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EFFECT OF CULTURE MEDIUM COMPOSITION ON PHEROMONE RECEPTOR LEVELS IN *ACHLYA AMBISEXUALIS*

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Summary—Sexual reproduction in the eukaryotic fungi *Achlya* is controlled by two steroid pheromones. Antheridiol is the steroid released by female cells that induces male sexual differentiation. The antheridiol-induced response of male cells has been shown to be influenced by the composition of the culture medium. The present study was designed to determine if the composition of the culture media might also affect the levels of antheridiol binding protein in the cytosol of male cells. The mycelial content of cytosolic steroid pheromone binding sites in *Achlya ambisexualis* E87 males was measured at daily intervals during 6 days of suspension culture in media containing different nitrogen sources. Levels of binding sites increased during the first 2 days in culture to a plateau that was maintained for the next 2–3 days. During the first 3 days in culture, levels were much lower in mycelia cultured in an enriched medium containing lactalbumin hydrolysate compared to mycelia cultured in defined media containing glutamic acid as the nitrogen source. The level of binding sites increased rapidly when mycelia were transferred from an enriched medium to a nutrient-free salt solution and decreased when mycelia were transferred from a defined to an enriched medium. The relative differences in cytosolic binding measured by *in vitro* radioligand saturation analysis were confirmed by *in vivo* uptake studies. It is concluded that the mycelial content of antheridiol binding sites can be experimentally manipulated by variations in the composition of the culture medium and/or the time period of incubation in the medium.

INTRODUCTION

The composition of the medium in which strains of *Achlya* are cultured in the laboratory has been shown to affect mycelial growth and reproduction [1–6]. Culture conditions that are favorable for vegetative growth adversely affect male sexual differentiation and development. The objectives of the present work were to determine if the media composition and/or culture age affect the mycelial levels of the antheridiol binding protein recently detected [7] in *Achlya ambisexualis* Raper strain E87. In the latter study [7], mycelia were obtained from 44-h cultures incubated in a defined minimal medium [8] containing glutamate as a nitrogen source. The nitrogen source in the medium has been shown to be one of the major factors modulating growth and sexual reproduction in *Achlya* [2, 3]. It was, therefore, of interest to determine and compare the levels of antheridiol binding protein in mycelia cultured within a relatively enriched medium containing an enzymatic hydrolysate of lactalbumin as the source of nitrogen with the levels measured in mycelia cultured within a minimal medium. Among studies concerning sexual reproduction, the difference in age of the *Achlya* cultures employed for analysis has not received much attention as an experimental variable despite the relationship between culture age, cell density, nutrition and growth in the closed systems used for *in vitro* culture of microorganisms. In the present study, the levels of antheridiol binding protein were measured at different time periods of incubation in a defined minimal medium and in enriched medium to determine the levels of binding protein in mycelia of various culture age.

We report that the mycelial content of antheridiol binding protein is dependent on the composition of the culture medium and the age of the culture. In addition, we demonstrate that the levels of binding protein can be increased or decreased by changing the composition of the culture medium.

EXPERIMENTAL

Chemicals

Bacto-peptone, Bacto-agar, Bacto-corn meal agar and yeast extract were purchased from DIFCO Laboratories (Detroit, Michigan). Lactalbumin hydrolysate was purchased from Gibco (Grand Island, New York). L-(−)-Methionine was purchased from Eastman Kodak Co. (Rochester, New York). L-α-aminoglutaric acid-monopotassium salt (potassium glutamate) was purchased from Sigma Chemical Co. (St Louis, Missouri). Nonradioactive antheridiol and its congeners were supplied by Dr Trevor C. McMorris, Department of Chemistry, University of California, San Diego. The radioactive analog of

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antheridiol, [1,2-^{3}H]7-deoxy-7-dihydro-antheridiol ([^{3}H]7dA*; sp. act. = 40 Ci/mmol), was prepared as will be described elsewhere (Meyer *et al.*, unpublished data).

Culture media

PYG medium [9]. 1.25 g/l Bacto-peptone/1.25 g/l Yeast Extract/2.5 g/l glucose/10 mM 1,4-piperazine-diethanesulphonic acid (pH 6.9).

PYG agar. 1.5% (w/v) Bacto-agar in PYG medium.

Corn meal agar. 1.5% (w/v) Bacto-cornmeal agar in distilled deionized water.

M1 medium [8]. 10 mM Hepes (pH 7.1)/2.6 mM potassium glutamate/0.1 mM L-methionine/16 mM glucose/2.0 mM KCl/1.5 mM KH_2PO_4/0.5 mM $CaCl_2$/ 0.5 mM $MgSO_4$/5.0 μM Na_2EDTA/4.5 μM $FeSO_4$/11 μM boric acid/1.8 μM $MnCl_2$/0.7 μM $CpCl_2$/0.36 μM $ZnSO_4$/0.32 μM $CuSO_4$/0.4 μM Na_2MoO_4.

M2 medium. Identical to the composition of M1 medium except 0.4 g/l lactalbumin hydrolysate was substituted for potassium glutamate.

M1 salts. M1 medium without potassium glutamate, methionine and glucose.

All broth media were compiled from appropriate aliquots of concentrated stock solutions of individual components and sterilized by filtration through Falcon 7105 bottle-top filter units (0.22 μm porosity) into sterile 2-l Erlenmeyer flasks. PYG medium for spore germination and media containing agar were autoclaved.

Culture conditions

Stock cultures of *Achlya ambisexualis* Raper strains E87 (male) and 734 (female) were obtained from T. C. McMorris and maintained on corn meal agar slant cultures under mineral oil at 4°C. At 1-year intervals, aliquots were removed and reinnoculated on fresh corn meal agar slants, grown for 6 days at 23°C, covered with mineral oil and stored as described.

Preparation of zoospores

Asexual spores were prepared after the method of Griffin and Breuker[5]. The spore suspension containing approx. $1–2 \cdot 10^5$ spores per ml was stored at 4°C for no longer than 1 month.

Mycelia growth in suspension culture

Aliquots (1 ml) of the spore stock were added to 100 ml of PYG medium in a siliconized 250 ml Erlenmeyer flask and incubated at 29°C, 150 rpm, for 24 h in an orbital shaker. The germinated spores and media were then added to 2 l of appropriate medium contained within a 2 l Erlenmeyer flask. Cultures were gassed with filtered (0.22 μm) air bubbled through the media at a rate of 1–2 l per min and incubated at 23°C for different time intervals.

Mycelia were transferred to 2 l of fresh media after collection in a sterile porcelain funnel and incubation continued for different time intervals.

Buffer solutions

The following solutions were employed for subcellular fractionation of the mycelia:

Buffer A: 50 mM Mops (pH 7.2)/25 mM Na_2MoO_4.

Buffer B: 50 mM Mops (pH 7.2)/25 mM Na_2MoO_4/100 mM KCl.

Buffer T: 50 mM Mops (pH 7.5)/25 mM Na_2MoO_4/100 mM KCl/10 mM 2-mercaptoethanol/ 0.5 mM phenylmethylsulfonylfluoride (PMSF).

Buffer B was prepared and held *in vacuo* with stirring for 24 h at 4°C and stored tightly capped at 4°C. On the day of use, an appropriate volume was sparged with nitrogen gas for 30 min prior to adding the mercaptoethanol and PMSF to form Buffer T. Buffer solutions were degassed and sparged with nitrogen gas to prevent development of a yellow coloration in the cytosol that reduced the efficiency of liquid scintillation counting (unpublished observation).

Preparation of cytosol

Mycelia were harvested by collection on a stainless steel strainer and resuspended in 500 ml Buffer A at 23°C and transferred to a fritted glass funnel (Pyrex No. 36060). After 5-min incubation in Buffer A, the mycelia were collected by vacuum filtration and transferred to 300 ml ice-cold Buffer B and incubated for approx. 2 min at 4°C.

The mycelia were then collected as several thin mats by successive transfers of enough of the suspension to cover the fritted glass portion of the funnel resulting in a mat of approx. 1 mm thickness after vacuum filtration. The mycelial mats were then weighed, wrapped in foil, and immersed in liquid nitrogen. Equal wet weights no greater than 25 g from each culture grown in different media and for different time periods were frozen for preparation of cytosol and subsequent assays.

Disruption of the mycelia was accomplished as follows: A 250 ml Waring stainless steel blender cup was precooled in liquid nitrogen and mounted on the blender base. The frozen mycelia mats were first fragmented in the foil pouch by pounding with a metal bar, transferred to the blender cup, and pulverized for 20 s. The resultant powder was transferred to a 100 ml homogenizer tube and 40 ml of Buffer T was added. The frozen powder and buffer were mixed with a spatula and thawed to 0–2°C in a warm water bath (37–40°C) with constant stirring. The brei was subjected to two strokes of a motor-driven Teflon pestle and filtered through two layers of Miracloth

Abbreviations: [^{3}H]7dA: [1,2-^{3}H]7-deoxy-7-dihydro-antheridiol. Hepes: 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid. Mops: 4-morpholinepropane-sulphonic acid.

(Calbiochem). The filtrate was centrifuged at 250,000 *g* for 1 h at 0–2°C and the supernatant clarified by filtration (0.45 μ). The resultant cytosol was pretreated with dextran-coated charcoal by adding 20–25 ml of cytosol to the pellet (2000 *g* for 10 min) of a 2.5 ml aliquot of 5% (w/v) Norit-A/0.5% (w/v) dextran T-70 suspension. After 15 min at 4°C, charcoal was removed from the cytosol by centrifugation at 12,000 *g* for 20 min and filtration of the decanted supernatant through a 0.22 μm membrane filter (Millipore). Cytosol was incubated with charcoal before the binding assays because preliminary studies revealed that the addition of phenylmethylsulfonylflouride (PMSF) resulted in an increase in specific binding of roughly 15% only if pretreatment with charcoal was performed. Without charcoal pretreatment, specific binding was inhibited in the presence of PMSF (unpublished observations). The pH of the resultant cytosol ranged from 6.8–7.2 depending upon the type of culture used and was routinely adjusted to pH 7.0 at 0°C before subsequent analysis. Preparation of cytosols by freezing was found to be superior in regard to the efficiency of mycelial breakage when compared to the Polytron method employed in a previous study [7]. No detectable difference in the binding of [^{3}H]7dA to the receptor could be detected in cytosols prepared by each method but the yield of receptor and other soluble protein was greater using the techniques and buffer solutions described above (unpublished observations). Protein equivalents were assayed by the modified Lowry procedure of Peterson[10] with ovalbumin substituted as the standard.

Radioligand binding assay: in vitro

All steroid stock solutions were stored at −20°C in 2-propanol and added to 12 × 75 mm conical polystyrene culture tubes immediately prior to addition of cytosol. The final concentration of 2-propanol in the assay volume was no greater than 1% (v/v). Incubation was routinely performed at 0°C in an ice–water slush for 2 h in the presence of a range of [^{3}H]7dA concentrations (0.3–25 nM). Unbound steroid was then adsorbed to dextran-coated charcoal over a 4-min period after the addition of a 200 μl aliquot of a concentrated dextran-coated charcoal suspension (5% (w/v) Norit-A/0.5% (w/v) dextran T-70/0.01% (w/v) NaN_3) to 1.0 ml of incubation volume and pelleted by centrifugation at 2000 *g* for 4 min.

The amount of bound tritium-labeled steroid was measured in 0.5 ml aliquots of the supernatant by liquid scintillation spectrophotometry in Beckman Ready Solv-HP cocktail at an efficiency of 39% determined by internal standardization. Nonspecific binding was defined as the amount of [^{3}H]7dA bound in the presence of a 7-fold molar excess of unlabeled antheridiol. Specific binding was calculated by subtracting the amount of nonspecific binding from the total amount of [^{3}H]7dA bound in the absence of unlabeled antheridiol. Alternatively, specific binding was determined by the method of Chamness and McGuire[11] or Rosenthal[12].

The total binding site content of mycelia harvested after various incubation periods in different media was determined as follows: Cytosol was prepared from each culture and assayed for the maximum [^{3}H]7dA binding capacity by Scatchard analysis of the equilibrium binding data [13]. The intercept on the horizontal axis of the Scatchard plot represents the maximum concentration of binding sites (mol l^{-1}) in the cytosol. This maximum concentration was multiplied by the total volume of cytosol (liter) to obtain the value for the total moles of cytosolic binding sites obtained from the wet weight of mycelium that was disrupted to produce the cytosol. This value was divided by the mycelial wet weight in grams to obtain the total moles of binding sites per gram of wet weight. Differences in the total number of binding sites observed among experimental groups were tested for significance by the Newman–Keuls multiple range analysis of variance test.

Radioligand binding in vivo

Mycelia were collected on a stainless steel strainer and 500 ml aliquots of the media in the filtrate were adjusted to contain 1.2 nM [^{3}H]7dA in the presence and absence of a 50-fold excess of unlabeled antheridiol. The total wet weight was divided and $\frac{1}{2}$ added to media containing only [^{3}H]7dA. The other half was added to media containing an equal concentration of [^{3}H]7dA and an additional 50-fold excess of antheridiol. Cultures were incubated at 23°C for 15–120 min then collected and processed for preparation of the cytosol as described above except that no pretreatment of the cytosol with dextran-coated charcoal was employed. The amount of specific [^{3}H]7dA binding in aliquots of the cytosol was determined in triplicate by the dextran-coated charcoal adsorption technique used for the *in vitro* binding assays.

RESULTS

The cytosolic protein (mg/g wet weight) and levels of antheridiol binding sites (pmol/g wet wt) of mycelia measured at 24 h intervals during 6 days of incubation in different media are shown in Figs 1A and 1B, respectively. Except for a decrease during the second day of incubation in M2 medium, the levels of cytosolic protein (Fig. 1A) were found to remain constant over a 6-day period. However, the cytosolic protein content of mycelia incubated in M2 medium (henceforth referred to as M2 cultures) were at all times greater than those in mycelia incubated in M1 medium (henceforth referred to as M1 cultures).

Because M2 cultures contained more cytosolic protein, a conventional method of expressing receptor binding capacities that are normalized on the basis of moles of specifically bound hormone per mass of cytosolic protein was not used in this study.

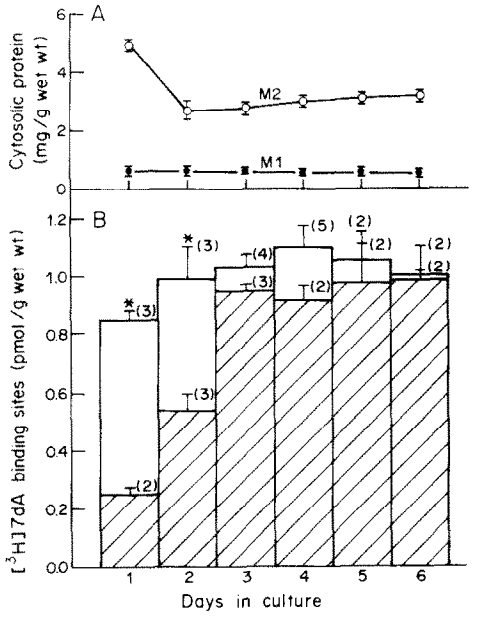

Fig. 1. Cytosolic content of total protein and [^{3}H]7dA binding sites of mycelia measured at 24 h intervals during 6 days of culture in different media. *Panel A*: Protein content (mg/g wet wt) in cytosol of mycelia cultured in M1 (●---●) and M2 (○---○) media. Each point and vertical bar represent the mean value and SE, respectively, of two separate experiments. *Panel B*: Cytosolic content of [^{3}H]7dA binding sites (pmol/g wet wt) in mycelia. Total height of bar (shaded and unshaded) represents the mean value for mycelia cultured in M1 media. The shaded portion of each bar represents the mean value for mycelia cultured in M2 media. Vertical lines represent the SE obtained from the number of experiments indicated parenthetically. Asterisk denotes significance ($P < 0.01$) between the difference in mean values (unshaded region) obtained from M1 and M2 cultures.

This method would result in values of receptor levels in M2 cultures that were apparently lower than those in M1 cultures simply because M2 cultures contain more cytosolic protein. We chose not to express the binding data on a DNA basis because the DNA content in *Achlya* mycelia is very low and difficult to measure with precision. Griffin *et al.*[2] have shown that the DNA content per unit of mycelia mass remains quite constant during growth in various media. Therefore, the changes in binding site concentration observed in our study are unlikely to be associated with changes in DNA concentration. Values for the maximum binding capacities were, therefore, normalized on the basis of the mass (wet weight) of mycelium used in preparation of the cytosol as described in Experimental. When expressed in this manner, the differences in maximum binding capacities of M1 and M2 cultures (Fig. 1B) were significant ($P < 0.01$) during the first 2 days of incubation. In both M1 and M2 cultures, the mean receptor levels increased to values that remained relatively constant for at least 6 days in culture. Although differences in maximum binding were observed, no significant differences in binding site affinity were detected among the various cultures. The mean value ($N = 24$) of the equilibrium dissociation constant was $2.3 \cdot 10^{-9}$ M $\pm$ 0.64 SD among all cultures.

In order to test the effect of the media composition on the number of binding sites independently from the age of the mycelia, cultures containing maximum levels of binding sites (3-day M1 cultures) were transferred to M2 or PYG media and mycelia containing low levels of binding sites (2-day M2 cultures) were transferred to a nutrient-free salt solution as described in the Experimental section. The results of measurements of the binding site content of these cultures at various time intervals after transfer are depicted in Figs 2A and 2B.

As shown in Fig. 2A, the transfer of 3-day M1 cultures to fresh M2 or PYG media resulted in a significant ($P < 0.01$) decrease in the quantity of binding sites after 3 h of incubation. The binding site content continued to decline until at 24 h post-transfer, the mean values were 28 and 10% of the mean value in 3-day M1 cultures for M2 and PYG transfers, respectively. In contrast, the mean levels measured 24 h after transfer of 3-day M1 cultures to fresh M1 media remained elevated at the plateau level observed for 2- to 6-day M1 cultures (Fig. 2A). The results of the transfer of 2-day M2 cultures to M1 salt solution are shown in Fig. 2B. Significantly ($P < 0.01$) greater levels of binding sites were measured at 12 h and at 24 h post-transfer. After 24 h, the mean value was equal to that of 4-day M1 cultures and greater than that in 3-day M2 cultures. The cytosolic protein content of mycelia increased in a

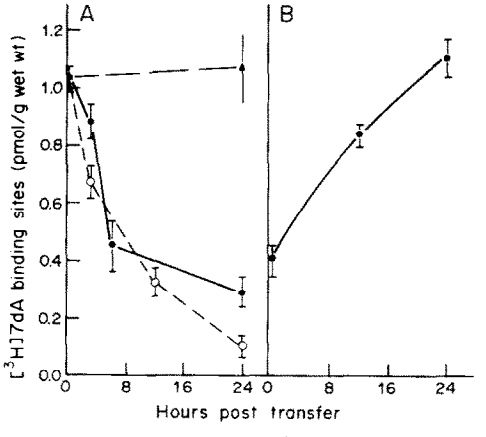

Fig. 2. Regulation of cytosolic [^{3}H]7dA binding sites by media composition. The mycelial content of [^{3}H]7dA binding sites (pmol/g wet wt) was measured at the time interval indicated on the abscissa after transfer of: *Panel A*: A 3-day M1 culture to M2 media (●---●) or to PYG media (○---○) and after transfer of: *Panel B*: a 2-day M2 culture to a nutrient-free salt solution (●---●). Each point and vertical line represent the mean and SE, respectively, of two separate experiments. Zero time points represent the control values for 3-day M1 and 2-day M2 cultures (reshown from Fig. 1) for comparison. The mean $\pm$ SE ($N = 2$) of a 3-day M1 culture transferred to fresh M1 media for 24 h is shown (●---▲) in Panel A.

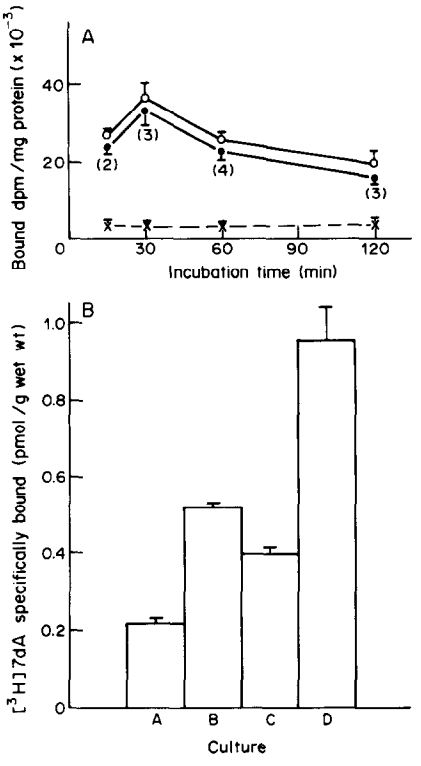

Fig. 3. *In vivo* uptake of [^{3}H]7dA. *Panel A*: 3-Day M1 cultures were exposed to 1.2 nM [^{3}H]7dA at zero time and the amount of bound [^{3}H]7dA (dpm/mg protein) in the cytosol determined at the time intervals indicated on the abscissa. Each point and vertical line represent the mean value ($N = 2$) and SE, respectively, for the amount bound in the presence (×---×) and absence (○---○) of a 50-fold excess of unlabeled antheridiol and the amount specifically bound (●---●) calculated as the difference between the two. *Panel B*: The amount of specifically bound [^{3}H]7dA (pmol/g wet wt) after 30 min of uptake in mycelia cultured in M1 media for 1 day (A) and 3 days (B); M2 media for 3 days (C) and M2 media for 2 days, then 1 day in M1 salts (D). Height of each bar and vertical line represent the mean value and SE, respectively, from two separate experiments.

time-dependent manner following transfer to M2 or PYG media and decreased after transfer to the nutrient-free salt solution (data not shown).

Cultures having different cytosolic levels of binding sites, as assessed by *in vitro* radioligand binding assays, were also tested for the quantity of binding sites that could be measured by *in vivo* uptake of [^{3}H]7dA as described in Experimental. The results of preliminary studies using 3-day M1 cultures to determine the time course of uptake are illustrated in Fig. 3A. The cytosolic level of specifically bound [^{3}H]7dA reached a peak value at 30 min of incubation and then declined over the following 1.5 h. Figure 3B shows the quantities of binding sites measured after 30 min of *in vivo* uptake by: 1-day and 3-day M1 cultures, 3-day M2 cultures and 2-day M2 cultures transferred to M1 salts for 24 h. The latter cultures exhibited the greatest amount of *in vivo* labeled binding sites among those tested. The amounts measured in 3-day M1 cultures were greater than those in 3-day M2 cultures and both of these cultures had greater amounts than 1-day M1 cultures. Although saturation analysis was not performed *in vivo*, the quantity of binding sites measured were greater than 50% of their respective saturation values determined *in vitro* indicating that a majority of the binding sites in each culture were detected by *in vivo* uptake.

In addition to M1 and M2 media, mycelia were also cultured in PYG medium, an even richer medium which is often used for spore germination [5–8]. The maximum binding capacitites in PYG cultures were too low for accurate measurement utilizing *in vitro* binding studies despite analysis by several different methods [11, 12]. Under *in vivo* labeling conditions, results with PYG cultures were variable and ranged from no detectable specific binding to detection in a 4-day culture of very low levels of receptor (0.016 pmol/g wet wt). As a negative control, the female strain 734, which does not respond to antheridiol, was cultured for 3–4 days in M1 medium and assayed for specific [^{3}H]7dA binding both *in vitro* and *in vivo*. No specific binding could be detected in the cytosol either *in vitro* or *in vivo* (data not shown).

DISCUSSION

The results of these experiments indicate that, under conventional laboratory culture conditions, the number of antheridiol binding sites in *A. ambisexualis* E87 mycelia is dependent on both the time period of incubation and the composition of the medium. Furthermore, the level of binding sites can be experimentally manipulated by transfer of mycelia previously grown in one type of medium to another medium of different composition.

The interrelationship of media composition and incubation time are illustrated in Fig. 1B. The levels of cytosolic binding in M1 and M2 cultures increase to the same maximum levels regardless of the difference in media composition. However, M1 cultures attain maximum levels after 2 days of incubation compared to 3 days for M2 cultures. That the absolute chronological age of the mycelium does not independently affect the content of binding sites is most clearly demonstrated by the results shown in Fig. 2. The mean receptor content of 3-day M1 incubates (which are maximum under the growth conditions employed in this study) rapidly decreases after transfer to M2 or PYG medium (Fig. 2A). After 24 h in M2 medium, the mean level is 28% of that measured in 4-day M1 cultures and approximates the levels measured in 1-day M2 cultures. Because the chronological age of 4-day M1 cultures and 3-day M1 plus 1-day M2 cultures are equal, the absolute age of the mycelium did not influence the quantity of binding sites.

Validation of the relative difference in cytosolic

maximum binding capacities as measured by *in vitro* saturation analyses was performed by measurements of the amount of [^{3}H]7dA bound to the receptor after 30 min of exposure to [^{3}H]7dA (1.2 nM) in the culture medium as shown in Fig. 3B. Cytosolic receptor levels measured by *in vivo* uptake exhibited the same relative differences as those measured *in vitro*. In addition, cytosols from 3-day M1 cultures and 2-day M2 cultures were mixed 1:1 (v/v) and the maximum binding capacity measured in the mixture. The results (data not shown) were consistent with a summation of the maximum binding capacities of each cytosol (diluted 1:1 (v/v) with buffer) indicating an absence of a factor(s) in cytosols from M2 cultures that could mask or degrade receptor sites and result in apparently lower receptor levels. Also, the quantities of receptor that were extracted from 2-day M2 and 3-day M1 cytosols by adsorption to DEAE-cellulose [14] were consistent with the difference in the cytosolic maximum binding capacities of these two cultures (data not shown).

The reasons for the observed effect of medium composition on mycelial levels of binding protein are unknown. One might logically speculate that the composition of the medium determines the time course and extent of metabolic depletion or repletion of intrahyphal nutrient supply which, in turn, affects the level of binding protein. However, it should be noted that in this study and in other laboratories, the germination of *Achlya* zoospores is accomplished by 12–24 h of incubation in the enriched PYG medium. The germlings and conditioned PYG media are then innoculated into a larger volume of culture medium. The preliminary germination in PGY medium and carry-over of conditioned medium would likely repress hormone binding activity in the young mycelia. Therefore, one cannot determine from the time-course study (Fig. 1) the relative contributions of nutrient depletion and mycelial age or development to the generation of binding activity. Nutrient depletion seems clearly to be an important factor, but there may also exist a critical period of development following spore germination that is required before binding activity is expressed. That down-regulation of binding activity was not observed after transfer to fresh M1 media (Fig. 2) indicates that the nature as well as the quantity of nitrogenous nutrients is also an important variable.

The increase in binding site content of 2-day M2 mycelia transferred to M1 salts occurred under conditions similar to those that induce sporangial differentiation in *Achlya* [5, 6, 15–17] and reproductive differentiation in other fungi according to the principles outlined by Klebs [see reference 15 for review]. It has been shown that transfer of *Achlya ambisexualis* E87 mycelia grown for 30 h in PYG media to distilled water is followed by changes in gene expression and the relative synthetic rate of certain proteins during sporangiogenesis [16]. Analysis of a homothallic *Achlya* strain with similar transfer studies identified two classes of proteins whose synthesis was affected [17].

It is not known if the results of this study have a direct relationship to sexual reproduction in *Achlya*. The mechanism of action of antheridiol, or any steroid hormone, depends upon the interaction of a number of cellular processes in which one component is the binding of the steroid to the cytosolic receptor. In this regard, preliminary studies have been performed that compared the response of male mycelia cultured under different conditions to exogenously added [^{3}H]7dA. In comparison to mycelia with low levels, the response of mycelia with high levels of binding sites was correlated with a reduced lag period before the appearance of branch initials and with a lower threshold concentration of [^{3}H]7dA that would elicit a response (data not shown). These results indicate that the culture history of a male mycelium should be considered as an important variable in the analysis of the effect of nutrition on sexual reproduction in *Achlya*. Additional studies are needed in which an objective measurement of the magnitude of the response can be employed to define a role for the antheridiol binding protein in sexual reproduction.

The manipulation of the levels of antheridiol binding protein that can be achieved by alterations in incubation time periods and media composition are significant to experiments designed to study the binding protein molecule *per se* and the mechanism of action of antheridiol. For purification purposes, these results indicate that a 3- or 4-day M1 culture (compared to M2 or PYG cultures) provides a cytosol in which the binding protein probably represents a greater percentage of the total cytosolic protein and would serve as an optimum starting material. A 2-day M2 culture transferred to M1 salts for 24 h should provide a similar cytosol. However, this transfer technique doubles the requirement for sterile media and glassware, increases the probability of contamination, and is inexpedient for routine use with the culture conditions described in this report. The technique may prove to be invaluable in future studies regarding the synthesis and regulation of the binding protein. If so, then the utility of the technique should outweigh its practical drawbacks.

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