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# MORPHOGENETIC CHANGES PROVOKED BY TWO ANTIMICROTUBULAR DRUGS WITH OPPOSITE EFFECTS DURING THE DEVELOPMENTAL CYCLE OF *ALLOMYCES ARBUSCULA*

BY

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## ABSTRACT

**Morphogenetic changes provoked by two antimicrotubular drugs with opposite effects during the developmental cycle of *Allomyces arbuscula*.**- Opposite morphogenetic changes have been provoked by growing *A. arbuscula* in the presence of two antimicrotubular agents: taxol maintained vegetative hyphal elongation growth and thereby prevented their apical differentiation into couples of male-female gametangia; nocodazole prevented germ tube outgrowth, induced emergence of epihyphal rhizoids and led to altered positioning of intrahyphal nuclei. Primary hyphal growth in the presence of taxol prevented the nocodazole-induced morphogenetic disturbances.

**Key-words:** Microtubules, *Allomyces*, Taxol, Nocodazole.

## INTRODUCTION

There is evidence that in fungal cells microtubules are involved in different fundamental processes including intracellular transport and appropriate positioning of organelles (MC KERRACHER & HEATH, 1987; HOWARD & AIST, 1980), migration of nuclei (JACOBS *et al.*, 1988), establishment and maintenance of hyphal polarity (TON THAT *et al.*, 1988; DOONAN *et al.*, 1988) and cell shaping and mechanical support (THOMPSON-COFFE *et al.*, 1992).

In this work, we describe the morphological changes induced at several developmental stages of the water mold *Allomyces arbuscula*, after the addition to its culture medium of nocodazole, known to block microtubules assembly by binding to tubuline dimers (YOKOYAMA *et al.*, 1990) or taxol which prevents microtubule depolymerisation and promotes its polymerisation (SCHIFF & HORWITZ, 1981; HERTH, 1983).

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## MATERIAL AND METHODS

The strain 73 of *Allomyces arbuscula* Butl. (gift from Dr C. Stumm, University of Leiden, Netherlands) was grown and maintained at 32°C on YpSs medium (EMERSON, 1941).

To study the germination and the vegetative growth of mycelium, a 40 ml of suspension of meiospores was inoculated into 1 l of GCY medium (TURIAN, 1963) in an Erlenmeyer flask and incubated at 32°C with aeration. The vegetative hyphae were induced to differentiate apical gametangia by the replacement, after 22h, of GCY medium by a diluted salt solution (DS, MACHLIS, 1953).

The inhibitors were added to GCY medium at the same time as the meiospore suspension or 22 h later for the germination and the hyphal growth. They were also present in the dilute salt solution at the beginning of the induction to differentiate gametangia.

The stock solution of 1mg nocodazole per 100  $\mu$ l (DMSO) was used at final concentrations of 5 nM or 10 nM during germination and at 10 to 20  $\mu$ M during vegetative growth.

Dimethyl sulfoxide (DMSO) was added to a stock solution of 5 mg taxol per 0,6 ml DMSO to give final concentrations of 5 to 10  $\mu$ M during germination and vegetative growth and 30  $\mu$ M during gametangial differentiation. DMSO was tested separately as control at the working concentrations.

The fungus was observed using an Olympus microscope equipped with an Olympus camera C-35 AB-4. Micrographs were taken on T-Max X-100 (Kodak, Basel) black and white films.

For immunofluorescent microscopy, according to SCHÖNENBERGER (1996), the vegetative hyphae grown were dried between two dry papers and fixed during 5 min with a solution of 3% paraformaldehyde in a microtubule stabilising buffer (MTSB) supplemented with 30  $\mu$ M taxol. After the incubation overnight in the 3% paraformaldehyde solution without taxol the hyphae were washed four times with the MTSB solution and then their walls digested during 1 hour at 37°C with a Sörensen solution. The digestion was stopped by washes with the Sörensen solution and the cytoplasmic membrane was permeabilized during 15 min at room temperature with 1% triton X 100 in the Sörensen solution with a pH of 7.4.

After 3 washes with the Sörensen solution the mycelium was loaded into slices after incubation with 500  $\mu$ g/ml of 4-6- diamino 2-phenylindol-2HCl (DAPI) (Serva) in a setting solution.

The nuclei were observed with a microscope Nikon Narishige coupled with a camera Narishige.

## RESULTS

### *Germination and growth culture*

Nocodazole drastically inhibited germination; the zoospores were enlarged and surrounded by numerous epihyphal rhizoids (Figs 1B and C). They died a few hours after the beginning of germination.

The addition of nocodazole to a 22 hours culture, greatly slowed down the rate of growth at the same time as laterals waves, buds and hairs appeared in subapical position. The entire hyphae formed abnormal swellings (Fig. 1E). If taxol was added 1 hour later to the same culture the effect of nocodazole was prevented (Fig. 1F).

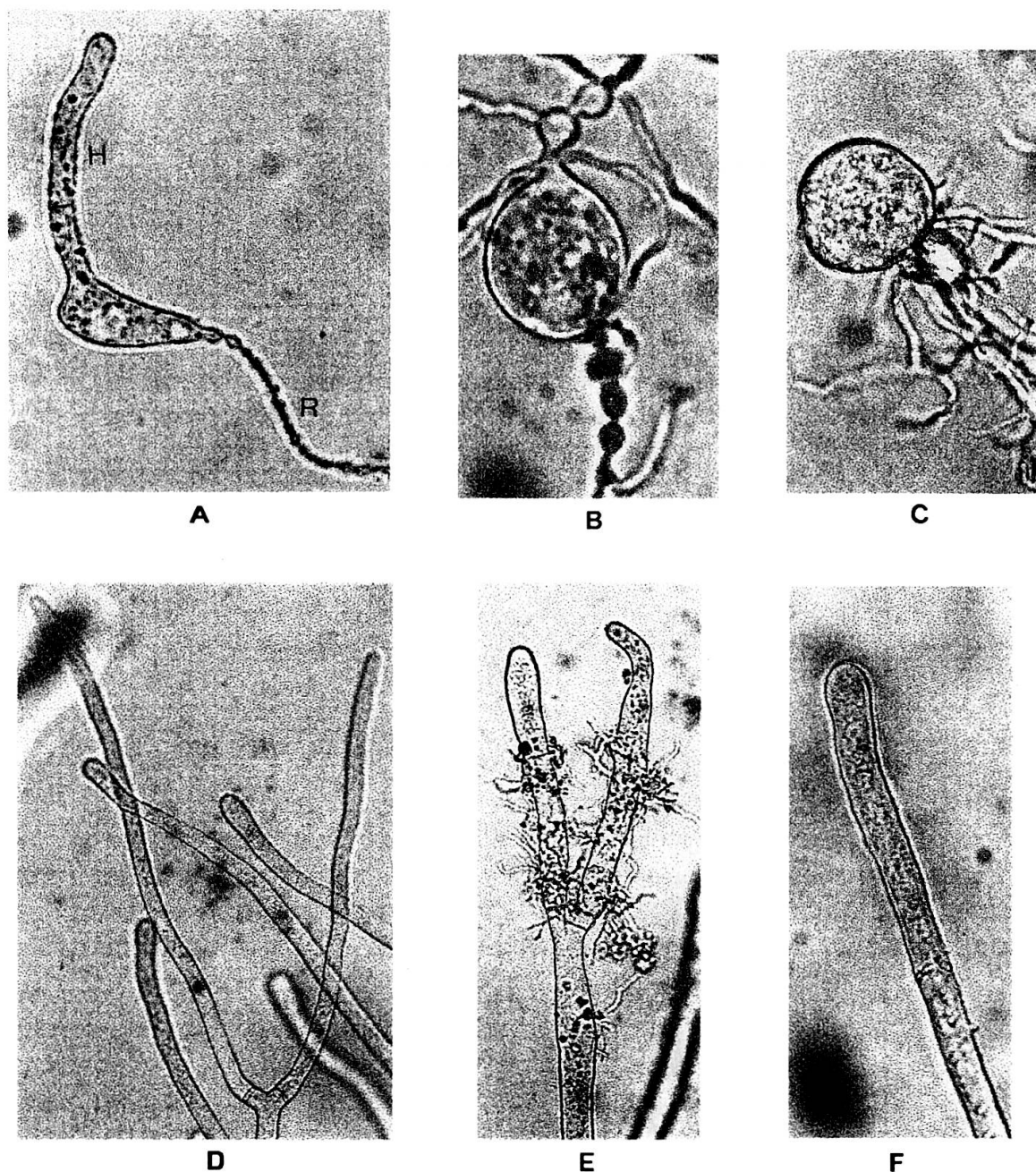


FIG. 1.

Germination and growth culture in the presence of nocodazole and taxol. A and D respective germination and hyphal growth of control cultures of *A. arbuscula*. Nocodazole added at the respective concentrations of 5 nM (B), 10 nM (C) and 20  $\mu$ M (E). Taxol present, in a culture supplemented with 20 M nocodazole, at the concentration of 15  $\mu$ M. (F).

A x 3500, B x 2400, C x 2200, D x 700, E and F x 1400.

Taxol allowed correct germination (Fig. 2A) and increased elongation of many hyphae (Fig. 2B).

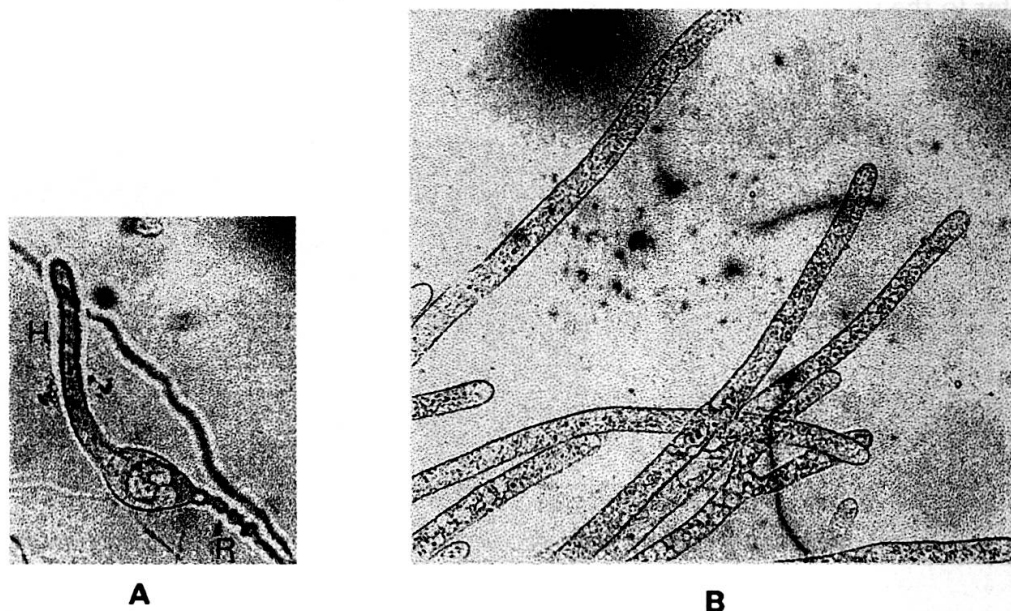


FIG. 2.

Germination and hyphal growth of *A. arbuscula* in the presence of 10  $\mu$ M taxol. A x 1800 and B x 700.

#### *Gametangial differentiation*

Nocodazole prevented any gametangial differentiation in the apices of hyphae which instead outgrew apical and sub-apical rhizoids (Fig. 3A). However, if the vegetative cultures were supplemented with taxol, around 50% of hyphae could develop a unique sexually undefined gametangium (Fig. 3D). This was also observed on 70% of the hyphae induced to differentiate in the presence of taxol alone (Fig. 3B).

The presence of taxol during hyphal growth restricted the normal gametangial formation of male and female gametangia to one gametangium of undefined sex per hypha independently of whether taxol was present (Fig. 3E) or not (Fig. 3C) at this moment.

#### *Positioning of nuclei in presence of nocodazole and taxol*

Using immunofluorescence techniques we have observed that the nuclei of the vegetative hyphae free of drugs were regularly positioned inside the hyphae as far as the apical dome (Fig. 4A and B). By contrast, the hyphae incubated during one hour with nocodazole have shown an accumulation of nuclei separated by intervals without any nucleus along the hyphae (Fig. 4D).

The nuclei of hyphae having germinated and grown in a medium supplemented with taxol were more regularly positioned than those observed in hyphae being incubated with nocodazole. However some regions of the hyphae were also devoid of nuclei. (Fig. 4F).



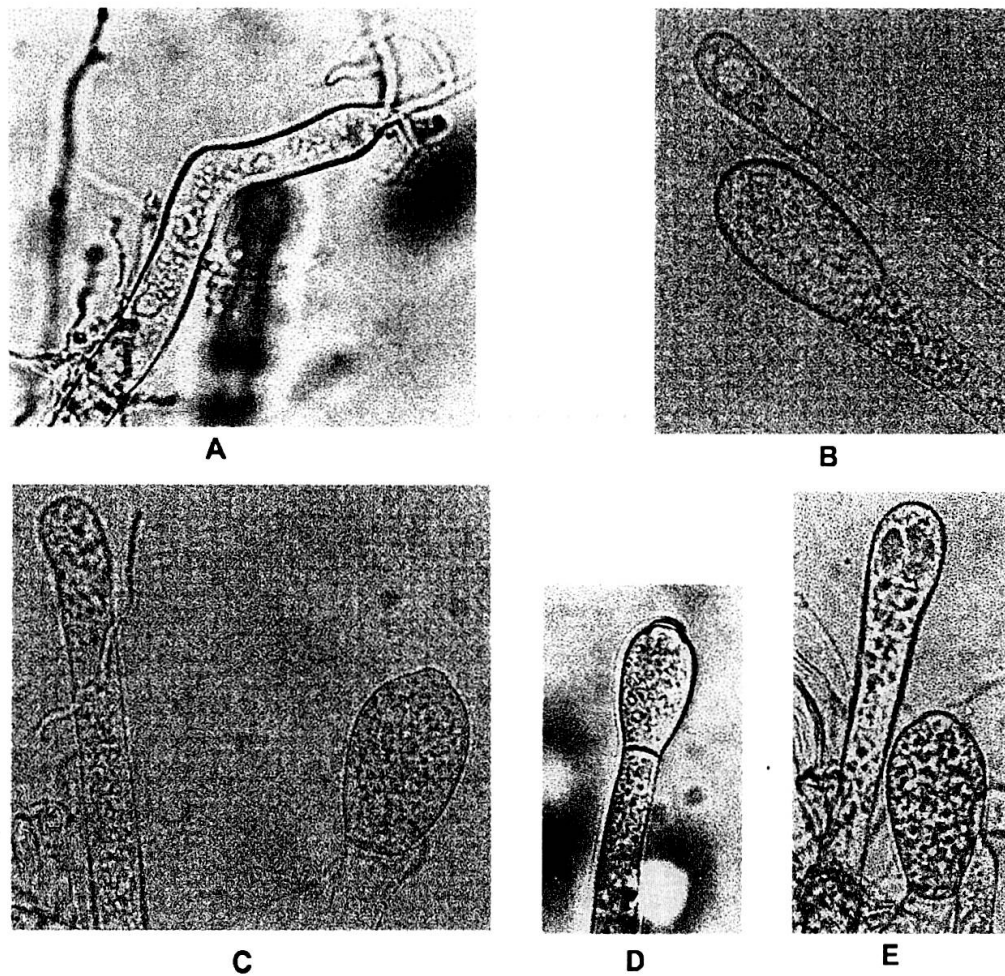


FIG. 3.

From a culture grown without inhibitors, sexual differentiation in the presence of nocodazole (A) or taxol (B). From a culture grown in the presence of 30  $\mu$ M taxol, sexual differentiation without any inhibitor (C) or in the presence of 20  $\mu$ M nocodazole (D) or 30  $\mu$ M Taxol (E). A, B, C, E x 1400 and D x 700.

## DISCUSSION

The experiments described in this report were designed to contribute to a better knowledge to the role of microtubules during the developmental cycle of *A. arbuscula*.

Nocodazole has stopped the previous mycelial growth when it was added to the culture medium. In the preformed hyphae the elbows and lateral rhizoïds observed suggest an inversion of the basoapical polarity, the rhizoïds being normally only present in the basal pole.

Microtubules are therefore essential for the elongation of the hyphae with both a constant diameter and rate of elongation.

The complete inhibition of germination in presence of nocodazole has confirmed that microtubules are necessary to germination. Our results confirmed that they are also implicated in the election of the outgrowth sites of the germinating tubes (CAPPUCCINELLI & MORRIS, 1982; TON THAT *et al.*, 1988; BARJA *et al.*, 1992).

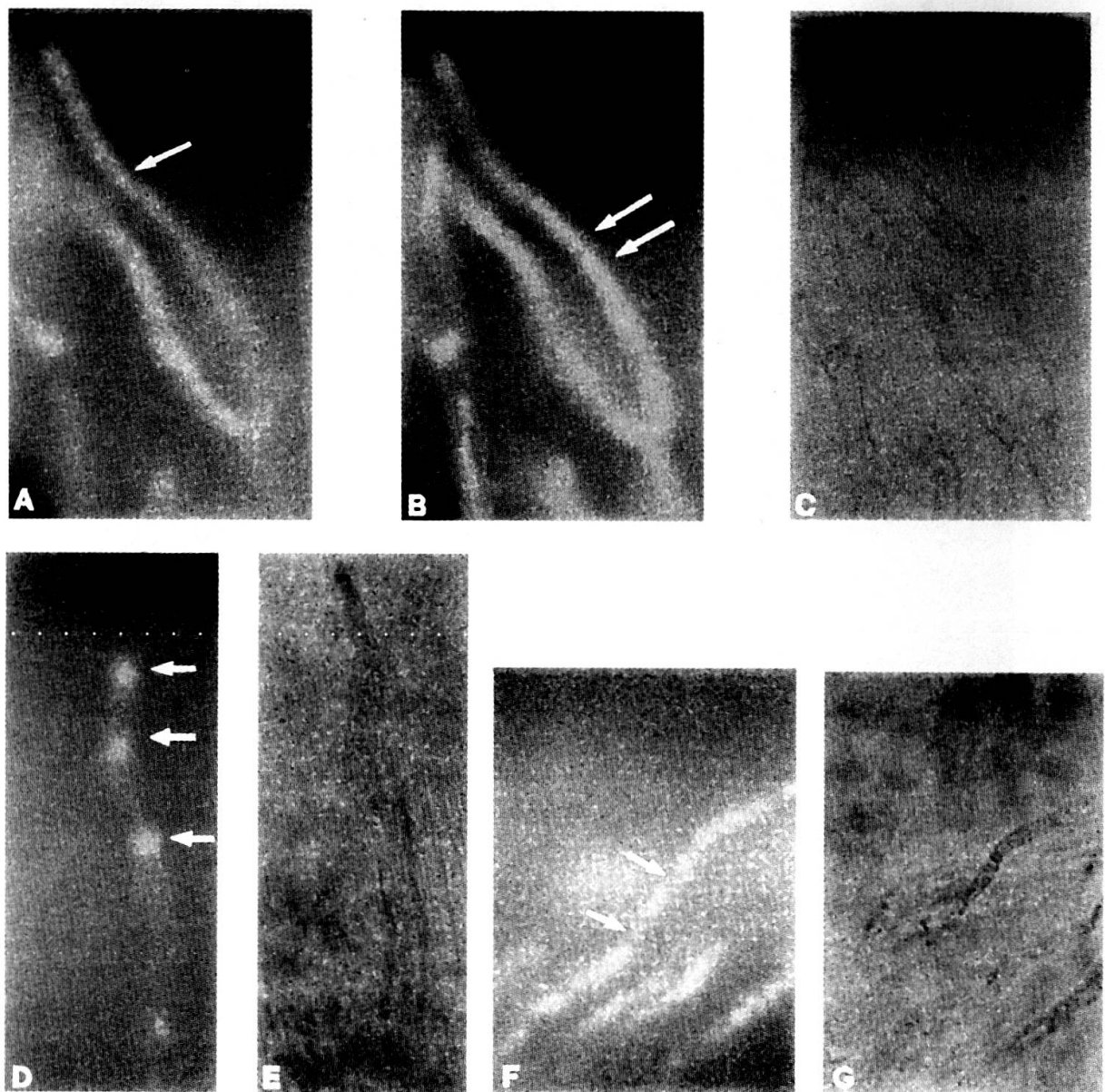


FIG. 4.

Immunofluorescent visualisation of nuclei in vegetative hyphae of *A. arbuscula* not treated (A to C) and treated with 10  $\mu$ M nocodazole (D and E) or 30  $\mu$ M taxol (F and G). X 700.

The large constrictions observed in the zoosporal germ tubes in the presence of nocodazole are very similar to those observed by ORTEGA PEREZ and TURIAN (1987) in the germinating tubes of conidia of *Neurospora crassa* treated by an anticalmodulin agents, suggesting that nocodazole could act indirectly by disturbing the intracellular gradient of calcium controlled by calmodulin.

The backward and supplementary addition of taxol to the culture medium already supplemented by nocodazole also did not allow germination of the zoospore. The effect of nocodazole was thus irreversible at this stage.

By contrast taxol alone allowed a normal germination suggesting that, in the zoospore, the important proportion of microtubules stabilized by the presence of taxol was not morphogenetically disturbing. The recent detection of the stabilizing acetylated isoform of tubulin in the *Allomyces* zoospore (SCHÖNENBERGER & TURIAN, 1993) confirms this beneficial effect of intact microtubules for germ tubes outgrowth as it has also been observed for elongating hyphae.

Finally, the observation by immunofluorescence of the disturbed positioning of nuclei, in the presence of nocodazole, confirms the required parallel role and interaction of microtubules-nuclei in the normal morphogenesis of the mold.

These results thus confirm that "dynamically stable" microtubules play an important role during the developmental cycle of *Allomyces* especially during germination and gametangial differentiation.

## RÉSUMÉ

### CHANGEMENTS MORPHOGÉNÉTIQUES PROVOQUÉS AU COURS DU CYCLE DE DÉVELOPPEMENT D'*ALLOMYCES ARBUSCULA* PAR DEUX AGENTS ANTIMICROTUBULES À EFFET OPPOSÉ.

Des changements morphogénétiques opposés ont été provoqués au cours de la croissance d'*Allomyces arbuscula* en présence de deux agents antimicrotubules: le taxol a maintenu la croissance hyphale par élongation et, de ce fait, a empêché leur différenciation apicale en un couple de gamétanges mâle-femelle; le nocodazole a empêché l'apparition des tubes germinatifs et a induit l'émergence de rhizoïdes épihyphaux ainsi qu'un positionnement différent des noyaux intrahyphaux. La croissance hyphale préliminaire en présence de taxol a empêché l'apparition des modifications morphogénétiques induites par le nocodazole.

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