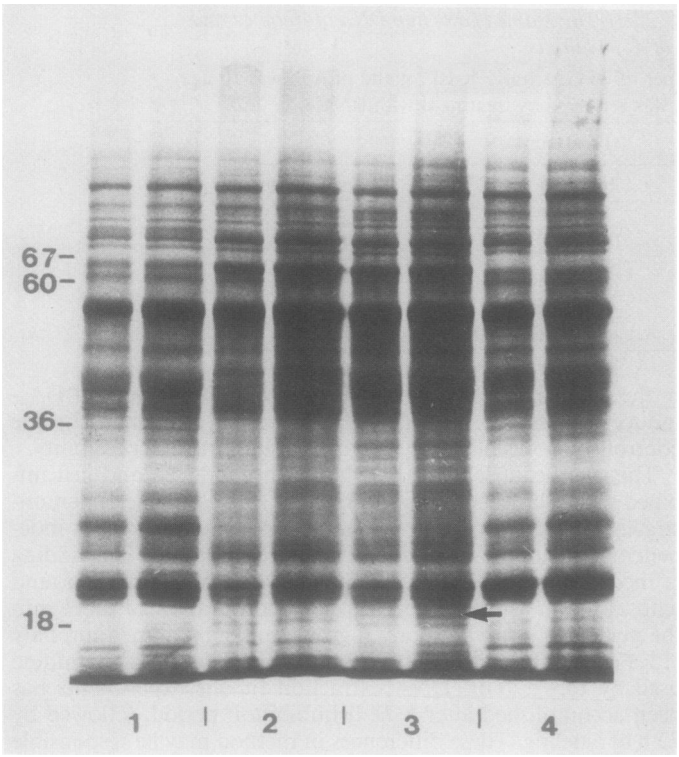

FIG. 3. Effect of aphidicolin (50 μg ml^{-1}) on protein synthesis. Fluorogram of the protein banding of *de novo* synthesized proteins, isolated from imbibed and germinating spores of *A. phyllitidis*. SDS-PAGE (12%) separation. 1, Imbibed spores (control); 2, induced spores in presence of aphidicolin; 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 proposed that the appearance of this protein is rather connected with 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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