Effects of Antheridiol on Growth, Branching and Electrical Currents of Hyphae of *Achlya ambisexualis*

By NEIL A. R. GOW AND GRAHAM W. GOODAY*

Department of Genetics and Microbiology, University of Aberdeen, Marischal College,
Aberdeen AB9 1AS, UK

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Treatment of male hyphae of the water mould *Achlya ambisexualis* with the female sex pheromone, antheridiol, caused them to stop extending. This response was accompanied by a marked decline in the characteristic inward current just behind the hyphal apex. After a hypha had stopped, a site of inward current developed some way behind the apex. Then an antheridial branch developed at this site. These responses only occurred in the absence of nutrients; in the presence of nutrients the hyphal extension rate and current pattern were unaffected by the addition of antheridiol.

INTRODUCTION

Female hyphae of the oomycete water moulds of the genus *Achlya* produce a sterol sex pheromone, antheridiol (McMorris, 1978). This causes male hyphae to show a series of responses: characteristic antheridial branching, chemotropic attraction of these branches and their development as antheridia with accompanying meiosis (Barksdale, 1963; Barksdale *et al.*, 1974). Biochemical responses include an increase in cellulase activity (Thomas & Mullins, 1969), an induced metabolism of antheridiol (Musgrave & Nieuwenhuis, 1975), the synthesis of the male pheromone, oogoniol (McMorris, 1978), a marked enhancement of synthesis of protein, rRNA and mRNA, of histone acetylation, and of transcription *in vitro* (Silver & Horgen, 1974; Timberlake, 1976) and changes in the pattern of secreted proteins (Brunt & Silver, 1986). The hyphae of *Achlya* recently have proved amenable to studies of bioelectricity, and currents produced by growing and vegetatively branching hyphae and by developing sporangia have been reported (Kropf, 1986; Kropf *et al.*, 1983, 1984; Armbruster & Weisenseel, 1984; Gow *et al.*, 1984; Gow, 1984). It is clear that vegetatively growing hyphae generate ion currents that enter their apices and exit sub-apically, and that sub-apical vegetative branching is associated with the formation of a localized inward-moving current (Kropf *et al.*, 1983). Here we report on the effect of antheridiol on extension growth, branching and current pattern, and the effect of nutrients on these responses.

METHODS

Fungus growth and conditions. *Achlya ambisexualis*, male, strain E87 was kindly supplied by Professor T. C. McMorris. It was grown on agar plates of the nutrient mating medium of Barksdale *et al.* (1974), modified by substituting casein hydrolysate for Edamin and sodium glycerophosphate for calcium glycerophosphate, with the addition of 60 mg $\text{CaCl}_2 \cdot 6\text{H}_2\text{O l}^{-1}$. All growth and experiments were at 25 °C.

For investigating the effects of antheridiol, the fungus was grown from a central hyphal inoculum for 3 d on agar plates. From each plate, two agar blocks, 2 × 0.5 cm, were excised from the younger part of the culture. Each was left for 30 min, stuck in a plastic Petri dish with silicone dental cement and just submerged either in artificial pond water (0.1 mM-KCl, 0.1 mM- CaCl_2 , 1.0 mM-NaCl, pH 6.0) or in the nutrient mating medium. The hyphae that grew out from the top surface were viewed with an inverted microscope. Large hyphae that were vigorously growing straight out from the block were chosen for further examination.

Fig. 1. Growth (●) and apical inward current (○) of three hyphae of *A. ambisexualis* before and after addition of antheridiol (at A, bold arrowhead). Hyphae (a) and (b) were growing into artificial pond water. Hypha (a) stopped growing 92 mins after addition (at S, solid arrow); branches formed 14, 15, 18 and 20 min later (at B, arrows). Hypha (b) stopped 63 min after addition (at S), but unusually no branches formed. Hypha (c) was growing into the nutrient mating medium, and did not stop growing or branch.

Electrical measurements. Electrical currents were measured with a one-dimensional vibrating probe system (Vibrating Probe Co., Davis, Calif., USA) successively at positions closely adjacent to a hypha and at reference positions at least 300 μm from the hypha (Kropf *et al.*, 1984; Gow, 1984). The electrode was vibrated at between 200 and 400 Hz with the vibration amplitude at 30 μm . Quadrature measurements were made at intervals with the lock-in amplifier 90° out of phase. These were in no case larger than 5% of the in-phase signal. The current density was calculated from the electrical field and the medium resistivity (Jaffe & Nuccitelli, 1974). The resistivity was determined from the conductivity of the bathing media, and did not change during experiments.

Effect of antheridiol. Pure synthetic antheridiol (kindly supplied by Professor T. C. McMorris) was stored in a solution of 100 $\mu\text{g ml}^{-1}$ in methanol at -20°C . When it was established that the hyphae were growing at a linear extension rate as measured with an eyepiece micrometer, and when steady state current flows had been recorded, antheridiol was added to give an initial nominal concentration of 50 ng ml^{-1} . We term this 'nominal' concentration as antheridiol adsorbs onto plastic surfaces (Barksdale, 1963). Control experiments had an equal volume of methanol.

RESULTS

Hyphae grew out from the block of nutrient agar into the non-nutrient artificial pond water medium at constant extension rates. If treated with antheridiol within the first 2 or 3 h, their extension rates slowed, and they stopped extending between 40 and 100 min later (Fig. 1 *a, b*). The first antheridial branches were formed sub-apically between 40 and 100 min later (Fig. 1 *a*). The first sign of these were slight bulges, which abruptly herniated and grew as contorted branches (Fig. 2). Very rarely, the stopped hypha did not branch (Fig. 1 *b*). In the absence of antheridiol, the hyphae grew out for several hours more, and then sporulated. If the agar blocks

Fig. 2. Antheridial branching of the hypha documented in Fig. 1(a). Four sub-apical branches have formed. The probe is vibrating alongside the hypha. Conditions as described in Fig. 1(a). Bar, 100 μm .

of *Achlya* were mounted in liquid nutrient medium, vegetative hyphae grew out without sporulating for several days. These hyphae showed no response when antheridiol was added (Fig. 1c). Control hyphae, i.e. with methanol added, grew normally without branching and eventually sporulated.

Current maps along the hyphae before addition of antheridiol showed the pattern typical of growing hyphae of *Achlya*, with the inward current of the apical region declining further back along the hypha, eventually giving way to outward current in the distal regions. Following addition of antheridiol, initially this current pattern was maintained, but with the inward apical currents rapidly showing the onset of steady decline to reach about a quarter of their former values by the time they had stopped extending, e.g. from 0.44 to 0.10 $\mu\text{A cm}^{-2}$ (Fig. 1a). Then new sites of inward current were detected, which increased in magnitude severalfold in a few minutes (Fig. 3). Observation at these sites showed that they became the sites of antheridial branches (Figs 2 and 3). Each new inward current site that was detected was followed by branching. Conversely, no branches were formed in regions not showing such currents or in the occasional hypha not showing any such new inward currents (e.g. that shown in Fig. 1b). Hyphae growing out into the nutrient mating medium showed no change in current map following addition of antheridiol.

DISCUSSION

The current maps of hyphae of *A. ambisexualis* E87 growing out into the nutrient or non-nutrient media, showing inward apical and outward distal currents, are similar to those of *Achlya debaryana* growing from hemp seeds into pond water (Armbruster & Weisenseel, 1983) and of *Achlya bisexualis* growing from agar into nutrient medium (Kropf *et al.*, 1983, 1984; Gow *et al.*, 1984; Gow, 1984).

The marked decline in apical current following addition of antheridiol can be compared with the abolition of inward current accompanying cessation of growth caused by pH change or removal of amino acids (Kropf *et al.*, 1984). A notable difference is that the hyphae stopped by antheridiol still retained a measurable inward apical current.

The observation of a new inward current preceding the formation of an antheridial branch and predicting its site of emergence can be compared with the report of Kropf *et al.* (1983) of a similar occurrence during vegetative branch formation. The antheridiol-induced response, however, forms a better system for study as, unlike vegetative branching, antheridial branching can be directly controlled.

The lack of electrical or branching response to antheridiol in the presence of nutrients is in accord with the study of Mullins & Warren (1975) who concluded 'in *Achlya* that exogenous nutrient conditions favourable for vegetative growth are unfavourable for sexual reproduction'. The cessation of growth of hyphae responding to antheridiol does not seem to have been reported before, but is in accord with the growth arrest of *Saccharomyces cerevisiae* and *Phycomyces blakesleeanus* in response to their specific sex pheromones (Betz & Duntze, 1979; Drinkard *et al.*, 1982). Here, we have only measured this growth arrest as cessation of apical growth rate and it is possible that increase in other growth parameters might continue unabated.

Fig. 3. Longitudinal current maps of the hypha shown in Figs 1(a) and 2 at five different times following addition of antheridiol: 78–83 min (▲), 92–95 min (△) when the hypha stopped elongating, 96–98 min (■), 98–104 min (□) and 104–112 min (●) when the four branches were formed. Positions of branches are indicated by arrows.

The biochemical responses to antheridiol outlined in the Introduction (Horgen, 1981; Gooday, 1983) are observed from about 30 min after exposure of *Achlya* to antheridiol and there is evidence for a cytosolic antheridiol-binding protein with properties similar to those of vertebrate steroid receptors (Riehl *et al.*, 1984). However, the speed of response of the apical current to the addition of antheridiol shown in Fig. 1(a) suggests that in addition there may be surface receptors for this sterol.

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