

ANDROGEN-RECEPTOR INTERACTION - AN OVERVIEW

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The discovery by Jensen and his associates (1) more than two decades ago that estrogen can be retained by estrogen-responsive tissues came about at the time when the molecular process of gene expression started to unravel. Since hormones can change the amounts of specific proteins in the target organs (2), other investigators also explored the possibility that steroid hormones may mediate organ functions through the regulation of RNA and protein synthesis. It was found that insect hormone can cause puffing of chromatin at selective loci where RNA is synthesized (3), and estradiol (4) and testosterone (5) can rapidly enhance the synthesis of RNA and accumulation of mRNA in the target cells (6,7).

Introduction of gradient centrifugation played a key role in showing the existence of an estradiol-protein complex (8). The subsequent finding of the selective retention of 5 α -dihydrotestosterone (DHT) (Fig. 1) by prostate cell nuclei *in vivo* (9,10) and *in vitro* (9,11) and the demonstration of the existence of DHT-receptor complexes and other steroid receptors (12) then provided necessary support to the view that a universal receptor mechanism may be operating in steroid hormone responsive cells. Subsequently, the study of steroid hormone receptors rapidly expanded (Fig. 2).

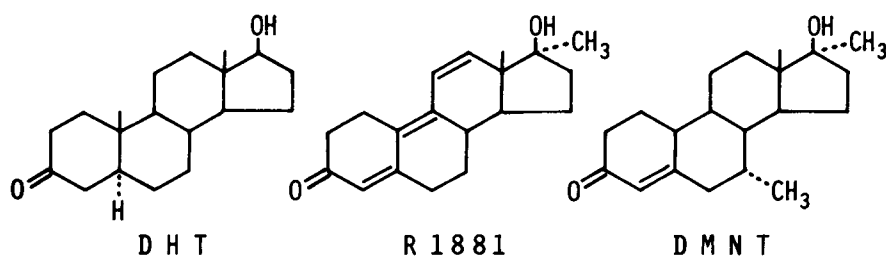


Fig. 1. Chemical structures of 5 α -dihydrotestosterone (DHT), 17 α -methyl-17 β -hydroxyestra-4,9,11-trien-3-one (methyltrienolone or R1881), and 7 α -17 α -dimethyl-19-nortestosterone (DMNT or mibolerone). The assay of androgen receptor in crude extracts has been carried out with radioactive R1881 or DMNT (synthetic androgens) that, unlike DHT, are metabolically stable and do not bind well to nonreceptor proteins such as testosterone-estradiol binding globulin (TeBG).

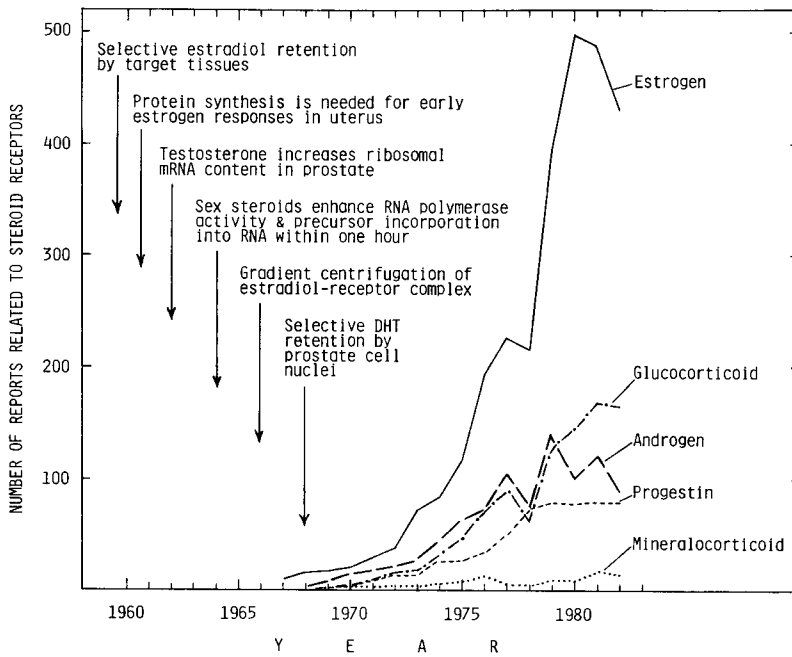


Fig. 2. Some of the major findings in 1960s and the number of published reports related to steroid receptors in 1970s.

Many natural steroid hormones enter target cells and bind to receptors without metabolic conversion. Testosterone, however, appears to act, in the prostate, mainly after its conversion by a 5α -reductase to DHT which in turn forms a complex with a specific receptor and binds tightly to the nuclear acceptor site(s). These studies triggered the increase in DHT research activity in the last 15 years (Fig. 3). The importance of the 5α -reductase is evident from observations that certain types of abnormal male sexual differentiation can be related to a 5α -reductase deficiency (13) and that reductase inhibitors can reduce nuclear retention of DHT and growth of the prostate (14,15). In some androgen target organs, such as kidney and muscle, where the reductase activity is feeble, testosterone may act as the active androgen and bind to an androgen receptor that may also bind DHT. It is not clear whether the DHT-receptor and testosterone-receptor complexes can function differently.

In the rat ventral prostate, a DHT-receptor complex can be *transformed* (also termed activation) and *translocated* to a nuclear acceptor site (Fig. 4). Precise molecular changes of the receptor complex in these processes have not been clearly elucidated. As in the case with other steroid hormone receptors, very little is known about the nuclear acceptor molecules responsible for interaction of the receptor complex

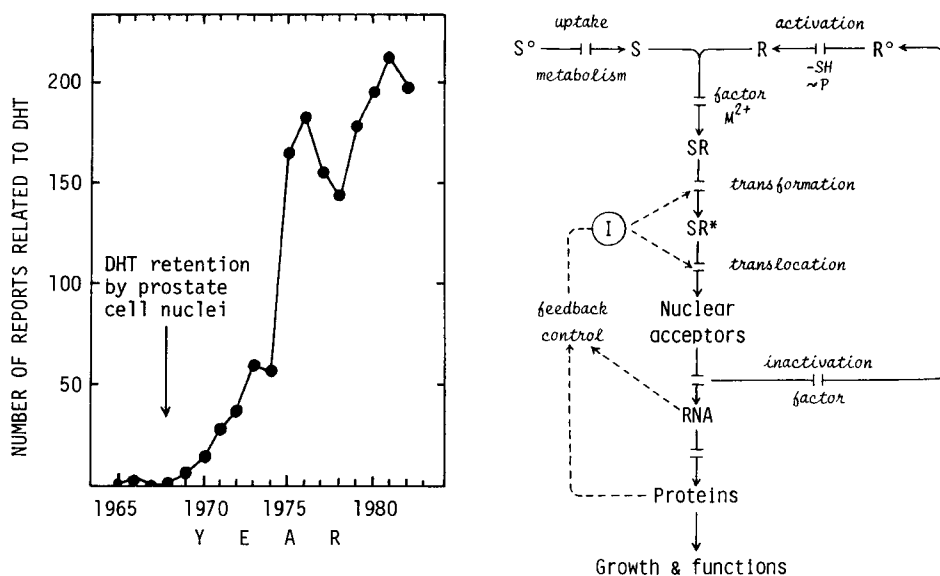
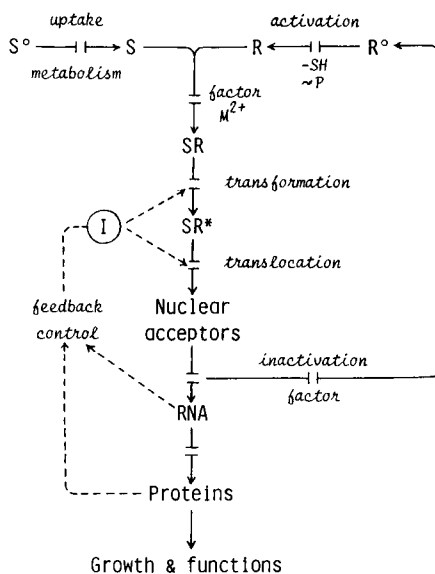


Fig. 3. The number of published reports related to 5 α -dihydrotestosterone (DHT) since 1965. The reports on the selective DHT retention by prostate cell nuclei appeared in 1968.

Fig. 4. A working model for the steps involved in intracellular cycling of the androgen receptor in target cells. In this hypothetical model, the receptor protein (R^0) is activated (or potentiated) by an energy-dependent process that is sensitive to respiratory poisons such as KCN, azide, and 2,4-dinitrophenol (DNP). The activated receptor (R) then binds an active androgen such as DHT (S) that is formed from a precursor (testosterone) (S^0). The androgen-receptor complex (SR) is transformed in a temperature-dependent step to the form that can be retained tightly by nuclear acceptor. This receptor-acceptor interaction may modulate the production of mRNA (and proteins) necessary for the growth and function of the prostate. Some protein factors may play a feed-back control in regulating the interaction of the receptor complex with chromatin. The term *activation* has also been used for the transformation step in which a steroid-receptor complex (rather than a receptor) is activated to the form that has higher DNA binding affinity.



with the nuclei, where an increase in the synthesis of RNA necessary for hormonal response takes place. As reviewed elsewhere (16), involvement of acidic and basic nuclear proteins, DNA, and RNA have been implicated, while recently nuclear matrix has also been suggested as the receptor accepting site (17). It is very likely that many of these nuclear components act cooperatively in providing structural specificities and functional requirements to bring about orderly hormonal responses in target cells.

The fate of the steroid-receptor complex after its interaction with nuclear acceptor components is not clear. The bound-nuclear receptor, associated factors, or the steroid may be modified in such a way that they no longer can associate with each other tightly, causing release of the receptor from the acceptor sites. Another possibility is that the receptor complex and other proteins may bind to nuclear RNA, thereby facilitating release of the complex from nuclei (Fig. 5). It was proposed that the receptor complex, in turn, may play an important role in the processing, stabilization, and/or utilization of RNA (18,19). Whether such a mechanism is involved in posttranscriptional control during protein induction by steroid hormone is an intriguing question.

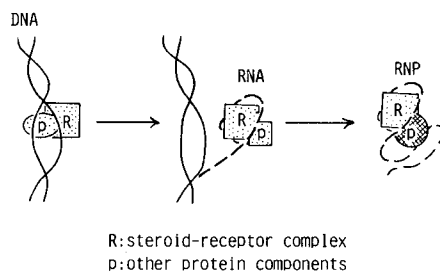


Fig. 5. A hypothetical role for RNA binding of a steroid-receptor complex. Simplified from Liao and Fang (18).

Direct evidence supporting this idea is still lacking, nevertheless, both the estrogen- and androgen-receptor complexes can bind to certain ribonucleoprotein particles in the uterus and prostate (20,21). More recently, we have shown by DNA-cellulose chromatography, gradient centrifugation, and nuclear incubation techniques that certain polyribonucleotides can effectively release the DHT-receptor complex from DNA or nuclei (22). The capability of these nucleotides to facilitate this release is clearly dependent on nucleotide sequence and size (possibly also the secondary structure) of the polymer. It is conceivable that, in vivo, certain RNA molecules having appropriate nucleotide sequence

may show high specificities toward different steroid-receptor complexes. Different RNA molecules (including rRNA) may contain, for example, identical or similar nucleotide sequences so that more than one RNA species can be selected, although with some preference, by the same steroid-receptor complex. Other binding proteins may also cooperate in providing a selective and/or diverse specificity.

In the last few years, binding of steroid-receptor complexes with RNA has gained attention. Interaction of RNA with androgen-receptor of mouse kidney (23), estrogen-receptor of rat uterus (24) and dexamethasone-receptor of rat liver (25-27) and human breast tumor (MCF-7) cells (28) have been examined. RNase treatment of steroid-receptor complexes has been shown to enhance the DNA binding activities and change the sedimentation profiles of estrogen-receptor and dexamethasone-receptor complexes (29,31).

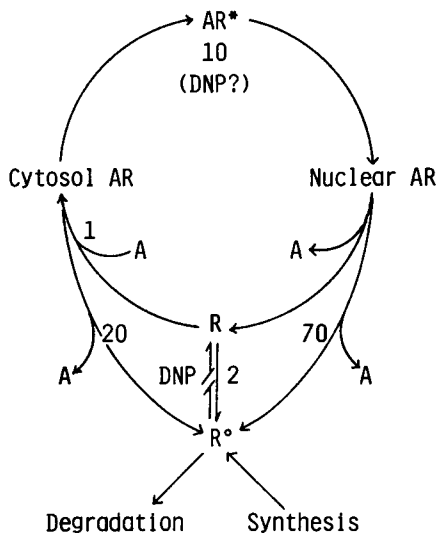


Fig. 6. Schematic representation of the androgen receptor recycling in prostate cells. Inactive receptor (R°) is activated to the androgen (A) binding form (R) by a DNP-sensitive, energy-dependent process. The cytosol androgen-receptor complex (AR) is transformed into a complex (AR*) that can be retained by the nuclei. The nuclear receptor released from the nuclei may be inactivated to the form that does not bind hormone. The numbers represent the apparent half lives, in minutes, for the receptor to disappear in the steps represented by arrows, or the time, in minutes, needed to reach one half of a maximum binding of androgen by the cytoplasmic receptor, or to achieve one half of the maximum binding of the transformed androgen-receptor complex by nuclei. DNP may have a secondary effect on the transformation of the receptor, or on the interaction of the receptor complex with the nuclear acceptor site.

The dynamic status of the androgen receptor in prostate cells was studied recently by incubation of rat ventral prostate with [^3H] DHT (Fig. 6). By pulse-chase techniques we have estimated that the nuclear acceptor-bound androgen-receptor complex has a half-life of about 70 minutes (32). This long half-life may reflect the involvement of the androgen-receptor complex in a time-consuming mechanism that is essential for orderly hormone responses. The androgen receptor released from nuclear acceptor sites appears to be rapidly deactivated (half-life: 2 minutes) in the presence of respiratory poisons such as 2,4-dinitrophenol or cyanide (18,32). Reactivation of the receptor can be achieved by removal of the poison even in the presence of cycloheximide, which should inhibit *de novo* synthesis of receptor. Purified prostate nuclei contain enzyme(s) that inactivate the steroid binding ability of the receptor protein. Whether this inactivating enzyme is a phosphatase, as has been suggested for other steroid receptor systems, is not known. Reactivation (also called *potentiation*) of glucocorticoid receptor (33,34) and estrogen receptor (35) requires ATP. No direct evidence is available to show whether it is the receptor itself or another closely related molecule that is phosphorylated or dephosphorylated during the inactivation-reativation processes. ATP and GTP, however, can stabilize DHT-receptor complex *in vitro* (18).

In prostate cells, the early effects (within one hour of androgen injection into castrated rats) of androgen on RNA synthesis (5) do not appear to require a depletion of a major portion of cellular receptors. Since the inactivation, reactivation, and nuclear retention processes are more rapid than receptor degradation, the androgen receptor appears to go through a dynamic process of recycling (32). For the prostate to grow and function normally this dynamic process must be regulated properly. A better understanding of the regulatory mechanism may lead to new approaches in the control of abnormal growth of the prostate.

The first protein factor found to interfere *in vitro* with the association of a steroid-receptor complex with nuclei or chromatin was isolated from rat ventral prostate in 1971 and named α -protein (36). This protein (also called prostate steroid binding protein, prostatein, or estramustine binding protein by other investigators) has four polypeptide chain components and binds cholesterol *in vivo* (37). If cholesterol is removed, the protein binds many different steroids (but not glucocorticoids) with a K_d of about 10^{-7} M. Of the 4 components only Component I can prevent binding or promote the release of androgen-receptor complex from chromatin. The effect is not due to irreversible destruction of the receptor complex, damage to nuclear acceptor site, or to steroid binding activity of α -protein (Component I does not bind steroid). Component I has 88 amino acids and its sequence has been determined (38). Whether a small section of Component I can be active is not clear. Other protein factors may affect the rate of

androgen association to the receptor (39), or regulate the alteration of receptor forms (40). These factors are yet to be purified. There is no direct evidence that α -protein or other protein factors act as regulators *in vivo* of prostatic cell function. It is not inconceivable, however, that an ultimate protein product of androgen action could exert feedback control at the genomic level by promoting the release of steroid-receptor complexes from their site of action (Fig. 4).

The effects of divalent cations on various steroid receptors have been described by several investigators. We have shown that the prostate cytosol DHT-receptor complex can interact with certain divalent metal ions rather specifically. The most striking effect was observed with Zn^{2+} , which is known to accumulate in the prostate of some species of animals. According to gradient centrifugation analysis, Zn^{2+} (3 mM) appears to interact with and shift the sedimentation property of the DHT-receptor complex and reduce the steroid binding affinity of the receptor (41).

The naked androgen receptor is more sensitive to the divalent cations than the androgen-receptor complex. For example, Zn^{2+} at 30 μ M can significantly inhibit the interaction of androgen and receptor from the cytosols of mouse (42) or rat (43) ventral prostates. This inhibition was apparently competitive with the steroid ligand. With the androgen receptor partially purified from the cytosol of the rat ventral prostate by DEAE-cellulose chromatography, $ZnCl_2$ (10 μ M), added prior to the addition of the radioactive androgen, inhibited about 50% of the androgen binding ability of the receptor. $CuSO_4$, $CdSO_4$, $HgCl_2$, and $AgNO_3$ also showed similar inhibition, but $CaCl_2$, $CoCl_2$, $MgCl_2$, $FeCl_2$, $MnCl_2$, and $AlCl_3$ were much less active or not inhibitory at concentrations up to 20 μ M. The inhibitory effect of $ZnCl_2$ and $CuSO_4$, up to 20 μ M, was not seen if the androgen was allowed to bind to the receptor prior to metal addition. Androgen binding to the receptor, however, did not significantly protect the receptor inactivation by $AgNO_3$ or $HgCl_2$. All the metal effects can be reversed by dithiothreitol (43).

These observations suggest several possibilities: (a) the androgen receptor may be a metalloprotein that requires an appropriate divalent cation such as Zn^{2+} for its normal function; (b) the cation may interact with the receptor at the site (possibly involving -SH groups) critical for hormone binding and/or a conformational change of the receptor protein; and (c) the effects of the cation and dithiothreitol may reflect the mechanism involved in the reversible inactivation/activation and recycling of the receptor in the intact prostate cells.

The steroid structure is important in determining not only the specificity and affinity of the hormone toward the receptor protein, but also the ability of the steroid-receptor complex to effectively interact with nuclear components and initiate cellular

response. The latter effect is apparently due to a steroid induced change in the receptor conformation. Thus, steroid structure is a major factor in deciding the effective concentrations of the hormone, as well as the type and duration of hormonal responses expected in the target cells.

An androgen molecule appears to be recognized from all sides by the isolated receptor protein as if the hormone is being 'enveloped' (44). A rigid structural specificity requirement at the ring A/B area is clear. In fact, testosterone may not be 'enveloped' by the receptor protein in the same way as DHT at the ring A/B area and, therefore, may not form a stable androgen-receptor complex, or promote a tight association of the receptor protein with the nuclear acceptor molecules.

Studies of the steroid specificity involved in receptor binding have largely been carried out with steroids having different side chain substituents (44-46). These studies indicate that the androgen receptor has openings for the 7α and 17α positions on a steroid and can accommodate a methyl group or a larger substitution. This view is in line with the fact that various steroid receptors can bind to steroids attached to immobilized materials through spacers at these positions.

Whether the steroid binding sites inside the receptor cavity have binding preference toward individual ring structure is not well understood. To explore this possibility, we have investigated the ability of various small molecules to compete with a radioactive androgen ($[^3\text{H}]$ R1881) for binding to the androgen receptor in cytosol fractions of rat ventral prostate. Of more than 60 hydrocarbons tested so far, some of the phenanthrene derivatives (Fig. 7) exhibited striking structural specificities at 20 μM to 2 mM, whereas anthracene derivatives were inactive at 2 mM. For example, 1,2,3,4,5,6,7,8-octahydrophenanthrene was at least 10 times more active than phenanthrene or naphthalene, whereas 9,10-dihydrophenanthrene was about 20 times more active than the octahydrophenanthrene or 1,2,3,4-tetrahydronaphthalene in competing with $[^3\text{H}]$ R1881 for binding to androgen receptor. We have tentatively determined that the two aromatic rings in 9,10-dihydrophenanthrene bind to receptor sites that are normally occupied by the B and D rings of the androgen. Interestingly, neither dihydrophenanthrene nor octahydrophenanthrene could compete with $[^3\text{H}]$ estradiol for binding to the estrogen receptor of rat uterus or MCF-7 cells.

These studies suggests that the steroid receptor can behave like a cavitand that can recognize individual ring structure in a steroid-like compound. This information may be useful in the development of affinity ligands for purification or characterization of the receptor. We believe that better understanding of the small ligand-cavitand interaction can also provide new knowledge helpful in designing novel steroidal or nonsteroidal hormones or antagonists.

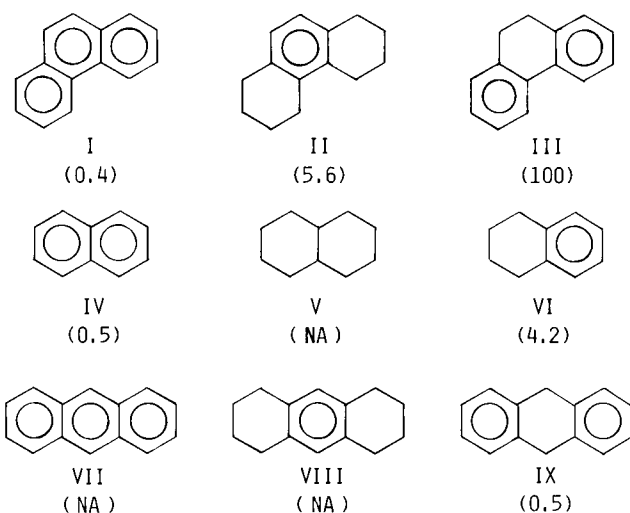


Fig. 7. The effect of naphthalene, anthracene, and phenanthrene related compounds on the androgen binding activity of the prostate receptor. Compounds were tested for their abilities to inhibit [^3H] R1881 (10 nM) binding by the cytosol androgen receptor of the ventral prostate of rats castrated 18 hours previously. The reciprocal of the concentrations needed for the test compounds to inhibit 50% of radioactive androgen binding were compared. The data were expressed (in parentheses) as percentages of the reciprocal number obtained for 9,10-dihydrophenanthrene (III) that showed 50% inhibition at 27 μM . Compounds that did not show significant inhibition at 5 mM were considered not active (NA). Other compounds shown are phenanthrene (I), 1,2,3,4,5,6,7,8-octahydrophenanthrene (II), naphthalene (IV), decahydronaphthalene (V), 1,2,3,4-tetrahydronaphthalene (VI), anthracene (VII), 1,2,3,4,5,6,7,8-octahydroanthracene (VIII), and 9,10-dihydroanthracene (IX).

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DISCUSSION

CLARK: With regard to your observations with DNP, Dr. Gorski observed many years ago that DNP had no effect on estrogen receptor concentrations in the rat uterus. My question relates to whether the inhibition that you have observed by the phenanthrenes is competitive or non-competitive mechanisms?

LIAO: There are some reports indicating that estrogen receptors can also go through an energy dependent activate process. The inhibition by 9,10-dihydrophenanthrene appears to be competitive.

THOMPSON: In the competition experiments with small γ -structured molecules, was assigned the maximum number of 100. How does this relate to the affinity of a true androgen ligand?

LIAO: The concentration of 9,10-dihydrophenanthrene needed to show 50% inhibition of radioactive methyltrienolone (^3H -R1881) binding by the prostate androgen receptor is 27 μM . Even the affinities of these small molecules are much lower than that of the natural androgens, the androgen receptor can recognize them specifically.