frontalin and racemic *exo*-brevicomin were used, can now be ascribed to the (−) enantiomer of frontalin and the (+) enantiomer of *exo*-brevicomin. Recently, Borden (*21*) has observed a related phenomenon for the ambrosia beetle, *Gnathotrichus sulcatus*, but in this case both enantiomers of the same attractant pheromone compound [6-methyl-5-hepten-2-ol (*22*)] have low activity and together they evoke a synergistic response. With both bark beetle species, only the enantiomers that occur in nature are active, at least at the concentrations tested. Therefore we expect both a chiral synthesis and chiral olfactory receptor system in these species.

Note added in proof: Recently, (*S*)-(−)-ipsenol has been reported to be much more attractive to *Ips grandicollis* than its antipode (*23*).

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Characterization of the Androgen Receptor from a Syrian Hamster Ductus Deferens Tumor Cell Line (DDT_1)

Abstract. *The hamster ductus deferens cloned tumor cell line (DDT_1) contains a complex steroid receptor protein that binds ^{3}H-labeled 5α-dihydrotestosterone. Diethylaminoethyl (DEAE) chromatography of cytosol from these cells yields two major receptor peaks of activity. Identification of this steroid binding protein as a cytoplasmic receptor was confirmed by salt dissociation on sucrose gradients, stability of the hormone-receptor complex at 0°C, and the retention patterns on phosphocellulose and DEAE cellulose. Multiple forms of the receptor exist in a single homogeneous cell type. The data support the theory that steroid hormones bind to a cytoplasmic protein receptor composed of dissimilar subunits as the initial step in steroid hormone action.*

Steroid hormones are believed to exert their effects on target tissues by a series of events beginning with the combination of the steroid with a specific cytoplasmic receptor in the cell. This hormone-receptor complex is then translocated into the cell nucleus where it binds to the genetic material and initiates the appropriate responses to the steroid such as cell growth (*1*).

Model systems for studying growth responses to steroid hormones in cell culture have proved exceedingly difficult to establish. We have developed the DDT_1 cell line (*2*), from a leiomyosarcoma induced in the ductus deferens of a male Syrian hamster by long-term administration of testosterone propionate and diethylstilbestrol (*3*). The DDT_1 cell line contains androgen receptors that translocate to the nucleus and exhibits a growth response to androgens. The order of binding affinity is 5α-dihydrotestosterone (DHT) > testosterone (T) > 17β-estradiol > progesterone. Cortisol and the synthetic glucocorticoids dexamethasone and triamcinolone acetonide do not inhibit ^{3}H-labeled DHT binding to cytoplasmic receptor. We now report further characterization of the cytoplasmic androgen receptor from this homogeneous cloned cell population.

DDT_1 cells, clone MF-2, were grown at 37.5°C in 6-liter suspension culture vessels to a density of 1.5×10^5 cell/ml in a medium consisting of 93 percent Hams-F12, 5 percent fetal bovine serum, penicillin and streptomycin (100 μg/ml), and Fungizone (2.5 μg/ml) (all from Gib-

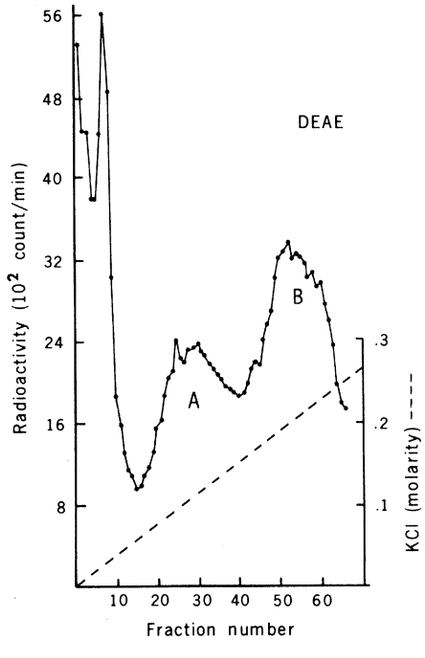

Fig. 1. DEAE-cellulose chromatography of 2 ml of DDT_1 cytosol labeled with [^{3}H]DHT and eluted with a 0 to 0.3*M* KCl gradient. Fractions (2 ml) were collected and the radioactivity in 0.5-ml samples were counted in 4 ml of Aquasol (New England Nuclear) at 25 percent efficiency (Beckman LS-233). Conductivities were determined with a radiometer conductivity cell and converted to KCl molarity. Two peaks of radioactivity were eluted: at 0.12*M* KCl (peak A) and at 0.23*M* KCl (peak B).

co). Cells were collected at 4°C, and all further procedures were carried out at 4°C; after collection, the cells were concentrated by centrifugation at 600*g*. The pellet of cells was then 1 : 1 in buffer A (tris, 0.01*M*; Na_2 EDTA, 0.0015*M*, 1-thioglycerol, 0.012*M*, *p*H 7.45) made up in 10 percent glycerol. The cells were allowed to swell for 15 minutes, and then sufficient buffer C (buffer A with 1.0*M* KCl) was added to reach a final KCl concentration of 0.15*M*. Homogenization was accomplished by 15 strokes of a Ten Broecke ground-glass homogenizer.

The homogenate was centrifuged at 600*g* for 10 minutes, and the lipid layer was removed. The remaining cytosol was centrifuged at 150,000*g* for 1 hour. Tritiated 5α-dihydrotestosterone (1,2,3,5,6,7-$^3H_6$) (5αDHT; Amersham/Searle; specific activity 175 c/mmole) was added to the cytosol at this time at concentrations specified in the text, and the solution was allowed to stand for a minimum of 1 hour.

Diethylaminoethyl (DEAE)-cellulose (Whatman DE-52, preswollen) chromatography was performed on cytosol that had been labeled with $5 \times 10^{-9}M$ [^{3}H]DHT. A column (1.3 by 7 cm) with an exchanger bed height of 3 to 4 cm was washed extensively with 30 to 50 ml of buffer B (buffer A without glycerol) until the conductivity of the eluate remained constant. The cytosol sample (2 ml) in buffer A was diluted with three volumes of buffer A, applied to the column, and washed with buffer A. Elution was accomplished by a 160-ml linear gradient of 0 to 0.3*M* KCl made up in buffer B.

As is the case for the chick oviduct progesterone receptor (*4*) and the rat ventral prostate androgen receptor (*5*), the DDT_1 cytosol eluted from the DEAE-cellulose column to yield two major peaks of radioactivity (Fig. 1). First, peak A eluted at 0.12*M* KCl; the second, peak B, eluted at 0.23*M* KCl. This chromatographic behavior indicated the heterogeneous nature of the cytosol androgen receptor in this cell line.

To ensure that both peaks of radioactivity were specific for ^{3}H-labeled 5αDHT, we performed competition studies by simultaneously adding a 250-fold excess of unlabeled 5αDHT, cyproterone acetate (CA), progesterone, 17β-estradiol (E_2), or triamcinolone acetonide (TA) and performed the DEAE chromatography as above. Binding was reduced by the following: unlabeled 5αDHT by 95.9 percent, CA by 83.8 percent, progesterone by 77.1 percent, E_2 by 86.5 percent, and TA not at all. Reduction of specific binding in the A and B peaks was always equivalent.

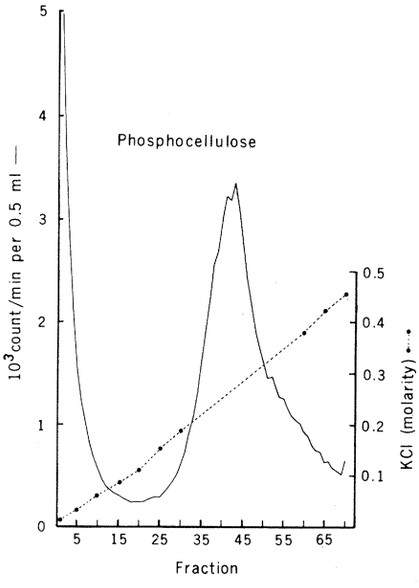

Fig. 2. Phosphocellulose chromatography of 5 ml of DDT_1 cytosol prepared in buffer A and labeled with [^{3}H]DHT. Radioactivity and KCl molarity were determined as in Fig. 1. A single peak of radioactivity was eluted at 0.26*M* KCl with a shoulder at 0.25*M* KCl.

In experiments with cytoplasmic androgen receptor proteins it is important to exclude other types of steroid binding proteins that might be present in cells or serum. Androgen binding protein (ABP) from the rat and rabbit epididymis and testosterone-estradiol binding protein from many serum sources bind 5αDHT (*6*). However, these proteins are not cytoplasmic receptors and do not translocate to the nucleus (*7*). Furthermore, these proteins have no apparent subunit structure, and are not retained on phosphocellulose (*8*), as is the case with cytoplasmic receptors.

To further characterize the DDT_1 cytoplasmic receptor, we performed phosphocellulose chromatography (*9*) (Fig. 2), using a column (1.3 by 7 cm) with an exchanger bed height of 5 cm. Cytosol (5 ml) labeled with $5 \times 10^{-9}M$ [^{3}H]-DHT was applied and eluted as described for DEAE chromatography, except that the salt gradient concentration was increased to 0.5*M* KCl. Under these conditions cytosol labeled with [^{3}H]DHT also revealed a receptor peak that was retained on the phosphocellulose and eluted at 0.26*M* KCl.

In the case of the progesterone receptor the A and B peaks elute separately on phosphocellulose (*9*); therefore in order to determine whether the DDT_1 A and B receptors coeluted from phosphocellulose producing the single peak of radioactivity, we separated the DDT_1 A and B peak on DEAE as in Fig. 1 and chromatographed these individually on phosphocellulose. Both peaks eluted at the same 0.26*M* KCl under these conditions.

We next examined the dissociation of

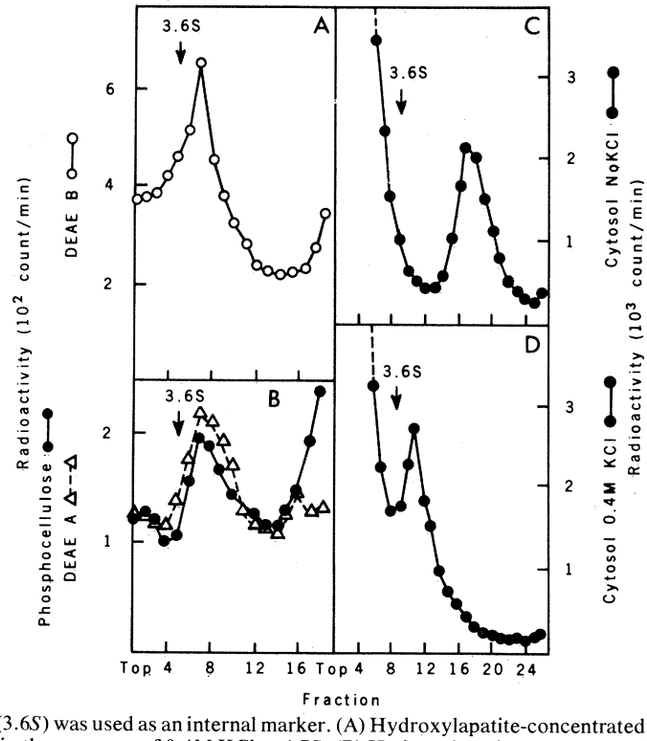

Fig. 3. Sucrose gradient analysis of DDT_1 cytosol receptors. The DEAE and phosphocellulose peaks were concentrated on hydroxylapatite and eluted with buffer A containing 0.5*M* phosphate (*12*). Samples (200 μl) of the concentrated receptors or cytosol receptors were centrifuged for 18 hours at 257,000*g* on linear sucrose gradients (5 to 20 percent) prepared in buffer A with or without 0.4*M* KCl (Beckman SW 65Ti rotor). Fractions of 0.3 ml (DEAE A, DEAE B, and phosphocellulose) or 0.2 ml (untreated and cytosol treated with 0.4*M* KCl) were diluted to 0.5 ml with H_2O and counted as described (Fig. 1). ^{14}C-Labeled ovalbumin (3.6*S*) was used as an internal marker. (A) Hydroxylapatite-concentrated DEAE peak B migrates in the presence of 0.4*M* KCl at 4.7*S*. (B) Hydroxylapatite-concentrated DEAE peak A and the phosphocellulose peak migrate as broad peaks at 4.8*S* in the presence of 0.4*M* KCl. (C) Untreated cytosol migrates at 7*S* in the absence of KCl. (D) Cytosol in the presence of 0.4*M* KCl migrates at 4.8*S*.

receptor-hormone complex. Excess (1000-fold) unlabeled hormone was added to prevent detectable recombinations of [^{3}H]DHT with receptor. Using the charcoal-dextran method of Korenman (*10*) we measured the half-life of the complexes under pseudo first-order conditions. The half-life was 74.5 hours at 0°C, with a rate constant of dissociation of 1.55×10^{-4} min^{-1}; at 0°C, all of the known extracellular androgen binding proteins, including the above-mentioned ABP and testosterone-estradiol binding globulin, have half-lives of dissociation in minutes (*6*). Using sucrose gradient centrifugation to separate bound and free steroid we measured the equilibrium constant (K_d) to be $2.99 \times 10^{-10}M$ (*2*).

In order to examine further the subunit structure of the DDT_1 cytoplasmic receptor, we utilized sucrose gradient analysis (*2*). Gradients (containing 0.4*M* KCl) analyzing the partially purified receptor peaks for DEAE and phosphocellulose or from cytosol treated with 0.4*M* KCl yielded receptor peaks sedimenting at 4.6 to 5*S* (Fig. 3). Similar gradients (without KCl) in similar cytosol preparations not treated with KCl revealed a peak sedimenting at 7*S* (Fig. 3). These data indicate that the 7*S* cytosol receptor in these cells has salt-dissociable 4.8*S* subunits that each bind androgen and are separable by DEAE chromatography. These results, in addition to our previous demonstration that this DDT_1 receptor enters the nucleus (*2*), exclude the possibility that the molecules are ABP's or other protein resembling the testosterone-estradiol binding globulin.

Our data indicate that the DDT_1 cytoplasmic receptor has several physical-chemical homologies with the highly purified chick oviduct progesterone receptor described by Schrader and O'Malley (*4*). The chick oviduct progesterone receptor has been suggested to have a subunit structure in which two 4*S* monomers combine to form a 6*S* dimer and also higher aggregates. The monomers appear to have diffferent functions in the nucleus, the A subunit binding preferentially to DNA and the B monomer binding to chromatin. This has led to the proposal (*11*) that the actual cytoplasmic form of the receptor is a 6*S* dimer, consisting of an A and B monomer, which moves into the nucleus and is bound to the chromatin acceptor site by the B subunit. The A subunit then dissociates from the B monomer and searches along adjacent chromatin regions until it locates and binds to a specific regulatory site on naked DNA. This model would explain the presence of subunits with different functions, but both binding steroid hormone.

We are not aware of a previous demonstration that the A and B monomers exist in only one cell type. Using DDT_1 cells, we have now shown that putative receptor subunits are present in a cloned cell line. This finding excludes the possibility that the multiple receptor forms from steroid target tissues are the result of mixing cytoplasm from different cell types during homogenization. These findings substantiate the hypothesis that the A and B monomers are subunits of a larger dimer existing within a single cell.

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Blue-Green Algae: Their Excretion of Iron-Selective Chelators Enables Them to Dominate Other Algae

Abstract. *During blue-green algal blooms, other algae can be completely suppressed. This ability of blue-green algae to suppress other algae may be determined by the availability of iron. Iron deprivation induces the production of hydroxamate chelators, which appear to be the agent suppressing other algae.*

The availability of iron for microbial uptake appears to be an important variable in determining the stability and composition of aquatic ecosystems. Twice in a eutrophic lake the sudden dominance of blue-green algae coincided with the production of strong iron chelators and the rapid uptake of iron. The increased demand for iron appears to result from high rates of nitrogen fixation. We believe that the *Anabaena* species, the dominant alga during these periods, excreted chelators that enhanced the growth of blue-green algae or directly restricted the growth of competing species (or both).

This type of competition between aerobic microbes has been intensively studied by terrestrial ecologists and pharmacologists. In microbes, the demand for iron often induces the excretion of siderochromes, which are trihydroxamates (or catechols) of low molecular weight that can selectively chelate ferric iron. Ferric iron is chelated by acetohydroxamic acid several orders of magnitude greater than are other cations (*1*). Neilands (*2*) believes that many of these chelators act as carrier molecules transporting iron across membranes. Species with stronger iron uptake systems would thus be able to preferentially utilize the available iron. This has been best documented in mammalian systems where the competition for iron between the microbial siderochromes and mammalian proteins, such as transferrins and lactoferrins, determines the virulence of the microbe (*3*). Some microbes produce antibiotics that are structural homologs of a competitor's siderochromes. These antibiotics bind iron and are inducible by low concentrations of iron (*3*). Because these antibiotics are actively taken up by the siderochrome uptake system and this toxicity results from intracellular reactions, the toxicity can be reduced or eliminated by the addition of another siderochrome. Thus, the ability of a species to survive a period of low iron availability may depend both on its ability to take up iron and its capacity to antagonize competing species.

Although the processes controlling the uptake of iron in aquatic ecosystems are not as well understood, there are many reports that are consistent with the results obtained in terrestrial systems. Iron often stimulates photosynthesis in many diverse aquatic ecosystems (*4, 5*). Also,