CYTOSOL-HORMONE STIMULATION OF TRANSCRIPTION IN THE AQUATIC FUNGUS, *Achlya ambisexualis*

by

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SUMMARY. Incubation of *Achlya* chromatin with antheridiol and the cytosol from male strain E87 increases the RNA synthetic capacity of the chromatin. Neither cytosol, nor antheridiol alone, nor mixtures of hormone with cytosol from the female strain 734 stimulated transcription of the chromatin. A temperature-dependent incubation of the steroid-cytosol complex and high ionic strength (0.05 - 0.12 M potassium chloride) was required for optimal hormone-cytosol stimulation of *in vitro* transcription. These results strongly suggest that complex hormonal control mechanisms similar to those found in animal systems exist in the primitive fungus *Achlya*.

INTRODUCTION. Animal hormone-dependent tissues contain steroid-binding macromolecules, or steroid receptors, with which the hormone interacts by a three stage mechanism (1). Hormone enters the cells and binds to specific extra-nuclear receptor proteins characteristic of the target tissue. The receptor-hormone complex is then translocated to the nucleus and on route encounters an alteration of the receptor protein, a phenomenon called receptor transformation (1). The steroid-protein complex then enters the nucleus where it associates with an acceptor site on the chromatin and affects RNA synthesis both in a quantitative and qualitative manner (2). These RNA synthetic changes have been related to changing levels of RNA polymerase activity (2-6), enhancement of chromatin template activity (2,3-6) and by elevating chromatin template capacity (2,8-11).

Are these cellular control mechanisms of gene activation unique to highly evolved animal systems? Steroid hormones play an integral role in the sexual reproductive processes of certain aquatic fungi. The Oömycete genus *Achlya* has at least two well defined steroid sex hormones, antheridiol and oögoniol (12,13). Female strains of *Achlya* continually secrete antheridiol which induces in the male strain both the production of sex organ initials

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and the secretion of a second hormone, oögoniol. This sterol acts upon the female partner by inducing the formation of female sex organs. *Achlya*, therefore, is perhaps the most primitive eukaryote to respond morphogenetically to steroid sex hormones.

The addition of antheridiol to undifferentiated cultures of *Achlya ambisexualis* (strain E87) elicits several changes in macromolecular metabolism (14-18). Silver and Horgen (16) reported that antheridiol stimulated the synthesis of total cellular RNA, poly(A+) RNA and protein. Furthermore, there is a temporal separation between the synthesis of ribosomal RNA and the synthesis of mRNA and protein (16,18). Changes in transcription induced by antheridiol may be responsible for the production of new gene products required for the morphological changes associated with the ontogeny of the male sex organ (16,17).

Do steroid-receptor interactions exist in this fungal system which selectively alter the transcription patterns in the nucleus? The experiments presented in this report show that antheridiol-cytosol complexes under defined ionic conditions stimulate the transcriptional capacity of chromatin isolated from non-hormone induced cultures. Furthermore, neither hormone nor cytosol alone stimulate *in vitro* chromatin transcription. These results demonstrate that hormone-target cell interactions exist in primitive eukaryotes, and that hormone-receptor-like interactions exist in *Achlya*.

MATERIALS AND METHODS. *Achlya ambisexualis*, E87 (male strain) and *A. ambisexualis*, 734 (female strain) were used for this study. Cultures were grown and harvested as described previously (16,18). Tissue from non-hormone treated *A. ambisexualis* (E87) was suspended in Tween 80 buffer (0.5 M sucrose, 2.5 mM $MgCl_2$, 10% v/v glycerol, 2% v/v Tween 80, 0.5 mM phenylmethyl sulfonylfluoride and 10.0 mM Tris-HCl, pH 7.9) and was homogenized at setting 8 for 15 sec with a Willems Polytron Homogenizer (Brinkman Instruments). The homogenate was filtered through 2 layers of Miracloth and centrifuged at 4080 g for 20 min. The crude nuclear pellet was then suspended in 0.14 M NaCl, 5.0 mM $MgCl_2$, 10.0 mM Tris-HCl, pH 7.6 and centrifuged at 4342 g for 10 min. The pellet was resuspended in the NaCl buffer and centrifuged a second time. Chromatin was isolated following the procedure of Rizzo and Nooden (19). Details of the isolation and characterization of *Achlya* chromatin will be described elsewhere (Sutherland and Horgen, manuscript in preparation).

Cytosol fractions were isolated by either suspending E87 or 734 mycelia in 10.0 mM Tris-HCl, pH 7.5, 1.5 mM EDTA. These mixtures were homogenized for 60 sec at the highest setting with a Willems Polytron Homogenizer and

Table 1. The Effects of Cytosol, Antheridiol, and Cytosol-Antheridiol Mixtures on Transcription of *Achlya* Chromatin

Chromatin preincubated with	Incubation Temperature °C	p moles incorporated per min per 50µg DNA	% of control
A. No Additions (control)	30	111 ± 4	100
E87 Cytosol	30	112 ± 6	101
Antheridiol	30	97 ± 2	86
E87 Cytosol + Antheridiol	30	415 ± 8	370
E87 Cytosol + Antheridiol preincubated at 0°C	0	27 ± 2	29
E87 Cytosol + Antheridiol preincubated at 25°C	0	76 ± 4	67
*E87 Cytosol + Antheridiol preheated to 90°C	30	103 ± 9	91
734 Cytosol + Antheridiol	30	117 ± 5	104
B. No Additions (control)	30	82 ± 2	100
E87 Cytosol + Antheridiol	30	271 ± 6	330
E87 Cytosol	30	85 ± 1	104
E87 Antheridiol	30	75 ± 4	92

A. Transcription with heterologous RNA polymerase. Fifty µg of chromatin DNA (Strain E87) was added along with 10 µg of *E. coli* RNA polymerase (Fraction IV containing Sigma factor, 4000 units per mg) to each reaction mixture. Either 1×10^{-9} g (2.1 nM) antheridiol or 1.25-1.50 mg of cytosol or a combination of both were added to each preincubation mixture. The final buffer concentrations in the 1 ml reaction mixture were 50 mM Tris-HCl (pH 8.0), 10 mM dithiothreitol, 10 mM $MgCl_2$, 20% glycerol, 0.1 M KCl, 200 mM ammonium sulphate and 0.4 mM K_2HPO_4. Unless otherwise indicated, these mixtures were preincubated for 15 min at 25°C. 0.4 mM UTP, 0.4 mM ATP, 0.4 mM CTP, 0.4 mM GTP and 10 µCi [^{3}H]UTP (specific activity 42 Ci/m mole) were added to begin the RNA synthetic reaction. Each reaction mixture was then incubated at the designated temperatures for 15 min. Reactions were terminated; the products were collected and the radioactivity determined as described by Horgen and Key (23). * In this experiment hormone-cytosol complexes were first heated to 90°C for 10 min before addition to the preincubation mixtures. *E. coli* RNA polymerase was purchased from Grand Island Biological Supplies.

B. Transcription with endogenous *Achlya* RNA polymerases. Preincubation mixtures were similar to A except 200 µg chromatin DNA was added and no *E. coli* RNA polymerase. All data presented represent an average of four replications. The data is expressed as p moles incorporated per min per 50 µg chromatin DNA.

filtered through Miracloth. The homogenates were centrifuged first at 27,138 g for 30 min; the supernatents were centrifuged at 50,000 RPM (Spinco 65 Rotor) for 60 min. The resultant supernatent fractions were considered the "cytosol" fractions.

Chromatin directed RNA synthesis was measured with either *Escherichia coli* RNA polymerase or endogenous *Achlya* RNA polymerases as described in Table 1. With 50 µg added chromatin DNA (heterologous polymerase experiments) very little endogenous polymerase activity could be measured. When 200 µg chromatin DNA (endogenous polymerase experiments) was incubated with the labeled nucleoside triphosphates, considerable endogenous activity was measured. RNA synthesis required added chromatin DNA and was dependent upon the presence of all four nucleoside triphosphates. RNA synthesis was inhibited by the addition of 50 µg/ml actinomycin D; the reaction product was sensitive to base hydrolysis and ribonuclease digestion.

RESULTS AND DISCUSSION. Nuclei and chromatin from animal target tissues are not affected by steroid hormone or cytosol alone, but show an increased incorporation of radioactive nucleotides into RNA only after addition of a preincubated mixture of hormone and target tissue cytosol containing the specific hormone-receptor complex (1,2,20,21). Table 1 shows the effect of preincubating *Achlya* chromatin with cytosol and/or hormone under a series of defined conditions. When assayed by addition of *E. coli* RNA polymerase to chromatin, neither added antheridiol nor cytosol alone significantly affects *in vitro* transcription. Preincubating chromatin with both hormone and cytosol from E87 (male), however, dramatically increases heterologous polymerase transcription of *Achlya* chromatin (Table 1). Cytosol from a female strain 734, which does not respond to antheridiol, preincubated with antheridiol and chromatin does not stimulate RNA synthesis. These results demonstrate that there is "target tissue" specificity associated with cytosol-hormone interactions in *Achlya* and that preincubation of *Achlya* chromatin with hormone and target tissue cytosol dramatically modifies the chromatin template activity available for transcription by heterologous polymerase. Estrogen administration to chicks *in vivo* increases the template activity of oviduct target tissue chromatin when assayed with either *E. coli* polymerase or homologous RNA polymerase B (22). In similar experiments we have demonstrated that antheridiol administration to growing cultures of *A. ambisexualis* (E87) increases the number of RNA polymerase binding sites on chromatin isolated from the hormone treated tissues (Sutherland and Horgen, manuscript in preparation).

Several reports on animal hormone-nuclear interactions suggest enhance-

ment of one or more of the nuclear RNA polymerase activities (2-6). A cytosol-hormone mixture also stimulates RNA synthesis in isolated chromatin directed by the endogenous *Achlya* polymerases (Table 1). Similarly, hormone or cytosol alone did not stimulate endogenous polymerase activities. While the experiments with heterologous polymerase as well as the experiments with endogenous polymerase are consistent with cytosol-hormone complexes interacting with chromatin and making more DNA available for transcription, the results with endogenous polymerase activities may also be related to hormone-cytosol stimulation of polymerase enzyme activities.

In mammalian systems a temperature dependent shift is required to convert the initial 4S hormone-receptor complex to an active 5S complex which then associates with the nuclear acceptor sites and activates transcription (1). More RNA synthesis occurs in *Achlya* if the cytosol-hormone complex is preincubated for 15 min at 25°C, than when the cytosol-hormone complex is preincubated at 0°C (Table 1). Controls where no additions to the chromatin or additions of cytosol or antheridiol alone were preincubated at 25°C gave values similar to cytosol-hormone preincubations at 0°C (data not shown). Therefore, temperature dependent interactions are necessary before hormone-cytosol complexes can activate *in vitro* RNA synthesis in *Achlya*. These assays were performed at 0°C to prevent any conversion of hormone-cytosol complexes during the incubation with the nucleoside triphosphates.

Binding of progesterone-receptor complexes to nuclei, chromatin or DNA of hen organs is largely determined on the ionic strength of the medium (24). Low ionic conditions (0.01 to 0.05 M KCl) result in nonspecific, nonsaturable binding; high ionic strength (0.15 to 0.2 M KCl) creates tissue-specific saturable binding (24). In *Achlya* potassium chloride concentrations from 0.05M to 0.12M gave maximal stimulation of RNA synthesis for chromatin preincubated with hormone-cytosol mixtures, whereas the salt had little effect on chromatin preincubated with cytosol alone (Fig. 1). In addition, incubating the salt with the chromatin by itself or with chromatin-hormone mixtures

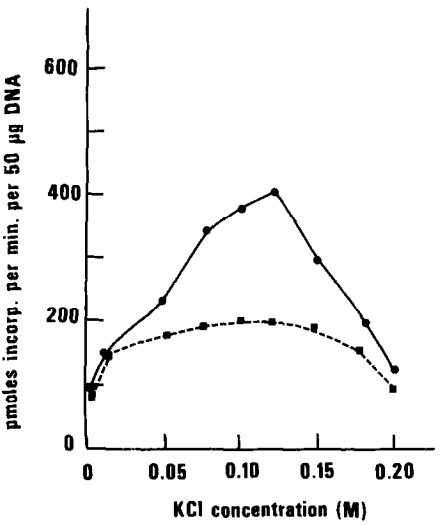

Fig. 1. The effect of KCl concentration on *in vitro* transcription by *Achlya* chromatin preincubated with antheridiol-cytosol complexes. Reaction mixtures were the same as described in Table 1 with the exception of KCl concentration. (●) , hormone-cytosol preincubation, (■), preincubation with cytosol alone.

did not stimulate *in vitro* transcription (data not shown). Ionic conditions similar to those that affect specific binding of hormone-receptors to pure hen DNA (24) affect specific cytosol-hormone stimulation of transcription in *Achlya*.

Achlya ambisexualis, perhaps phylogenetically the simplest eukaryote known to respond morphogenetically to steroid hormones, does so in a manner similar to higher eukaryotes (12-18). In this report data are presented which suggests that hormone-cytosol interactions similar to the hormone-receptor mechanisms operable in complex animal systems exist in the aquatic fungus *Achlya*.

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