# PREFACE

The products of the testes have been recognized as essential to the development and maintenance of male physique and virility since ancient times. However, only in the past few decades has the entire spectrum of influence and action of testosterone, its metabolites, and its analogs begun to be fully investigated and appreciated. As we understand more about how androgens act and how they influence a myriad of physiological functions, we are able to use androgens in a wider variety of clinical settings. Unfortunately, use is frequently accompanied by abuse; over the last 20 years, abuse of anabolic agents has become widespread in some communities of athletes, sometimes with untoward consequences.

In *Androgens in Health and Disease,* we have brought together reviews of our latest understanding of andrology in both basic science and clinical medicine. In the first section of the book, the biology of androgens, androgen metabolites, and androgen receptors is presented from several different perspectives. We hope that clinicians as well as basic scientists will find this section useful. The next two sections explore the roles of androgens in development and in the modulation of a wide variety of physiologic systems. Although the lay public tends to view androgens primarily as agents of virility and muscularity, androgens actually regulate nearly every physiologic system in some way, as these chapters demonstrate.

In the last two sections of the book, several newer uses of androgens are presented. Most endocrinologists and urologists, as well as some practitioners with broader scopes of practice, are familiar with the use of androgens in delayed puberty and in young men with acquired hypogonadism. However, fewer clinicians are aware that testosterone can be a useful adjunct to the treatment of many other disease states, particularly those in which catabolism and loss of muscle mass occur. In addition, it is becoming increasingly apparent that many older men experience "andropause," analogous but certainly not identical to menopause. Although not appropriate for all older men, androgens may improve physical function and enhance the quality of life for many individuals. As with hormone replacement, the development of hormonal male contraception lags far behind that which is available for women. However, several recent studies have demonstrated the potential utility of androgens, often combined with other hormonal agents, in suppressing spermatogenesis consistently and reversibly. These potential indications are much more prevalent than is "classical" hypogonadism, and thus the potential future uses of androgens as therapeutic agents are extensive.

The last section of the book presents recent developments in the role of androgens in regulating female physiology. Excessive androgens, either in the serum or at the tissue level, can cause a wide range of symptoms and physical findings in women, and hyperandrogenism may also be associated with an increased risk of cardiovascular disease. Conversely, androgen deficiency in women is not often recognized, but it too may impact the physical and emotional well-being of women, particularly after menopause.

We hope *Androgens in Health and Disease* will serve as a valuable resource to researchers and clinicians alike and will broaden the reader's concept of how and why androgens exert their effects. We are grateful to all of the authors for contributing their time and expertise to this endeavor. We offer sincere thanks to Ms. Tetana Oguara for her expert and enthusiastic support in coordinating and compiling the manuscripts.

***Carrie Bagatell,*** *MD*
***William J. Bremner,*** *MD, PhD*

# 2 Androgen Action

*Terry R. Brown, PhD*

***CONTENTS***



## INTRODUCTION

Androgen action in human male subjects is predominantly the result of the biological activities of testosterone and 5α-dihydrotestosterone (DHT) (*see* Fig. 1). Although the former steroid is the predominant androgen present in the peripheral circulation at a 10-fold to 12-fold greater concentration, the local tissue concentration of DHT may be greater, as is its biological potency. Testosterone is synthesized within Leydig cells and secreted by the testes, whereas DHT is formed primarily as a metabolic product in peripheral tissues expressing steroid 5α-reductase activity. The equilibrium kinetic properties of lipophilic steroids suggest that testosterone can enter cells by passive diffusion across the cell membrane. However, only 1–2% of the testosterone present in the blood is free to diffuse into tissues, because the vast majority of the steroid is bound to sex hormone-binding globulin (SHBG; 40–50%) and to albumin (50–60%). Normal physiologic levels of circulating testosterone are necessary for adequate androgen biologic activity; however, some actions of androgens within tissues require the local conversion of testosterone to its more biologically active metabolite, DHT. A single molecular form of the androgen receptor (AR) exists within androgen target cells. The inactive cytoplasmic AR is a member of a large macromolecular chaperone complex, which dissociates upon the binding of testosterone or DHT to its receptor. The binding of steroid produces a conformational (or allosteric) change to an activated receptor complex that translocates into the nucleus and binds with high affinity as homodimers

From: *Contemporary Endocrinology: Androgens in Health and Disease*
Edited by: C. Bagatell and W. J. Bremner © Humana Press Inc., Totowa, NJ

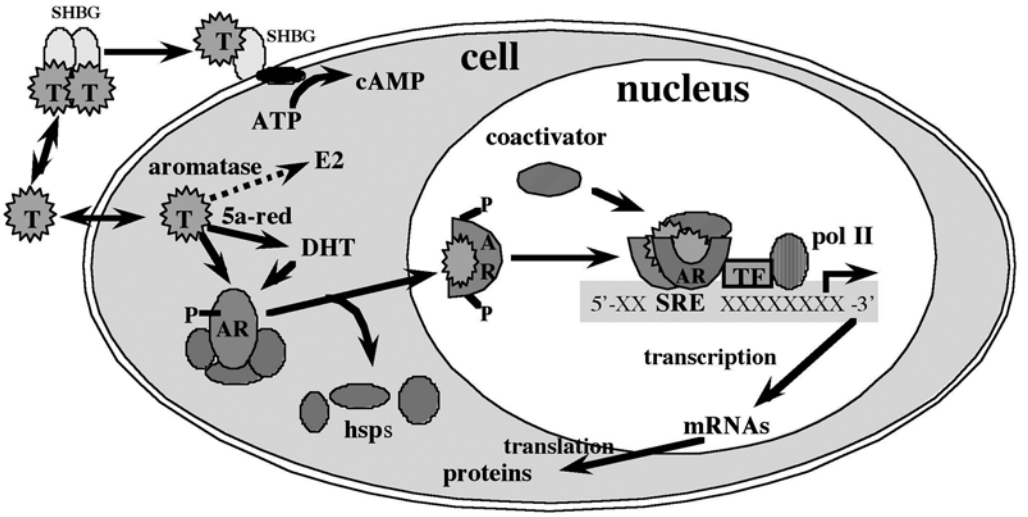


**Fig. 1.** Mechanism for androgen action in target organs. Testosterone (T) circulates in the blood predominantly bound to carrier proteins, such as the dimers formed by sex hormone-binding globulin (SHBG). SHBG–T or SHBG–DHT complexes can bind to receptors on the cell surface and activate the cyclic AMP-dependent protein kinase A pathways. Alternatively, free T enters cells by passive diffusion. Within the target cell, T may act by itself or be converted to its active metabolites, dihydrotestosterone (DHT) by steroid 5α-reductase or estradiol ($E_2$) by the aromatase pathway. $E_2$ acts through the estrogen receptor pathway (not shown). The cytoplasmic androgen receptor (AR) is a phosphoprotein (P) that forms a large macromolecular complex with various chaperone molecules, including heat shock proteins (hsps). Upon binding of androgen, T, or DHT, the receptor undergoes a conformational change and hsps are released. The AR complex undergoes further phosphorylation and acquires increased avidity for binding to DNA. The activated AR–T/DHT steroid complexes bind as dimers to specific steroid response elements (SREs) defined by DNA nucleotide sequences in regulatory regions of androgen-responsive genes. The chromatin-bound receptors are complexed with other nuclear proteins that function as coactivators (or corepressors) of gene transcription that may act to modify the chromatin structure (histone acetylation/deacetylation) and/or interact with the transcriptional initiation complex composed of various transcription factors (TFs) and RNA polymerase II (pol II). This complex acts to facilitate or repress transcription of specific mRNAs, which are subsequently translated into cellular/secretory proteins.

to specific binding sites on nuclear chromatin adjoining androgen-responsive genes. ARs function as transcription factors and form larger complexes via interactions with additional nuclear proteins that serve as transcriptional coactivators or adaptors, some of which possess intrinsic kinase, methyltransferase, or histone acetyltransferase activities. These multifactorial transcriptional complexes activate (or repress) androgen-regulated gene transcription by RNA polymerase, which alters the levels of specific mRNAs. The translation of mRNAs on cytoplasmic ribosomes directs the synthesis of proteins responsible for androgen-induced changes in cell function, growth, or differentiation.

It is important to remember that testosterone also serves as a precursor for estrogen biosynthesis. Therefore, estrogenic actions can occur in men following aromatization of testosterone to estradiol and binding of the latter steroid to nuclear estrogen receptors.

Hence, the local tissue metabolism and concentration of testosterone, DHT, or estradiol will determine the specific hormonal responses that are observed.

This chapter will address the biochemical events and molecular biology of androgen action, with a particular emphasis on the molecular mechanisms of androgen action at the cellular level. The androgen receptor is the key intracellular molecule involved in mediating androgen action and much of this chapter describes the multiple dimensions of its molecular structure and biologic function. Various human pathologic conditions associated with abnormal androgen action are discussed briefly, with emphasis on how studies of these conditions have increased our overall understanding of androgen action.

## ANDROGENS AND ANTIANDROGENS

Androgens are steroid hormones that induce the differentiation and maturation of the male reproductive organs, the development of male secondary sex characteristics, and the behavioral manifestations consistent with the male role in reproduction *(1)*. The two most important endogenous steroid hormones of the adult male that manifest both androgenic and anabolic activities are testosterone and DHT. Synthetic analogs of testosterone, such as nandrolone decanoate, oxandrolone, and stanozolol, produce primarily anabolic actions on muscle and skeleton and are often substances abused by bodybuilders and athletes. Antiandrogens are synthetic compounds that compete with androgens for binding to AR, but do not generate androgenic effects *(2)*. These compounds are used primarily in the treatment of prostate cancer and include cyproterone acetate, flutamide, and bicalutamide. Although cyproterone acetate acts as an inhibitor of androgen action, it is also a strong progestin with central antigonadotropic effects and is a weak glucocorticoid. Flutamide and bicalutamide are pure antiandrogens with no inherent glucocorticoid, progestational, androgenic, or estrogenic activities. Bicalutamide has an affinity for AR approximately fourfold greater than hydroxyflutamide, the active metabolite of flutamide. Bicalutamide also has a significantly longer half-life that is compatible with single daily administration.

## SEX HORMONE-BINDING GLOBULIN

Sex hormone-binding globulin (SHBG) is synthesized in the liver and forms a homodimer that is glycosylated and secreted into the blood as a 95-kDa β-globulin *(3)*. Several different isoforms of SHBG, represented by differential posttranslational glycosylation, normally appear in blood and a variant allele of the gene is present in a subpopulation of individuals. Each of the protein subunits is capable of binding a molecule of steroid near its dimeric interface *(4)*. SHBG binds testosterone, DHT, and, more weakly, estradiol *(5)*. Under physiologic conditions, about 40–50% of testosterone is bound with higher affinity to SHBG. Some evidence suggests that the actual in vivo bioavailable testosterone includes both the free steroid as well as the lower-affinity, readily dissociable albumin-bound steroid, or about half of the total testosterone *(6)*. The level of SHBG in blood is increased by estrogens, but decreased by androgens. The suppression of SHBG levels following exogenous administration of testosterone has been employed clinically as an index of human androgen sensitivity in cases of suspected androgen-insensitivity syndrome resulting from mutations in the AR gene. Recent studies have also demonstrated that SHBG–steroid complexes may be biologically active via their binding to cell surface SHBG receptors that evoke an

increase in intracellular cAMP levels *(7)*. Androgen-binding protein (ABP) is a product of the same gene that is expressed in testicular Sertoli cells and secreted into the seminiferous tubules, where it binds a significant proportion of the testosterone and/or DHT present in the intratubular fluid of the testes and epididymides. This may explain, in part, the apparent requirement for high intratesticular levels of testosterone in the maintenance of spermatogenesis.

## ANDROGEN METABOLISM

Testosterone is, itself, an active steroid with both androgenic actions to promote spermatogenesis in the testes and anabolic actions to increase tissue mass in muscle. In sexual tissues, such as the skin and prostate, testosterone is irreversibly converted primarily to the active metabolite DHT *(8)*. DHT can be further reduced to 5α-androstane-3α-, 17β-diol (3α-diol) and subsequently conjugated with glucuronide to form 3α-diol glucuronide *(9)*. These latter reactions are reversible such that 3α-diol, or its glucuronide, can be converted back to DHT. Most of the DHT, 3α-diol, and their glucuronides that appear in blood are derived from extrasplanchnic metabolism. Inactivation of testosterone occurs primarily in the liver and involves the formation of 17-ketosteroids via oxidation of the 17-OH group, reduction of the A ring, and reduction of the 3-keto group with the formation of polar metabolites that include diols, triols, and their conjugates *(10)*. Testosterone and its synthetic precursor androstenedione can be aromatized to estradiol and estrone, respectively, by the cytochrome P450 aromatase enzyme, designated CYP19 *(11)*. The enzyme complex catalyzes a multistep reaction leading to removal of the methyl group as formic acid and the rearrangement of the steroid A ring to an aromatic structure. This reaction requires NADPH as a cofactor for NADPH–cytochrome P450 reductase to enable the transfer of reducing equivalents to the enzyme, with 3 mol of oxygen being consumed in the sequence of hydroxylation reactions. Approximately 60–70 μg of estradiol are formed each day in normal men, primarily within adipose tissue, and this serves to further diversify the biological actions derived from androgens at the individual tissue level.

## STEROID 5α-REDUCTASE ENZYME

The conversion of testosterone to a variety of 5α- and 5β-reduced metabolites was known prior to the discovery that the 5α-reduced metabolite DHT was the principal intracellular androgen concentrated within the nuclei of many androgen-responsive target tissues, such as the prostate *(8)*. DHT proved to be twice as potent as testosterone in bioassays. Its physiologic importance was confirmed in human subjects with abnormal male sex differentiation and decreased serum concentrations of DHT resulting from genetic deficiency of steroid 5α-reductase enzyme activity *(12)*. Russell and Wilson *(8)* discovered that steroid 5α-reductase was present as two isoforms encoded by different genes, each containing 5 exons and having 50% identity of their nucleotide sequences. The 28- to 29-kDa enzyme proteins are localized to the endoplasmic reticulum and nuclear membranes, bind testosterone as a substrate, and require NADPH as a cofactor. The human type 1 isoform is present at low levels in the prostate but is the predominant isozyme expressed in the skin and liver. It is encoded by a gene on the short arm of chromosome 5, has an optimal activity across a broad pH range from 6.5 to 8.0, has a high $K_m$ (1–5 μ*M*) for testosterone, and is relatively insensitive ($K_i$ = 300–500 n*M*) to the

4-azasteroid inhibitor finasteride. The type 2 isozyme is encoded by its gene on the short arm of chromosome 2, has an acidic pH (5.0) optimum, and a low $K_m$ (0.1–1.0 μ*M*) for testosterone and is sensitive to inhibition by finasteride ($K_i$ = 3–5 n*M*). As discussed in a later chapter, molecular defects in the type 2 isozyme are responsible for the reduced serum and tissue DHT concentrations and inadequate virilization of the urogenital sinus and external genitalia observed in some infants with male pseudohermaphroditism because of steroid 5α-reductase enzyme deficiency.

## ANDROGEN RECEPTOR

The AR is a member of the larger superfamily of nuclear ligand-dependent transcription factors that includes all of the steroid receptors among its members *(13)*. Thus, the AR gene and protein share homology and conservation of structure and function with the other steroid receptors *(14)* (*see* Fig. 2). The human AR gene locus resides on the X chromosome between q11 and q12 and is estimated to span a region of approx 90 kb *(15,16)*. The gene encodes eight exons and transcription is initiated from one of two sites within a 13-bp region of a single promoter that lacks a TATA or a CCAAT box *(17)*. The AR promoter contains a GC box near the initiation site and an adjacent upstream homopurine/homopyrimidine stretch. Transcription from the initiation site at +13, but not +1, is dependent on binding of Sp1 to the GC box. The purine/pyrimidine region can bind Sp1 in its normal double-stranded B-DNA conformation, but a novel single-strand binding protein may also be involved *(18)*. Relative to the rather large size of the AR gene, the predominant mRNA transcript is 10.6 kb, with a minor 8.5-kb transcript also detected in various tissues. The 10.6-kb transcript includes a 1.1-kb 5' untranslated region (UTR), the 2.7-kb open reading frame (ORF) and a relatively long 3' UTR of 6.8 kb, whereas the shorter, alternative 8.5-kb mRNA transcript arises from differential splicing in the 3' UTR. Recently, unique androgen-responsive regulatory regions within exons 4 and 5 have been postulated to mediate upregulation of AR mRNA transcription that occurs in several cell types in response to androgen *(19)*. A portion of the 5' UTR of AR mRNA is capable of forming a stem-loop secondary structure that has been suggested to play a role in the induction of AR translation *(20)*.

Variations in the coding region of the cDNA clones for the human AR arise as a result of polymorphisms in two stretches of the nucleotide sequence that encode amino acid polymeric repeats for glutamine and glycine within the amino terminus of the approx 110-kDa protein. Hence, the number of amino acids reported in the literature for AR vary between 910 and 919. All numeric references to amino acid residues in this chapter are derived from the original AR cDNA cloned by Lubahn et al. *(21)* and have been adopted as the international reference standard for the Human Androgen Receptor Mutation Database maintained at McGill University *(22)*.

Posttranslational modification of AR occurs via phosphorylation on several serine residues and results in a shift in the apparent molecular weight (MW) to 112–114 kDa *(23,24)*. The translation of an 87-kDa (AR-A) minor isoform of the receptor has been reported to occur from an alternative initiation methionine codon at position 189, but this isoform generally represents less than 20% of the total AR protein expressed in tissues *(26)*. Interestingly, cell transfection studies showed that transcriptional activation by the amino truncated AR-A isoform could not be distinguished from the full-length AR-B isoform. More recent studies, however, suggest that the entire amino terminus plays a

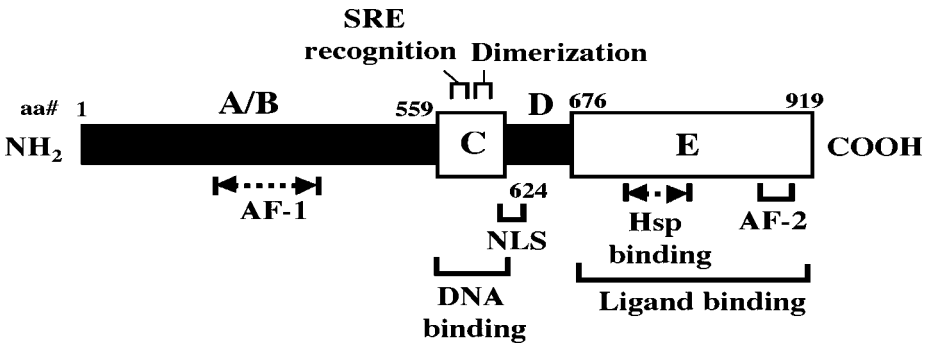


**Fig. 2.** Structural and functional domains of the human androgen receptor. The human AR is a 919-amino-acid protein with homology of structure and function with other members of the nuclear steroid-receptor family. Five representative structural and functional domains indicated by the letters A–E are conserved within these receptor proteins. The amino-terminal A/B region is characterized by significant divergence of amino acid sequence and number among the various receptors presenting differences in epitope recognition and in properties related to transcriptional activation. A strong transcriptional activating function (AF-1) resides within the A/B domain of AR. The central C region contains the two zinc fingers required for DNA binding, with the first zinc finger containing the "P-box" for specific recognition of steroid response elements (SREs) on DNA and the second zinc finger containing the "D box" that promotes receptor dimerization. A nuclear localization signal (NLS) sequence resides in the region linking the DNA-binding and hinge (D) domains. The hinge region provides conformational flexibility within the molecule between the DNA-binding and ligand-binding (E) domains. The E domain also contains putative sites for binding of chaperones, such as the heat shock proteins (hsps), and a second, ligand-dependent activating function (AF-2) where coactivators may bind to AR.

critical role in the conformation and stability of the ligand-bound receptor. Like other nuclear steroid receptors and as discussed in the forthcoming subsections, the AR shares a molecular architecture that includes a poorly conserved amino-terminal transactivation domain, a highly conserved DNA-binding domain, a connecting hinge region, and a discrete ligand-binding domain (*see* Fig. 2).

### *Amino-Terminal Transactivation Domain*

The amino terminus is the least conserved region and varies significantly in length among members of the steroid-receptor superfamily. The relatively long amino terminus of the AR includes 538 amino acids and is entirely encoded by the first exon of the AR gene *(26)*. Assays of transcriptional activity that examined the effects of deleting different portions of the AR cDNA, initially ascribed a transcriptional activation domain to the amino terminus of AR *(14)*. However, only recently have more detailed structural analyses revealed additional significant biological functions of this region. The entire amino-terminal domain is required for full activity of the receptor *(27,28)*. More specifically, a core region was defined between amino acids 101 and 360, termed tau-1, that contributes 50% of the activity. However, in the absence of the carboxyl-terminal ligand-binding domain, a different region, termed tau-5 (residues 370–494), can also mediate activation. A functional interaction occurs between the amino-terminal domain, which contains the so-called activation function (AF)-1, and the carboxy-terminal ligand-binding domain,

which contains a second activation domain referred to as AF-2. Within the amino terminus of AR, the subregions encompassing amino acid residues 14–36 and 371–494 were subsequently implicated in these interactions with the ligand-binding domain. Most recently, the FXXLF motif represented by the amino acid sequence FQNLF (aa 23–27) and the WXXLF motif represented by the amino acid sequence WHTLF (aa 433–437) have been identified as the specific regions within the amino terminus of AR that interact with other regions within the carboxy-terminal ligand-binding domain *(29)*. The increased stability of the AR that is observed when it is bound to androgen has been attributed to the intramolecular interactions that occur between the amino- and carboxy-terminal regions of AR. Moreover, the conformational change imposed by such intramolecular interaction may interfere with the recruitment and interaction of the commonly recognized LXXLL motifs that reside within most nuclear coactivators and their recognition by the AF-2 domain found in most steroid receptors, including AR (discussed in more detail under the headings Ligand-Binding Domain and Coactivators). Recent reports have inferred that AR interaction with nuclear coactivators relies less upon the AF-2 domain and more on the AF-1 domain for enhanced transcriptional activity *(29–31)*. Furthermore, the AF-1 domain to which coactivators binds appears to be distinct from the region involved in the amino- and carboxy-terminal interaction within the AR protein.

A unique, polymorphic polyglutamine stretch encoded by (CAG)nCAA and polymorphic polyglycine sequence encoded by (GGN)n, are present in the human AR amino terminus, in addition to polyalanine and polyproline amino acid repeats *(21)*. Within the normal population, the number of glutamine repeats varies from 9–33 residues and the glycine stretch ranges between 16 and 27 residues *(32,33)*. The polymorphic nature of these amino acid repeats accounts for natural variations in the length of the cDNAs that were cloned for the human AR and in the assignment of amino acid numbers encoded within the protein. Acidic polyproline and polyglutamine sequence motifs are generally believed to confer a transcriptional activation function when present in various proteins. Indeed, fewer numbers of glutamine residues in this region of AR are associated with higher levels of gene transactivation than are longer repeat lengths *(32)*. Moreover, genetic variations in the length of the polyglutamine stretch are implicated in the progressive nature of prostate cancer *(34,35)* and in the neuromuscular degenerative disease described by Kennedy, otherwise known as spinal bulbar muscular atrophy *(36)*. In prostate cancer, the more transcriptionally active AR with fewer polyglutamine residues is associated with higher incidence and faster progression of disease, whereas in spinal bulbar muscular atrophy, abnormally long repeats that exceed 40 glutamine residues are associated with neuronal degeneration.

### *DNA-Binding Domain*

The DNA-binding domain represents a highly conserved, cysteine-rich region that occupies a central location within the structural organization of the various members of the steroid-receptor superfamily (*see* Fig. 3). In the AR, this 66-amino-acid domain is encoded by exons 2 and 3 and shares 70–80% homology of amino acid sequence with the related glucocorticoid (GR) and progesterone (PR) receptors *(26)*. Derivation of the molecular structure of the AR DNA-binding domain is based on previous nuclear magnetic resonance (NMR) and X-ray crystallographic data for the GR. The DNA-binding domain of AR consists of three $\alpha$-helices organized into two zinc fingers within which four cysteine residues at the base of each finger coordinate zinc in a tetrahedral

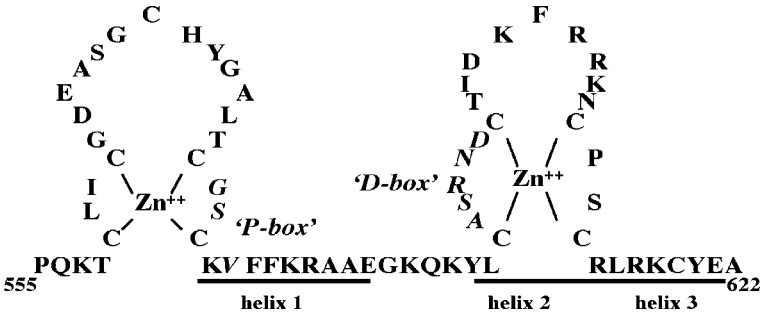


**Fig. 3.** Amino acid sequence of the human androgen-receptor DNA-binding domain. The single-letter amino acid code is shown for the sequence within the two zinc fingers formed by tetrahedral coordination of zinc by each of four cysteines in the androgen receptor DNA-binding domain. The proximal "P box," comprised of the amino acids G, S, and V, for specific recognition of the androgen response element on DNA is indicated in the first finger and the distal "D box," composed of A, S, R, N, and D, involved in dimerization is indicated in the second finger. Three helices are formed within the structure of the DNA-binding domain, with helices 1 and 3 arranged in perpendicular plane to the DNA.

array *(37)*. The first zinc finger incorporates a perpendicularly oriented α-helix where the amino acid residues gly577, ser578, and val581 at its carboxy terminus form the proximal (P) box, which is conserved in AR, GR, and PR. Mutational analyses showed that this conserved sequence is responsible for the common recognition and binding of these three receptors to a similar DNA steroid response element (SRE) consisting of the consensus palindromic nucleotide sequence, -AGAACAnnnTGTTCT-. The α-helix containing the P box is positioned in the major groove of DNA where the three conserved amino acid residues make base-specific contacts with DNA. The second zinc finger has two α-helices, the first of which forms the distal (D) box, consisting of five amino acids (ala-ser-arg-asn-asp) that form a symmetric interface for homodimerization of the receptor that accompanies its binding to a palindromic SRE, as previously demonstrated for GR. The first and third helices within the DNA-binding domain are perpendicular to each other and form numerous hydrogen bonds.

The critical features of protein–DNA binding that differentially determine the specificity of AR, GR, and PR binding to DNA regulatory sequences, and hence their discriminatory function in steroid-specific gene regulation, are now under scrutiny. Recent experiments have identified genes that are specifically responsive to androgens and analysis of the AR-binding sites in their promoters has revealed SRE motifs that resemble direct nucleotide repeat sequences rather than palindromic, inverted nucleotide repeat sequences *(38)* (*see* Table 1). Further analysis of the binding of AR to these direct-repeat nucleotide binding sites suggested that the dimerization interface within the AR homodimers differed from that observed on inverted-repeat DNA-binding sites. These findings predicted that dimerization of the AR occurred in a head-to-tail orientation of the monomeric AR molecules and that the intermolecular interactions at the dimerization interface involved amino acids in the second zinc finger and the adjoining hinge region *(38)*. Subsequent mutation analysis revealed that three specific amino acids, thr585, gly610, and leu617, are involved in the recognition of the direct nucleotide repeat

**Table 1**
**Classification of Androgen Response Elements**

| | |
|---|---|
| A. High affinity, nonspecific | |
| GRE177 | GTTACA aac TGTTCT |
| C3(1) ARE | AGTACT tga TGTTCT |
| GRE2 TAT | TGTACA gga TGTTCT |
| PSA ARE1 | AGCACT tgc TGTTCT |
| SLP-HRE-3 | GAAACA gcc TGTTCT |
| B. High affinity, AR specific | |
| PB-ARE-2 | GGTTCT tgg AGTACT |
| SLP-HRE-2 | TGGTCA ggc AGTTCT |
| SC ARE1.2 | GGCTCT ttc AGTTCT |
| C. Low affinity, nonspecific | |
| PB-ARE-1 | ATAGCA tct TGTTCT |
| MVDP pARE | TGAAGT tcc TGTTCT |
| GPX5 | ATCCTA tgt TGTTCT |
| CRP2 | AGAACA aaa TGTACA |
| D. Low affinity, AR specific | |
| SC ARE | AGCAGG ctg TGTCCC |

*Note:* Four classes of androgen response elements have been defined by Claessens et al. *(38)* based on gel-shift assays of protein–DNA binding with AR or GR and by transfection experiments in which transcription of an ARE-reporter gene is activated by either AR and/or GR. The androgen response elements (AREs) and glucocorticoid response elements (GREs) are derived from the promoter sequences: GRE177 from the MMTV-LTR; C3(1) ARE from the C3 subunit of the rat prostate prostatein gene; GRE TAT from the mouse liver tyrosine aminotransferase gene; PSA ARE1 from the human prostate-specific antigen gene; SLP-HRE-2 and -3 from the mouse sex-limited protein gene; PB-ARE-1 and -2 from the rat prostate probasin gene; SC ARE1.2 from the human secretory component gene; VDP pARE from the mouse vas deferens protein gene; GPX5 from the mouse epididymal glutathione peroxidase 5 gene; and CRP2 from the cystatin-related protein gene.

that serves as an AR-specific binding site in the rat probasin gene promoter. Further support for this hypothesis will evolve as the AR-binding sites are identified within the promoters of other androgen-responsive genes. In addition, the proximity of binding sites on the DNA of gene promoters for other transcription factors can also modify the specificity for AR binding to adjacent SREs; hence, the transcriptional activity of AR is also dependent on the context of individual promoter elements *(39)*.

### *Nuclear Localization Signal and the Hinge Region*

In the absence of ligand, the AR is primarily localized to the cytoplasm, whereas the addition of androgen induces its rapid translocation to the nucleus *(14)*. A bipartite nuclear targeting signal sequence encoded at the junction between exons 3 and 4 func-

tions to shuttle the AR through nuclear pores. The signal sequence consists of 2 clusters of basic amino acids, separated by 10 amino acids, that reside within the region that joins the DNA-binding domain and the hinge region *(40)*. Amino acid substitutions or deletions in this region bounded by residues 617–633 cause an almost complete cytoplasmic localization of the receptor.

Recent advances in imaging techniques and the in vivo expression of chimeric AR tagged with a modified version of green fluorescent protein have revealed a number of important properties associated with ligand-dependent nuclear translocation of the receptor in live cells *(41)*. First, in the absence of ligand, the AR is predominantly localized to the cellular cytoplasm. Second, upon the addition of androgen, such as DHT, the nuclear translocation of AR is apparent within minutes and is nearly complete within 30 min. The apparent rate and extent of nuclear import is reduced with ligands that display reduced affinity for the receptor or antagonist activity. In the presence of androgens that act as agonists and activate transcription, the nuclear AR appears tightly associated with chromatin and organized within distinct subnuclear compartments. By comparison, when cells are exposed to antagonists, such as the antiandrogens flutamide or bicalutamide, the AR exhibits ligand-dependent migration into the nucleus but does not display a pattern of subnuclear compartmentalization as observed in the presence of androgen. Furthermore, androgen withdrawal releases the receptor from its chromatin association and exports it back into the cytoplasmic compartment for recycling when the steroid is reintroduced. The small nuclear ring finger protein SNURF, which interacts with the AR through a region overlapping the bipartite nuclear localization signal, facilitates AR nuclear import and retards its export upon hormone withdrawal *(42)*. Further details regarding the processes of nuclear import and export are likely to become apparent from additional experiments using sophisticated imaging techniques.

The hinge region has been associated with conformational flexibility induced by the binding of ligand to the carboxy-terminal domain and the binding of the ligand-activated receptor to DNA. Interestingly, a repressor activity is associated with the hinge region in AR. Deletion of amino acid residues 628–646 from the hinge region of AR created a mutant receptor with a twofold higher ligand-dependent transactivation activity than the wild-type receptor *(43)*. Moreover, interaction of the mutant receptor with the p160 coactivator TIF2 was significantly enhanced, as was its effect on AR transactivation. Mutations of the AR involving single-amino-acid substitutions within the hinge region also occur in prostate cancer. Recently, several of these mutant ARs were shown to have increased transcriptional activity when compared with the wild-type AR *(44)*.

### *Ligand-Binding Domain*

Androgen agonists and antagonists bind to the carboxy-terminal ligand-binding domain of AR encoded by exons 4–8 *(26)*. In the absence of ligand, some steroid receptors actually repress gene transactivation by occupying nuclear chromatin sites. By contrast, the AR is predominantly localized to the cytoplasm in the absence of ligand and exists within a large macromolecular complex of chaperone proteins that includes several members of the heat shock family *(45)*. In this state, the AR is maintained in an inactive conformation that is not capable of binding to DNA. Deletion of the ligand-binding domain relieves its repressive function such that the truncated AR protein is able to bind to DNA and is constitutively active as a result of expression of its intrinsic AF-1 activation domain *(14)*. Alternatively, the binding of an androgen agonist causes the

inactive macromolecular chaperone complex to dissociate and promotes the nuclear translocation and DNA binding of AR *(46)*. Agonist binding also activates the transactivation function of AF-2 and this coincides with alteration of the AR molecular conformation. The active conformation of the receptor is stabilized by the presence of androgen and AR homodimers bind in the nucleus to androgen response elements (AREs) on DNA *(47)*. Androgens bind to either the nonphosphorylated or phosphorylated forms of AR. Although the significance of posttranslational modification of AR by phosphorylation has not been clearly defined, its role in ligand-independent activation via alternative signal transduction pathways has been inferred from a number of recent studies.

A series of 12 conserved helices form the ligand-binding domain of steroid receptors as revealed by X-ray crystallographic analyses of these receptors conducted in the presence and absence of agonists and antagonists *(48,49)*. The AR ligand-binding-domain crystal structure in the presence of the synthetic ligand, R1881, contains nine α-helices, two $3_{10}$-helices, and four short β-strands associated in two antiparallel β-sheets *(50,51)* (*see* Fig. 4). The AR, like PR, has no helix 2, but its helix 12 is longer than those of some other steroid receptors and has an extended carboxy-terminal amino acid chain. The helices are arranged in a "helical sandwich" pattern and helices 4 and 5, and 10 and 11, are contiguous. In the case of AR, helices 10 and 11 are interrupted by a proline residue at position 868 which causes a kink between the helices. As in PR, there is a very short β-sheet formed by the loop between helices 8 and 9 and the carboxy-terminus, which holds helix 12 in the closed agonist conformation, close to and capping the ligand-binding site (*see* Fig. 5). The androgen ligand DHT interacts with helices 3, 5, and 11 within the ligand-binding domain. The AF-2 region resides within helix 12 of the AR and other receptors. When the receptor is occupied by a steroid agonist, helix 12 closes over the ligand-binding pocket to form a surface for interaction with transcriptional coactivators; by contrast, binding of antagonists prevents this interface from forming. Conformational changes that accompany binding of steroid agonists also promote the intramolecular interactions that occur between the amino-terminal transactivation (AF-1) and carboxy-terminal ligand-binding (AF-2) domains in AR, interactions that are critical for receptor transactivation *(29)*. The relatively weaker transactivation effect of the AF-2 domain in AR has been associated with a competition between the internal AF-1 domain and coactivators for interaction with AF-2. It has been suggested that the p160 family of coactivators interact with AR by forming a bridge that links the AF-1 and AF-2 domains *(28–31,52)*.

### *Steroid Response Elements in DNA*

Steroid response elements minimally contain a core recognition motif of six nucleotide basepairs, generally consisting of two core motifs (half-sites) separated by a spacer of variable length *(53,54)* (*see* Table 1). The nucleotide sequence of the core motif is relatively specific for subgroups of receptors; the AR, GR, and PR all bind to hexamer half-sites with the consensus sequence TGTTCT. However, an ARE sequence differs from that for the estrogen receptor, TGACCT, at positions 3 and 4, which determines receptor-specific recognition. The consensus SRE for AR is a 15-bp sequence, AGAACAnnnTGTTCT, organized as an inverted or palindromic repeat of core motifs, but which fails in most circumstances to discriminate among AR, GR, and PR. More recently, the identification of synthetic and naturally occurring direct repeats that specify binding of AR, rather than GR, or PR, have suggested that regulatory regions in the

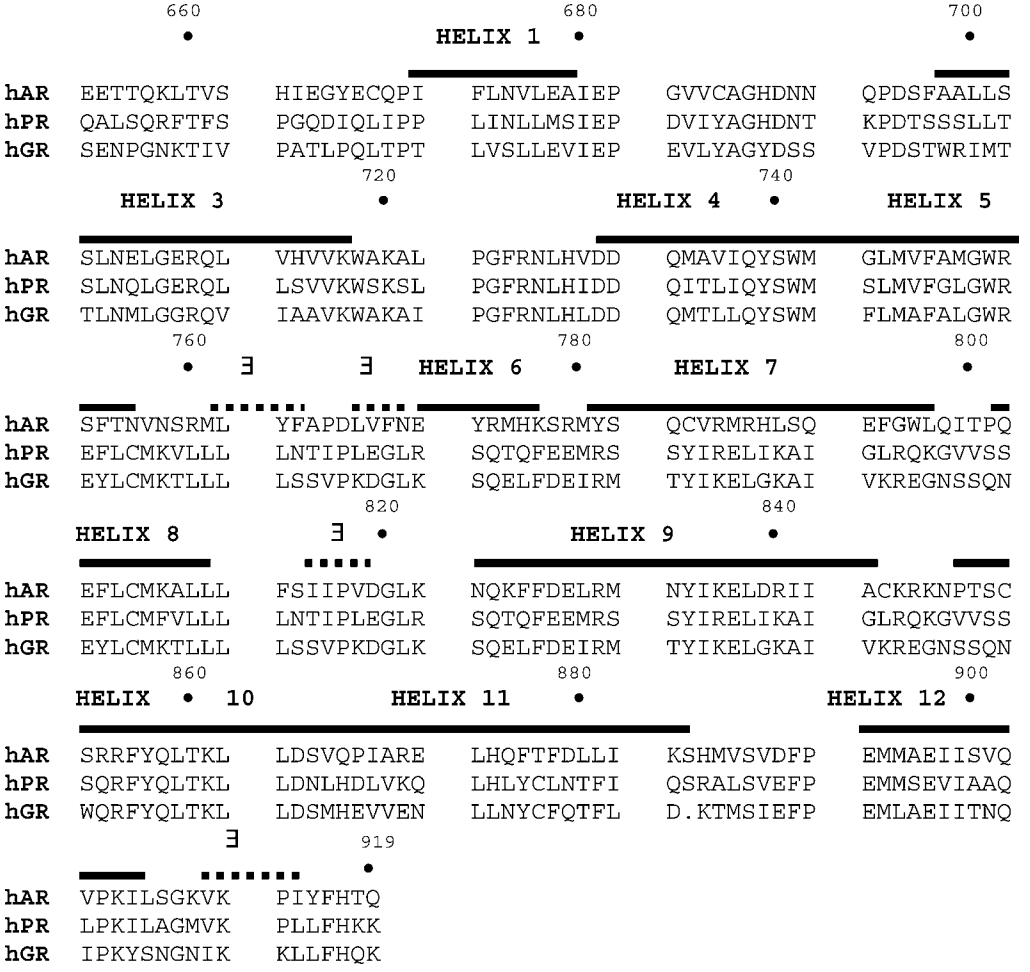


**Fig. 4.** Amino acid primary sequence of the human androgen-receptor ligand-binding domain and its homology with the ligand-binding domains of the human progesterone and glucocorticoid receptors. The single-amino-acid code is shown for each of the receptors and the amino acid residue number is indicated (*) for the androgen receptor. The positions of each of the conserved 11 α-helices (H1–H12) and the 4 β-strands in the AR secondary structure are indicated by the solid and dashed lines, respectively, based on the recently published crystal structures of the androgen receptor. (From refs. *50* and *51*.)

promoters of androgen-responsive genes may utilize these sequence-specific binding sites *(38,55)* (*see* Table 1). As mentioned previously, the dimerization of AR monomers in an antiparallel configuration when bound to DNA may also direct AR-specific transactivation *(38)*.

### *Transcriptional Activation*

Sequence-specific DNA-binding transcription factors, such as AR, interact with other general transcription factors in the control of gene activation. These general factors, in turn, interact with the core promoter elements to induce basal transcription. RNA polymerase II and the general transcription factors form the transcription initiation complex

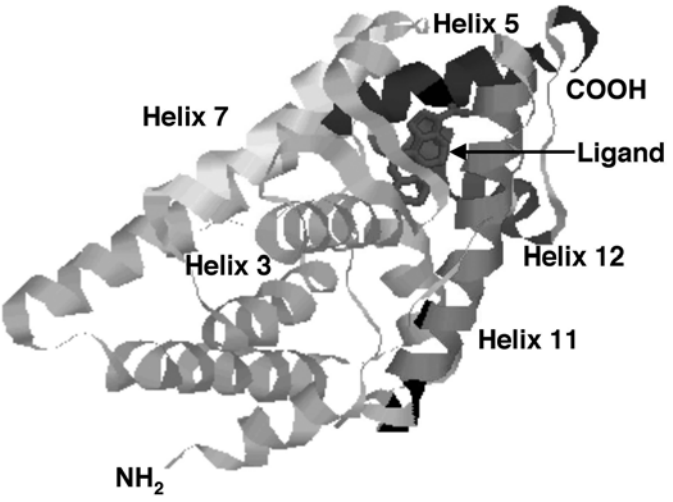


**Fig. 5.** Tertiary ribbon structure of the human AR ligand-binding domain in the presence of its ligand based on its crystal structure. The ligand, methyltrienolone (R1881), which makes contact with helices 3, 5, and 11 is shown within the binding pocket of the AR, which is capped by helix 12 in the presence of androgen agonists. The AR crystal structure was published by Matias et al. (ref. *50*) and can be viewed at the internet website http://ww.rcsb.org/pdb/ in the Protein Data Bank (PDB) Structure Explorer under the molecule reference number 1E3G.

in conjunction with the TATA-box binding proteins, TATA-binding protein (TBP) and TBP-associated factors ($TAF_{II}$s). Steroid receptors may enhance basal transcription, either by direct interaction with the basal transcription machinery or with $TAF_{II}$s *(56)*. For example, interaction of the amino terminus of AR with $TF_{II}F$ has been reported *(57)*. Most such interactions between AR and the transcriptional machinery imply direct binding of AR to chromatin. However, AR may not necessarily bind to DNA but rather may bind directly with other sequence specific DNA-binding transcription factors, such as AP-1 and nuclear factor (NF)-κB to modulate gene transcription *(58,59)*.

### *Coactivators and Corepressors*

Coactivators are cellular proteins that interact with the agonist-activated steroid receptor complexes to enhance transactivation of target genes, whereas, in most cases, corepressors have an inverse effect through interaction with antagonist-bound or ligand-free receptors *(60–62)*. A general model for coactivator and corepressor function has evolved. Initially, the repressor protein associates with the receptor on chromatin to maintain a transcriptionally inactive structure by tethering of histone deacetylases to the DNA at sites close to the responsive element for the receptor. Subsequently, binding of hormone by the receptor causes release of the corepressor and recruitment of acetyltransferases, which disrupt the chromatin template by acetylation of histones. Finally, interaction between the activation domains of both the receptor and the recruited coactivator with the basal transcription factors results in gene transcription. Whereas the aforementioned mechanisms may be generally applicable, the ligand-free AR exists predominantly in the cytoplasm in a complex of chaperone proteins and is probably not involved in interactions with corepressors that possess histone deacetylase activity as

part of an inactive chromatin-bound complex. However, when steroid antagonists bind to receptors, these complexes can bind to DNA, yet they fail to activate gene transcription. This effect may be the result of failure of coactivators to interact with the antagonist-bound form of the receptor or to the hypothetical binding of a corepressor and associated histone deacetylase. Coactivators may have several roles, including expression of intrinsic histone acetyltransferase (HAT) activity, recruitment of other proteins with HAT activity, and as integrators that enable regulatory molecules to be recruited and assembled at sites of transcriptional activity *(60,61)*.

A number of coactivators are either directly or indirectly involved in chromatin remodeling *(60,61)*. The so-called p160 family of coactivators have been subdivided into three subgroups, represented by SRC-1 (steroid-receptor coactivator-1), SRC-2 which includes TIF2 (transcriptional intermediary factor 2) and GRIP-1 (glucocorticoid-receptor interacting protein 1) and SRC-3, which includes TRAM-1 (thyroid-receptor activator molecule 1), ACTR, and AIB1 (amplified in breast cancer 1). In general, these coactivators interact with the ligand-activated AF-2 regions of different steroid receptors to enhance transcriptional activity. However, for AR, the interaction of these coactivators appears to be predominantly with the AF-1 region and much less with the AF-2 region *(30,31)*. The coactivators possess one or more receptor interaction domains represented by the amino acid sequence motif LXXLL. The coactivators like SRC-1 also interact with integrators such as p300/CBP (cAMP-response element binding [CREB]-binding protein) and pCAF (p300/CBP-associated factor), proteins that possess intrinsic HAT activity, with the overall effect being synergistic for transcriptional activation *(60,61)*.

Other proteins have been identified as coactivators or effectors of transcriptional activity through their specific interaction with AR. Among these are a series of proteins of varying molecular weight that have been reported by Chang et al. The most well-characterized of these proteins is ARA-70 (AR-associated protein, 70 kDa), which interacts in a ligand-dependent manner with AR to increase its transcriptional activity *(63)*. Other members of this group include ARA24, ARA54, ARA55, and ARA160 *(64–66)*. The interaction between AR and other putative coregulator proteins has been reported *(67–75)*. These proteins include ARIP3 (androgen-receptor interacting protein)/PIAS 1 (protein inhibitor of activated STAT), SNURF, ANPK (androgen nuclear protein kinase), BRCA1 (breast cancer susceptibility), BAG-1, Smad3, cyclin E, Ubc9 (ubiquitin E2-conjugating enzyme), and HBO1 (MYST family, human origin recognition complex). The encyclopedia of proteins continues to grow, but the biological relevance of individual protein interactions with AR will require further investigation.

By contrast to molecules that function as coactivators, the corepressors act to repress basal promoter activity in the absence of hormone. Studies of the corepressors, NCo-R (nuclear-receptor corepressor), SMRT (silencing mediator for retinoids and thyroid hormone), and RIP140 (receptor interacting protein, 140 kDa) have provided some general insights into the activities of these molecules *(62)*. The relative activity and interaction of general corepressors on AR transcriptional activity have not been reported, but the PIASy isoform functions in transrepression of AR activity *(75)*.

### *Ligand-Independent Activation*

Steroid receptors, including AR, can also be stimulated to activate gene transcription in the absence of ligand by growth factors, such as insulin-like growth factor I (IGF-I), keratinocyte growth factor (KGF), epidermal growth factor (EGF), luteinizing hor-

mone-releasing hormone (LHRH), neuropeptides, HER-2/neu, interleukin-6 (IL-6) and other agents that directly or indirectly increase intracellular kinase activity or diminish phosphatase activity *(76,77)*. Although the AR is a phosphoprotein, it is unclear whether peptide-activated kinases directly phosphorylate the AR or whether this results in receptor activation, possibly through enhanced binding to DNA. In addition to the AR itself, phosphorylation of coactivators and basal transcription factors could explain the AR ligand-independent transcriptional activity. Importantly, peptide activation of AR activity is synergistic with minimal levels of androgens, thus lowering the androgen threshold concentration required for full AR function. These observations are particularly relevant for androgen-deprivation therapy of patients with prostate cancer, in whom the level of androgen is decreased but not completely ablated, and in androgen-independent tumors, where AR expression is present.

Protein kinase A (PKA) can induce prostate-specific antigen (PSA) mRNA and androgen-responsive reporters in prostate cancer cells, an effect that is blocked by antiandrogens *(78)*. Forskolin directly activates AR transcriptional activity *(79)*. Interestingly, the binding of AR to ARE sequences is increased when nuclear extracts are prepared from forskolin-treated cells, despite the observation that nuclear levels of AR are 10-fold higher in androgen-treated cells. Both cAMP and the activator of protein kinase C (PKC), TPA, act synergistically to enhance androgen-stimulated AR transcriptional activity *(80,81)*. 8-Bromo-cAMP has also been shown to induce phosphorylation of SRC-1 and to facilitate ligand-independent activation *(82)*. IL-6 activates AR in prostate cancer cells through a mechanism that involves crosstalk between AR and PKA, PKC, and/or MAPK via ErbB2 *(83)*. AP-1 is a complex of transcription factors encoded by c-fos and c-jun protooncogenes and has been implicated in cell growth, differentiation, and development with its activity modulated by growth factors, cytokines, oncogenes, and activation of PKC by tumor promoters. The fact that c-jun inhibits AR action in some prostate cells and activates it in others emphasizes possible cell- and/or promoter-specific responses that may be explained by differences in the requirements of a particular promoter for coactivators or by differences between cells in the availability of required coactivators *(78,84,85)*. The HER2/Neu protooncogene has been linked to proliferation of prostate cancer cells and can induce AR transactivation of the PSA gene by the mitogen-activated protein (MAP) kinase-dependent phosphorylation of AR *(86,87)*. Inactivation of the PTEN tumor suppressor gene in prostate cancer allows the AKT/PKB pathway to be constitutively active. Alternatively, activation of PI3 kinase by IGF-I can lead to activation of AKT/PKB *(88)*. AKT binds to and phosphorylates AR. Taken together, these results show that multiple kinase-dependent phosphorylation pathways can be activated in prostate cancer cells, suggesting that phosphorylation events affecting AR, related transcription factors, or coactivators may lead to ligand-independent activation of AR.

## ANDROGEN RECEPTOR AND HUMAN PATHOLOGY

### *Androgen Insensitivity Syndrome*

The AR is required for normal male sex differentiation and development. Much of our knowledge regarding AR function is derived from studies of human subjects with mutations of the AR gene and defects in the biological action of androgens that underlie the androgen-insensitivity syndrome (AIS) *(89,90)*. AIS can be either complete in which the phenotype of

the external genitalia is female, or partial, in which the external genitalia are phenotypically ambiguous. Although hypospadias is a frequent phenotypic feature of partial AIS, isolated hypospadias is rarely associated with an AR gene mutation *(91)*. Numerous AR gene mutations have been identified in AIS and consist primarily of single nucleotide substitutions that result in amino acid substitutions, termination codons, frame shifts, or alterations of mRNA splicing and relatively few deletions varying from a few nucleotides to the entire gene *(89,90)*. Mutations leading to varying degrees of androgen insensitivity have been reported in each of the eight exons of the AR gene. However, most mutations have been identified in the regions that encode the DNA- and ligand-binding domains.

### *Prostate Cancer*

The AR has been the focus of intensive investigation for its role in the mechanisms that regulate the growth and progression of prostate cancer. AR expression is present in prostate tumors at all stages of the disease and tumor growth often continues despite androgen ablation therapy that includes inhibition of gonadotropin secretion and testicular androgen synthesis, antiandrogens that antagonize AR function, and even castration. Recent research has centered on the possibility that AR mutations may occur in more advanced stages of prostate cancer and contribute to the progression of disease via alteration of cell function and proliferation. Thus, AR mutations may lead to gain of function such that the AR is exquisitely sensitive to very low levels of androgen *(92)*, the ligand specificity of the receptor is altered *(93,94)*, or the receptor is activated in the absence of ligand *(76,77)*. Although somatic mutations of the AR have been identified in prostate tumor specimens, reports on the frequency of such mutations have been highly variable between laboratories and study populations, ranging from less than 10% to almost 50% *(95–97)*. In general, the frequency of mutations appears to increase in tumors from later stages of prostate cancer as disease progresses and in metastatic lesions. Another mechanism involves AR gene amplification that has been detected in up to 30% of advanced cancer specimens and may lead to enhanced levels of AR expression *(98)*. As previously mentioned, ligand-independent activation of AR may result from crosstalk with other signal transduction pathways in the absence of androgen. In addition, alterations in the size of the polyglutamine repeat may affect the transactivation of AR and influence androgen action in prostate cancer *(35,99)*. Finally, enhanced expression or phosphorylation of coactivators may lead to their affect to amplify AR function, especially in an environment of reduced endogenous androgen levels following ablation therapy *(100)*.

### *Spinal Bulbar Muscular Atrophy*

An abnormal expansion of the polyglutamine stretch, encoded by (CAG)nCAA, was identified as the molecular basis for spinal bulbar muscular atrophy (SBMA; Kennedy's disease) *(36)*. In normal individuals, the (CAG)nCAA repeat contains 9–33 CAG-like triplets, whereas 38–75 CAG codons are generally found in the AR genes of subjects with SBMA. Increasing disease severity is correlated with greater length of this repeat. As a disease, SBMA is characterized by progressive muscle weakness and atrophy with clinical symptoms manifest in the third to fifth decade of life *(32,33,36)*. The pathology is associated with a severe depletion of lower motornuclei in the spinal cord and brainstem and distal axonopathy of the dorsal root ganglion cells. In addition, subjects with SBMA frequently exhibit endocrine abnormalities, including testicular atrophy, reduced or

absent fertility, gynecomastia, and elevated follicle-stimulating hormone (FSH), LH, and estradiol levels, similar to what is observed in mild forms of AIS. Sex differentiation occurs normally and characteristics of mild androgen insensitivity only appear later in life. This may be related to the combination of reduced AR expression and decreased testosterone level in older men. SBMA is an X-linked disease and occurs only in men. At present it is not known whether disease progression involves the ligand-activated or ligand-free AR. In two cases, an extended period of exogenous testosterone administration had little effect on clinical symptoms.

In transfection studies, the length of the CAG repeat was inversely proportional to the ability of the expressed AR to transactivate an androgen-regulated reporter gene *(32)*. However, other investigators have suggested that reduced transcriptional activity was secondary to reduced stability of AR mRNA and decreased AR protein synthesis *(101)*. The reduction in AR function has also been related to the reduced interaction between coactivators and AR *(102)*. Interestingly, AR with an expanded CAG repeat are more resistant to proteolytic degradation and, in particular, are less susceptible to cleavage by caspase-3 *(103,104)*. The increased length of the polyglutamine tract also results in the formation of nuclear inclusions. These aggregates accumulate in the cytoplasm and nucleus and sequester other cellular proteins such as SRC-1, NEDD8, and Hsp70 and Hsp90 *(105)*. These aggregates appear to be the result of misfolding of protein and defects in proteolytic degradation involving the ubiquitin–proteasome pathway.

### *Male Infertility*

Male infertility has been associated with an abnormal androgen receptor in subjects with mild forms of androgen insensitivity and absence of other phenotypic abnormalities of the external genitalia *(105)*. Mutations in the AR gene have been associated with only a very few cases of isolated azoospermia *(107–109)*. However, recent studies have demonstrated that the glutamine repeat length may be increased in some men with oligospermia and azoospermia *(110)*. Hence, men with longer CAG repeats centered around the norm of 20 glutamine repeats have a greater risk for infertility.

## SUMMARY

Androgens play a key role in male sex differentiation and development and in the maintenance of male reproductive function, and the effects of these hormones are an important component in the development of several pathologic conditions. Testosterone and its 5α-reduced metabolite DHT are potent androgens that act upon target cells to initiate and maintain the masculine phenotype. Germline mutations in the androgen receptor and steroid 5α-reductase genes cause the androgen insensitivity syndromes and 5α-reductase deficiency, respectively. The effects of these genetic mutations on male sex differentiation and development have played a key role in elucidating the pathways of androgen action. Androgen receptors transduce the steroid signal within cells, but attempts to correlate differences in receptor levels with various disease states have been relatively unsuccessful. However, molecular studies of AR gene structure have recently provided new insights toward defining a molecular and genetic basis for the pathology associated with diseases—including spinal bulbar muscular atrophy, prostate cancer, and male infertility—affecting middle-aged and older men. Further studies at the molecular level to define the steroid- and DNA-binding properties of androgen recep-

tors, as well as the transcriptional activity and interactions of the receptor with coactivators, corepressors, and integrators within the transcriptional complex, will provide additional insight into the complex nature of androgen action. Moreover, epidemiologic data and molecular genetic analyses of gene structure have led to a new understanding of the interrelationships between environmental and genetic factors that may impact on the incidence of certain pathologic conditions in men.

## REFERENCES


1. Griffin JE, Wilson JD. Disorders of the testes and the male reproductive tract. In: Wilson JD, Foster DW, Kronenberg HM, et al., eds. Williams Textbook of Endocrinology. WB Saunders, Philadelphia, PA, 1998, pp. 819–875.
2. Fuh VL, Stoner E. Androgen inhibitors/antiandrogens. In: Knobil E, Neill JD, eds. Encyclopedia of Reproduction. Academic, San Diego, CA, 1998, pp. 166–173.
3. Hammond GL, Bocchinfuso WP. Sex hormone-binding globulin/androgen binding protein: steroid binding and dimerization domains. J Steroid Biochem Mol Biol 1995;53:1–6.
4. Avvakumov GV, Grishkovskaya I, Muller YA, Hammond GL. Resolution of the human sex hormone-binding globulin dimer interface and evidence for two steroid-binding sites per homodimer. J Biol Chem 2001;276:34453–34457.
5. Dunn JF, Nisula BC, Rodbard D. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab 1981;53:58–68.
6. Pardridge WM. Serum bioavailability of sex steroid hormones. Clin Endocrinol Metab 1986;15: 259–278.
7. Rosner W, Hryb DJ, Khan MS, et al. Sex hormone-binding globulin. Binding to cell membranes and generation of a second messenger. J Androl 1992;13:101–106.
8. Russell DW, Wilson JD. Steroid 5α-reductase: two genes/two enzymes. Annu Rev Biochem 1994;63:25–61.
9. Morimoto I, Hawks ED, Horton R. Studies on the origin of androstanediol and androstanediol glucuronide in young and elderly men. J Clin Endocrinol Metab 1981;52:772–778.
10. Brooks, RV. Androgens. Clin Endocrinol Metab 1975;4:503–520.
11. Simpson ER, Zhao Y, Agarwal VR, et al. Aromatase expression in health and disease. Recent Prog Horm Res 1997;52:185–213.
12. Wilson JD, Griffin JE, Russell DW. Steroid 5α-reductase 2 deficiency. Endocr Rev 1993;14:577–593.
13. Zhou Z-X, Wong C-I, Sar M., Wilson EM. The androgen receptor: an overview. Recent Prog Horm Res 1994;49:249–274.
14. Jenster G, vander Korput HAGM, van Vroonhoven C, et al. Domains of the human androgen receptor involved in steroid binding, transcriptional activation and subcellular localization. Mol Endocrinol 1991;5:1396–1404.
15. Lubahn DB, Joseph DR, Sullivan PM, et al. Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 1988;240:327–330.
16. Kuiper GGJM, Faber PW, van Rooij HCJ, et al. Structural organization of the human androgen receptor gene. J Mol Endocrinol 1989;2:R1–R4.
17. Faber PW, van Rooij HCJ, Schipper HJ, et al. Two different, overlapping pathways of transcription initiation are active on the TATA-less human androgen receptor promoter. J Biol Chem 1993;268:9296–9301.
18. Chen S, Supakar PC, Vellanoweth RL, et al. Functional role of a conformationally flexible homopurine/homopyrimidine domain of the androgen receptor gene promoter interacting with SP1 and a pyrimidine single strand DNA-binding protein. Mol Endocrinol 1997;11:3–15.
19. Grad JM, Dai JL, Wu S, Burnstein KL. Multiple androgen response elements and a myc consensus site in the androgen receptor (AR) coding region are involved in androgen-mediated up-regulation of AR messenger RNA. Mol Endocrinol 1999;13:1896–1911.
20. Mizokami A, Chang C. Induction of translation by the 5'-untranslated region of human androgen receptor mRNA. J Biol Chem 1994;269:25655–25659.
21. Lubahn DB, Joseph DR, Sar M, et al. The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis, and gene expression in prostate. Mol Endocrinol 1998;2:1265–1275.

22. Gottlieb B, Beitel LK, Trifiro MA. Variable expressivity and mutation databases: the androgen receptor gene mutations database. Hum Mutat 2001;17:382–388.
23. Kemppainen JA, Lane MV, Sar M, Wilson EM. Androgen receptor phosphorylation, turnover, nuclear transport, and transcriptional activation. J Biol Chem 1992;267:968–974.
24. Blok LJ de Ruiter PE, Brinkmann AO. Androgen receptor phosphorylation. Endocr Res 1996;58:569–575.
25. Gao T, McPhaul MJ. Functional activities of the A and B forms of the human androgen receptor in response to androgen receptor agonists and antagonists. Mol Endocrinol 1998;12:654–663.
26. Lubahn DB, Brown TR, Simental JA, et al. Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. Proc Natl Acad Sci USA 1989;86:9534–9538.
27. Doesburg P, Kuil CW, Berrevoets CA, et al. Functional in vivo interaction between the amino-terminal, transactivation domain and the ligand binding domain of the androgen receptor. Biochemistry 1997;36:1052–1064.
28. He B, Kemppainen JA, Voegel JJ, et al. Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH2-terminal domain. J Biol Chem 1999;274:37219–37225.
29. He B, Kemppainen JA, Wilson EM. FXXLF and WXXLF sequences mediate the NH2- terminal interaction with the ligand binding domain of the androgen receptor. J Biol Chem 2000;275:22986–22994.
30. Alen P, Claessens F, Verhoeven G. et al. The androgen receptor amino- terminal domain plays a key role in p160 coactivator-stimulated gene transcription. Mol Cell Biol 1999;19:6085–6097.
31. Bevan Cl, Hoare S, Claessens F, et al. The AF1 and AF2 domains of the androgen receptor interact with distinct regions of SRC1. Mol Cell Biol 1999;19:8383–8392.
32. Trifiro MA, Kazemi-Esfarjani P, Pinsky L. X-Linked muscular atrophy and the androgen receptor. Trends Endocrinol Metab 1994;5:416–421.
33. Choong CS, Wilson EM. Trinucleotide repeats in the human androgen receptor: a molecular basis for disease. J Mol Endocrinol 1998;21:2235–2257.
34. Irvine RA, Yu MC, Ross RK, Coetzee GA. The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. Cancer Res 1995;55:1937–1940.
35. Giovannucci E, Stampfer MJ, Krithivas K, et al. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. Proc Natl Acad Sci USA 1997;94:3320–3323.
36. LaSpada AR, Wilson EM, Lubahn DB. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 1991;352:77–79.
37. Freedman LP. Anatomy of the steroid receptor zinc finger region. Endocr Rev 1992;3:29–145.
38. Claessens F. Verrijdt G, Schoenmakers E, et al. Selective DNA binding by the androgen receptor as a mechanism for hormone-specific gene regulation. J Steroid Biochem Mol Biol 2001;76:23–30.
39. Gonzales MI, Robins D. Oct-1 preferentially interacts with androgen receptor in a DNA- dependent manner that facilitates recruitment of SRC-1. J Biol Chem 2001;276:6420–6428.
40. Zhou Z-X, Sar M, Simental JA, et al. Ligand-dependent bipartite nuclear targeting signal in the human androgen receptor. J Biol Chem 1994;269:13115–13123.
41. Tyagi RK, Lavrovsky Y, Ahn SC, et al. Dynamics of intracellular movement and nucleocytoplasmic recycling of the ligand-activated androgen receptor in living cells. Mol Endocrinol 2000;14:1162–1174.
42. Poukka H, Karvonen U, Yoshikawa N, et al. The RING finger protein SNURF modulates nuclear trafficking of the androgen receptor. J Cell Sci 2000;113:2991–3001.
43. Wang Q, Lu J, Yong EL. Ligand- and coactivator-mediated transactivation function (AF2) of the androgen receptor ligand-binding domain are inhibited by the cognate hinge region. J Biol Chem 2001;276:7493–7499.
44. Buchanan G, Yan M, Harris JM, et al. Mutations at the boundary of the hinge and ligand binding domain of the androgen receptor confer increased transactivation function. Mol Endocrinol 2001;15:46–56.
45. Pratt WB. Role of heat-shock proteins in steroid receptor function. In: Parker M, ed. Frontiers in Molecular Biology: Steroid Hormone Action. IRL, Oxford, 1993, pp. 64–93.
46. Wong CI, Zhou Z-X, Sar M, Wilson EM. Steroid requirement for androgen receptor dimerization and DNA binding. J Biol Chem 1993;268:19004–19012.
47. Langley E, Zhou Z-X, Wilson EM. Evidence for an anti-parallel orientation of the ligand- activated human androgen receptor dimer. J Biol Chem 1994;270:29983–29990.
48. Brzozowski AM, Pike ACW, Dauter Z, et al. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 1997;389:753–758.

49. Williams SP, Sigler PB. Atomic structure of progesterone complexed with its receptor. Nature 1998;393:392–395.
50. Matias PM, Donner P, Coelho R, et al. Structural evidence for ligand specificity in the binding domain of the human androgen receptor. J Biol Chem 2000;275: 26164–26171.
51. Sack JS, Kish KF, Wang C, et al. Crystallographic structures of the ligand-binding domains of the androgen receptor and its T877A mutant complexed with the natural agonist dihydrotestosterone. Proc Natl Acad Sci USA 2001;98:4904–4909.
52. Berrevoets CA, Doesburg P, Sketetee K, et al. Functional interactions of the AF-2 domain core region of the human androgen receptor with the amino-terminal domain and with the transcriptional coactivator TIF-2 (transcriptional intermediary factor-2). Mol Endocrinol 1998;12:1172–1183.
53. Roche PJ, Hoare SA, Parker MA. A consensus DNA-binding site for the androgen receptor. Mol Endocrinol 1992;6:2229–2235.
54. Nelson CC, Hendy SC, Shukin RJ, et al. Determinants of DNA sequence specificity of the androgen, progesterone, and glucocorticoid receptors: evidence for differential steroid receptor response elements. Mol Endocrinol 1999;13:2090–2107.
55. Zhou Z, Corden JL, Brown TR. Identification and characterization of a novel androgen response element composed of a direct repeat. J Biol Chem 1997;272:8227–8235.
56. Ing N, Beekman JM, Tsai SY, et al. Members of the steroid hormone superfamily interact with TFIIB (S300-II). J Biol Chem 1992;267:17617–17623.
57. McEwan I, Gustafsson J-A. Interaction of the human androgen receptor transactivation function with the general transcription factor TFIIF. Proc Natl Acad Sci USA 1997;94:8485–8490.
58. Bubulya A, Wise SC, Shen X-Q, et al. c-Jun can mediate androgen receptor-induced transactivation. J Biol Chem 1996;271:24583–24589.
59. Kallio PJ, Poukka H, Moilanen A, et al. Androgen-receptor mediated transcriptional regulation in the absence of direct interaction with a specific DNA element. Mol Endocrinol 1995;9:1017–1028.
60. Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 2000;14:121–141.
61. Lee KC, Kraus WL. Nuclear receptors, coactivators and chromatin: new approaches, new insights. Trends Endocrinol Metab 2001;12:191–197.
62. Perissi V, Staszewski LM, McInerney EM, et al. Molecular determinants of nuclear receptor–corepressor interaction. Genes Dev 1999;13:3198–3208.
63. Yeh S, Chang C. Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 1996;93:5517–5521.
64. Fujimoto N, Yeh S, Kang HY, et al. Cloning and characterization of androgen receptor coactivator, ARA55, in human prostate. J Biol Chem 1999;274:8316–8321.
65. Kang HY, Yeh S, Fujimoto N, Chang C. Cloning and characterization of human prostate coactivator, ARA54, a novel protein that associates with the androgen receptor. J Biol Chem 1999;274:8570–8576.
66. Hsiao PW, Chang C. Isolation and characterization of ARA160 as the first androgen receptor N-terminal-associated coactivator in human prostate cells. J Biol Chem 1999;274:22373–22379.
67. Kotaja N, Aittomaki S, Silvennoinen O, et al. ARIP3 (androgen receptor- interacting protein 3) and other PIAS (protein inhibitor of activated STAT) proteins differ in their ability to modulate steroid receptor-dependent transcriptional activation. Mol Endocrinol 2000;14:1986–2000.
68. Moilanen AM, Karvonen U, Poukka H, et al. Activation of androgen receptor function by a novel nuclear protein kinase. Mol Biol Cell 1998;9:2527–2543.
69. Yeh S, Hu YC, Rahman M, et al. Increase of androgen-induced cell death and androgen receptor transactivation by BRCA1 in prostate cancer cells. Proc Natl Acad Sci USA 2000;97:11256–11261.
70. Froesch BA, Takayama S, Reed JC. BAG-1L protein enhances androgen receptor function. J Biol Chem 1998;273:11660–11666.
71. Kang HY, Lin HK, Hu YC, et al. From transforming growth factor-β signaling to androgen action: identification of Smad3 as an androgen receptor coregulator in prostate cancer cells. Proc Natl Acad Sci USA 2001;98:3018–3023.
72. Yamamoto A, Hashimoto Y, Kohri K, et al. Cyclin E as a coactivator of the androgen receptor. J Cell Biol 2000;150:873–879.
73. Poukka H, Aarnisalo P, Karvonen U, et al. Ubc9 interacts with the androgen receptor and activates receptor-dependent transcription. J Biol Chem 1999;274:19441–19446.
74. Sharma M, Zarnegar M, Li X, et al. Androgen receptor interacts with a novel MYST protein, HBO1. J Biol Chem 2000;275:35200–35208.

75. Gross M, Liu B, Tan JA, et al. Distinct effects of PIAS proteins on androgen-mediated gene activation in prostate cancer cells. Oncogene 2001;20:3880–3887.
76. Klocker H, Culig Z, Ider IE, et al. Mechanism of androgen receptor activation and possible implications for chemoprevention trials. Eur Urol 1999;35:413–419.
77. Culig Z, Hobisch A, Bartsch G, Klocker H. Androgen receptor—an update of mechanisms of action in prostate cancer. Urol Res 2000;28:211–219.
78. Sadar MD. Androgen-independent induction of prostate specific antigen gene expression by cross-talk between the androgen receptor and protein kinase A signal transduction pathways. J Biol Chem 1999;274:7777–7783.
79. Nazareth LV, Weigel NL. Activation of the human androgen receptor through a protein kinase A pathway. J Biol Chem 1996;268:19900–19907.
80. DeRuiter PE, Teuwen R, Trapman J, et al. Synergism between androgens and protein kinase-C on androgen regulated gene expression. Mol Cell Endocrinol 1995;110:1–6.
81. Ikonen T, Palvimo JJ, Kallio PJ, et al. Stimulation of androgen- regulated transactivation by modulators of protein phosphorylation. Endocrinology 1994;135:1359–1366.
82. Rowan BG, Garrison N, Weigel NL, O'Malley BW. 8-Bromo cAMP induces phosphorylation of two sites on SRC-1 that facilitate ligand independent activation of the chicken progesterone receptor and are critical for functional cooperation between SRC-1 and CBP. Mol Cell Biol 2000;20:8720–8730.
83. Qui Y, Ravi L, Kung HJ. Requirement of ErbB2 for signaling by interleukin-6 in prostate carcinoma cells. Nature 1998;393:83–85.
84. Shemshedini L, Knauthe R, Sassone-Corsi P, et al. Cell-specific inhibitory and stimulatory effects of Fos and Jun on transcription activation by nuclear receptors. EMBO J 1991;10:3839–3843.
85. Bubulaya A, Wise SC, Shen X-Q, et al. c-Jun can mediate AR- induced transactivation. J Biol Chem 1996;271:24583–24589.
86. Craft N, Shostak Y, Carey M, Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. Nature Med 1999;5:280–285.
87. Yeh S, Lin HK, Kang HY, et al. From HER-2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cells. Proc Natl Acad Sci USA 1999;96:5458–5463.
88. Li P, Nicosia SV, Bai W. Antagonism between PTEN/MMAC1/TEP-1 and androgen receptor in growth and apoptosis of prostate cancer cells. J Biol Chem 2001;276:20444–20550.
89. Quigley CA, DeBellis A, Marschke KB, et al. Androgen receptor defects: historical, clinical and molecular perspectives. Endocr Rev 1995;16:271–321.
90. Brown TR. Androgen insensitivity syndrome. J Androl 1995;16:299–303.
91. Sutherland RW, Wiener JS, Hicks JP, et al. Androgen receptor gene mutations are rarely associated with isolated penile hypospadias. J Urol 1996;156:828–831.
92. Gregory CW, He B, Johnson RT, et al. Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res 2001;61:2892–2898.
93. Veldscholte J, Berrevoets CA, Ris-Staplers C, et al. The androgen receptor in LNCaP cells contains a mutation in the ligand binding domain which affects steroid binding characteristics and response to antiandrogens. J Steroid Biochem1992;41:665–669.
94. Tan J, Sharief Y, Hamil KG, et al. Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human cancer xenograft CRW22 and LNCaP cells. Mol Endocrinol 1997;11:450–459.
95. Tilley WD, Buchanan G, Hickey TE, Bentel JM. Mutations in the androgen receptor gene are associated with progression of human prostate cancer to androgen independence. Clin Cancer Res 1996;2:277–285.
96. Taplin ME, Bubley GJ, Ko YJ, et al. Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. Cancer Res 1999;59:2511–2515.
97. Marcelli M, Ittmann M, Mariani S. et al. Androgen receptor mutations in prostate cancer. Cancer Res 2000;60:944–999.
98. Koivisto PA, Kononen J. Palmberg C, et al. Androgen receptor gene amplification: a possible mechanism for androgen deprivation therapy failure in prostate cancer. Cancer Res 1997;57: 314–319.
99. Stanford JL, Just JJ, Gibbs M, et al. Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. Cancer Res 1997;57:1194–1198.

100. Gregory CW, He B, Johnson RT, et al. A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. Cancer Res 2001;61:4315–4319.
101. Choong CS, Kemppainen JA, Zhou Z-X, Wilson EM. Reduced androgen receptor gene expression with first exon CAG repeat expansion. Mol Endocrinol 1996;10:1527–1535.
102. Irvine RA, Ma H, Yu MC, et al. Inhibition of p160- mediated coactivation with increasing androgen receptor polyglutamine length. Hum Mol Genet 2000;9:267–274.
103. Abdullah A. Trifiro MA, Panet-Raymond V, et al. Spinobulbar muscular atrophy: polyglutamine-expanded androgen receptor is proteolytically resistant in vitro and processed abnormally in transfected cells. Hum Mol Genet 1998;7:379–384.
104. Kobayashi Y, Miwa S, Merry DE, et al. Caspase-3 cleaves the expanded androgen receptor protein of spinal and bulbar muscular atrophy in a polyglutamine repeat length-dependent manner. Biochem Biophys Res Commun 1998;252:145–150.
105. Stenoien DL, Cummings CJ, Adams HP, et al. Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. Hum Mol Genet 1999;8:731–741.
106. Aiman J, Griffin JE, Cushard WG, Wilson JD. Androgen insensitivity as a cause of infertility in otherwise normal men. N Engl J Med 1998;300:223–227.
107. Akin JW, Behzadian A, Tho SPT, McDonough PG. Evidence for a partial deletion in the androgen receptor gene in a phenotypic male with azoospermia. Am J Obstet Gynecol 1991;165:1891–1894.
108. Yong EL, Ng SC, Roy AC, et al. Pregnancy after hormonal correction of severe spermatogenic defect due to mutation in androgen receptor gene. Lancet 1994;344:8925–8927.
109. Ghadessy FJ, Lim J, Abdullah AA, et al. Oligospermic infertility associated with an androgen receptor mutation that disrupts interdomain and coactivator (TIF2) interactions. J Clin Invest 1999;103:1517–1525.
110. Tut TG, Ghadessy FJ, Trifiro MA, et al. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab 1997;82:3777–3782.