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REVIEW ARTICLE

Androgen receptor and the mechanism of androgen action

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During the past two decades, a great deal of information has accumulated on the structure of androgen receptor (AR) and the mechanism by which it forms a complex with a steroid hormone and then interacts with DNA to regulate gene expression. Steroid hormones enter the cells by passive diffusion and activate their related receptors. The activated receptor binds to specific *cis*-acting enhancer sequences usually present in the 5'-flanking region of target genes and regulates transcription through interaction of the receptor with DNA, proteins and other transcription factors. Precursor mRNAs are synthesized, processed and translated to produce new proteins. As a result, the cellular function changes. The present review summarizes our current knowledge of the structure of AR and its interaction with DNA to regulate the expression of specific gene(s).

ANDROGEN is involved in growth, development, differentiation and reproduction. The hormonal signal is mediated by the intracellular androgen receptor (AR) protein, which belongs to a superfamily of ligand-dependent transcription factors (TF). This family also includes other steroid hormones, thyroid hormone and retinoic acid receptors[1-4]. The subcellular localization of the unliganded progesterone, estradiol, glucocorticoid and androgen receptor is still debatable[5]. Immunocytochemical studies using intact and castrated rats reveal the presence of AR in the nucleus[6]. On the contrary, AR transiently overexpressed in monkey kidney COS cells is found to be localized in the cytoplasm or distributed over the cytoplasm and nucleus in the absence of ligand, and to be exclusively nuclear in the presence of androgen[7, 8].

After synthesis in the cytoplasm, steroid hormone receptors (SR) form transient complexes with a variety of proteins such as 90 and 70 kDa heat shock protein (hsp). This may promote proper folding and stability of the receptor molecule. Hsp 90 binding may also be closely related to receptor location. For instance, the SR associated with hsp 90 are recovered in the cytosol following the homogenization of tissue in a hypotonic solution but the receptors for thyroid hormone, retinoic acid and vitamin D_3, which are not complexed with hsp 90, are primarily present in the nucleus. Other proteins such as 59 and 23 kDa are also bound to nontransformed SR, although their role in the receptor function is not known. SR complexed with hsp and other proteins are usually unable to interact with DNA. However, the specific binding of the corresponding hormones dissociates receptors from hsp and/or other proteins[9]. Antihormones also promote such disaggregation but they do not activate target genes[10]. The binding of SR to their cognate ligands exhibits great specificity. For example, AR has higher affinity for androgen and antiandrogen than for estrogen, progesterone and corticosteroids[11]. Following the removal of hsp and other bound proteins, the receptor is ready for phosphorylation. The modified receptor ultimately binds tightly to DNA and a variety of other proteins and modulates the expression of genes[12, 13].

Androgen receptor structure

The structure of the AR gene and protein has been studied in detail with the help of advanced techniques of molecular and cell biology. AR has been located as a single-copy gene on the X-chromosome at X_q11-12 locus[14, 15]. Though it spans over 90 kbp of length, less than 5% is translated into protein. It consists of eight exons and seven intervening sequences. The exons code for different domains of the receptor. The promoter of AR is characterized by a short GC box (–59/–32) and a long homopurine stretch (–117/–60). Two major transcription initiation sites (TIS) are located in a 13 bp region – TIS I (+1/2/3) and TIS II (+12/13). A single Spl binding sequence is present in the GC box. It is essential for initiation of transcription from the second site[16, 17]. The cDNA sequence of human AR reveals an open reading frame of 2730 nucleotides encoding a protein of 910 amino acid residues with a molecular weight of 98.5 kDa[18–20]. The mRNA has a relatively long 6.8 kb 3′-untranslated region (UTR). Whereas 5′-UTR is involved in high-level expression of AR, the alternative splicing in 3′-UTR generates two mRNAs of 11.0 and 8.5 kb sizes.

Functional domains

Amino acid sequence comparison and deletion mutagenesis studies of AR have shown six domains (A–F) of varying homology (Figure 1). Three major domains are highly conserved: A or amino-terminal domain, ATD (87%), C or central DNA-binding domain, DBD (100%) and E or hormone-binding domain, HBD (94%). Three other domains are less conserved: B (56%), D (38%) and F (41%). ATD is hydrophilic whereas HBD is hydrophobic. Both HBD and DBD are the most highly conserved between the same receptors from different species and between different SR of the same species[21, 22].

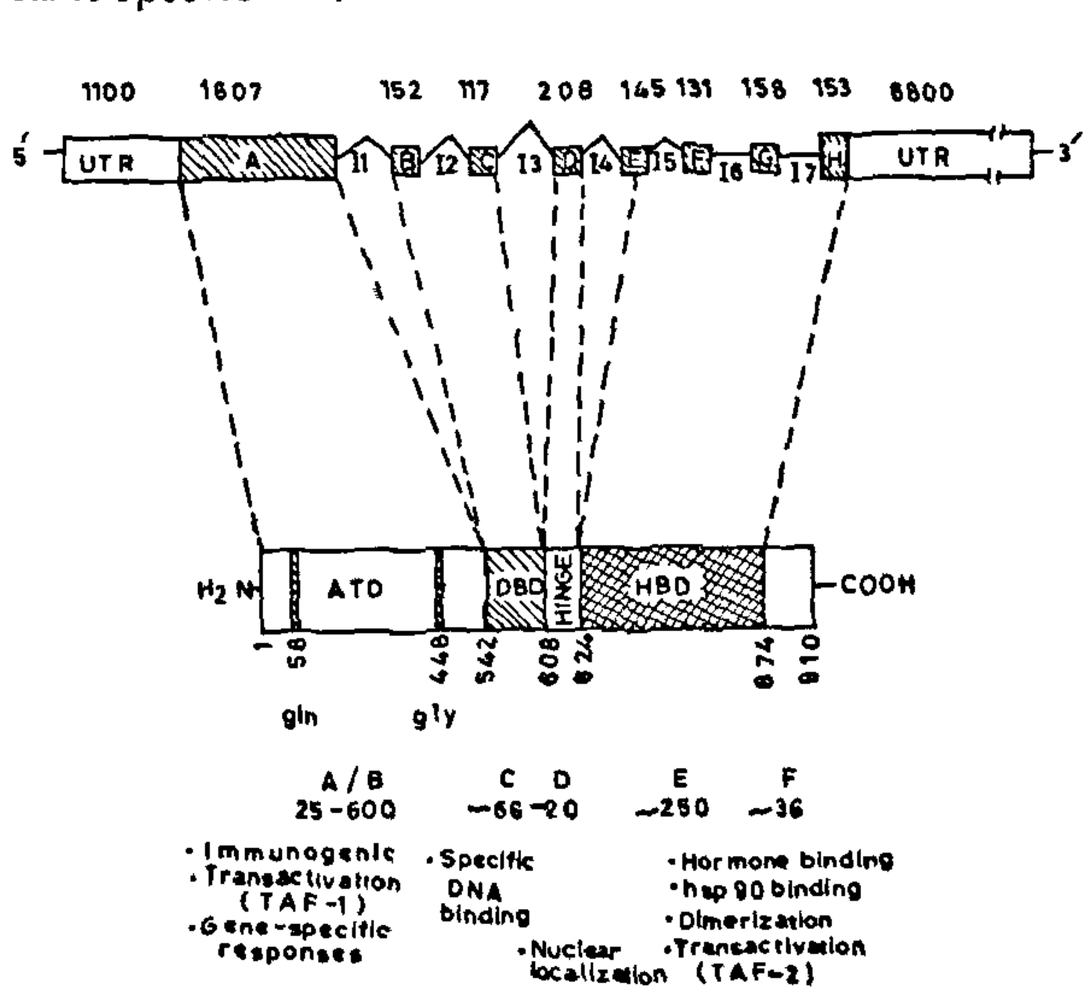

Figure 1. Schematic representation of the organization of androgen receptor gene (upper panel) The eight exons (A–H) are shown by hatched boxes and seven introns (I1–I7) by lines, 5′- and 3′-untranslated regions (UTR) are located at both ends The lengths of the exons (in nucleotides) are shown above the boxes Approximate correspondence of the gene regions to different domains of androgen receptor and their functions (lower panel) is depicted The figures below the line indicate the positions of amino acid residues and the figures below the regions (A–F) indicate the approximate numbers of amino acid residues in each domain Two stretches of polyglutamine (*gln*) and polyglycine (*gly*) residues are shown in amino-terminal domain ATD = amino-terminal domain, DBD = DNA-binding domain, HBD = hormone-binding domain

Amino-terminal domain

This region is coded by a large 1589 bp exon E1. Its size and amino acid sequence are highly variable among different domains of the receptor. Perhaps diversity in the structure of ATD enables the receptor to interact with a specific subset of TF. It is important since only a limited degree of specificity can be rendered by DBD. This might explain why glucocorticoid, progesterone, androgen and mineralocorticoid receptors all recognize the same response element but bring about diverse effects *in vivo*. Thus, ATD has an important role in determining the specificity of gene activation. This domain consists of several homopolymeric amino acid stretches, e.g. three polyglutamine stretches with variable lengths, a long polyglycine stretch, a polyproline stretch of eight residues and a polyalanine stretch of five residues[23]. The exact function of these amino acid repeats is unknown, though glutamine stretches are also present in proteins which are involved in developmental control and/or regulation of gene expression[24].

ATD is involved in the transactivation function (TAF-1) of the receptor[25]. However, the exact nature and boundaries of putative transactivation domains are not well-defined. Using a series of deletion mutants of rat AR, transient expression condition in monkey kidney CV-1 cells and *in vitro* DNA-binding studies, Palvimo *et al.*[26] have shown that a region of ATD (residues 147–296) is mandatory for transactivation. Receptors with deletions (residues 147–408) in ATD but with intact DBD and HBD interact *in vitro* with androgen-responsive element (ARE) albeit with affinities lower than that of the wild-type receptor. Thus, ATD has two important functions, viz. to provide gene specificity and to maximize the transactivation capability of the receptor.

DNA-binding domain

This is the most intensively studied domain. It consists of 66–68 amino acid residues and is coded by 152 bp exon E2 and 117 bp E3. Human AR reveals a strong homology (~80%) with glucocorticoid receptor (GR) and progesterone receptor (PR). It is characterized by a high content of basic amino acid residues and nine conserved cysteine residues. It has a compact globular structure with two finger-like motifs. Both fingers contain one central Zn atom which interacts via coordination bonds with four cysteine residues. The two zinc clusters are structurally and functionally different and are encoded by two different exons (E2 and E3). The loop of the finger consists of 12–13 amino acids and the two fingers are joined through a linker region of 15–17 amino acids (Figure 2).

Mutational studies have shown that three amino acid residues of the first zinc finger are responsible for specific recognition of the DNA sequence of the responsive-element sequences[27]. These three amino acid residues (*gly* 568, *ser* 569 and *val* 572) are identical in AR, PR and GR but different in estrogen receptor (ER). Because of this similarity, AR, PR and GR recognize the same responsive element. Each zinc finger is important for high-affinity binding to target DNA sequences, though the first finger plays a greater role in specific sequence recognition. The second zinc finger is probably also involved in AR dimerization[28]. Recently, Dedhar *et al.*[29] have demonstrated that DBD of AR and other known SR contains a conserved amino acid sequence KXFFKR (where X is G, A or V) which interacts with the amino terminus of a calcium-binding storage protein called calreticulin. This interaction prevents the SR from binding to their specific response element. Thus, the modulation of the DNA-binding activity of SR by a calcium storage protein adds a new dimension to the mechanism of steroid hormone action.

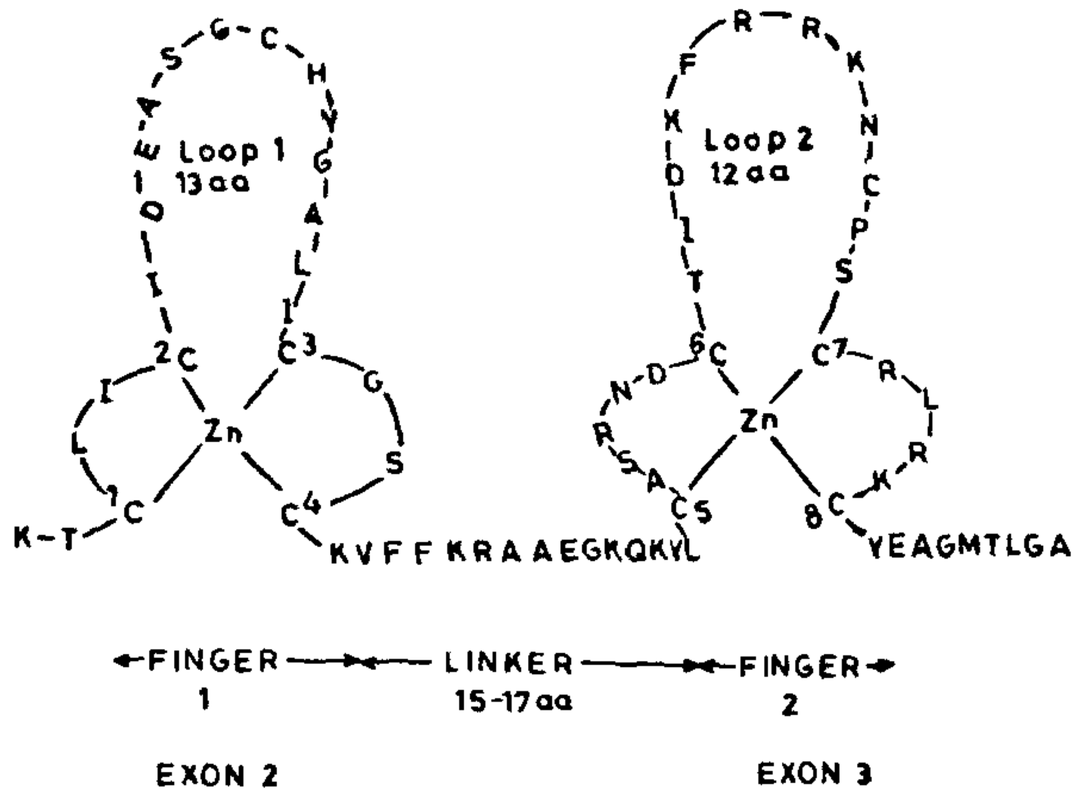

Figure 2. Amino acid sequence of two zinc fingers in the DNA-binding domain of androgen receptor. Two zinc ions (Zn) are chelated in a tetrahedral coordination by two clusters of four cysteines (C1–C4 and C5–C8) forming two zinc fingers (finger 1 and finger 2), separated by 15–17 amino acid residues (linker).

Hormone-binding domain

It consists of approximately 250 amino acid residues in the carboxyl-terminal region. The coding information is distributed over five exons which vary in size from 131 to 288 bp (part of E4, complete E5, E6 and E7, and part of E8). HBD is completely conserved between the human, mouse and rat AR. This indicates that most amino acids in HBD are very important for AR function.

It is suggested that a region in HBD is important for receptor dimerization. Leucine-rich regions, known as leucine zippers, are possible sites of protein dimerization[30]. Such a structure is found at amino and carboxyl

termini of SR. However, their involvement in receptor dimerization is presently unclear. In the absence of hormone, HBD acts as a repressor of transactivation function. Deletions in ATD and DBD do not affect hormone binding. By performing deletional analysis and using chimeras in which human ER HBD is linked to GAL4 DBD, HBD has been shown to contain a hormone-inducible transcription activation function (TAF-2)[31]. Human ER TAF-1 and TAF-2 act in a promoter- and cell-specific manner and activate transcription independently and synergistically. Domain-swapping experiments suggest that GR and ER HBD can be exchanged, resulting in regulated expression of the target gene by an appropriate steroid and its respective receptor HBD[32].

Mechanism of androgen action

The action of androgen is mediated by AR, which is a signal-transducing protein. Following the activation of AR by binding to ligand, or by dissociation of hsp and other inhibitory proteins complexed to it, or by covalent modification like phosphorylation, the receptor undergoes conformational changes.

Phosphorylation

Phosphorylation of SR is an important means for the regulation of steroid hormone action. There is ample evidence suggesting that progesterone, glucocorticoid, estrogen, androgen and vitamin D_3 receptors exist as phosphoproteins in intact cells. Using partial proteolysis of AR and probing of phosphorylated fragments with polyclonal antisera raised against different epitopes in the amino terminus, Kuiper *et al.*[33] found all the phosphorylation sites between amino acid residues 1 and 300 outside DBD and HBD. This region is also essential for the transactivation function of AR. Thus, it is likely that phosphorylation plays a role in transcription regulation. Furthermore, it has been demonstrated that the androgen-binding activity of rat ventral prostate AR is lost in ATP-depleted cells but is regained when ATP levels are restored[34]. In addition, the inhibition of endogenous phosphatase activity is correlated with increased hormone-binding activity of AR[35]. These observations suggest that AR phosphorylation is required for hormone binding. However, the protein kinase catalysing the phosphorylation of AR has not yet been identified.

Nuclear translocation

Changes in the conformation of SR ultimately lead to translocation of the receptor into the nucleus. To pass through the nuclear membrane, SR harbour a nucleoplasmin-like bipartite nuclear localization signal (NLS) in their hinge region (D) between DBD and HBD (residues 608–624). NLS is highly conserved among different members of the nuclear receptor family[36]. Zhou *et al.*[37] have recently mapped the NLS and established the minimal amino acid sequence required to detect nuclear import of AR. Site-directed mutagenesis of human AR reveals a bipartite sequence that spans DNA binding and hinge regions from amino acids 617 to 633. The sequence. RKCYEAGMTLGARKLKK, consists of two clusters of basic amino acids separated by a spacer of 10 amino acid residues. Two mutations in each domain or three mutations in the right basic half result in undetectable nuclear transport of the receptor. In contrast, single amino acid changes or small insertions within the spacer region do not block nuclear targeting, suggesting that the spacer sequence is not crucial to target signal function. The presence of NLS is important for nuclear import of PR, ER, GR and AR[38]. However, deletion of NLS does not result in the exclusive cytoplasmic localization of PR and AR in the presence of hormone, indicating the complexity of NLS and/or the presence of additional signals[39].

There are two mechanisms by which the ligand can control the transport of SR across the nuclear membrane. Firstly, phosphorylation in or near NLS could be involved in NLS–nuclear-pore interaction and consequently nuclear import. Secondly, hormone-induced dissociation of heteromeric complexes of the receptor with hsps could expose the NLS, resulting in nuclear translocation.

Steroid-responsive elements

Following activation and translocation of SR into the nucleus, the receptor becomes capable of binding to specific sequences of DNA called steroid-responsive elements (SRE). In general, SRE are 15 bp consensus palindromic sequences composed of two half-sites of 6 bp arms each arranged in a dyad axis of symmetry (inverted repeats) and separated by a central 3 bp spacer of random composition. Roche *et al.*[40] used a DNA-binding site selection assay to determine a consensus binding sequence for AR. A purified fusion protein containing DBD of AR was incubated with a pool of a random sequence of oligonucleotides, and complexes were isolated by gel mobility shift assays. Individually selected sites were characterized by nucleotide sequencing and compiled to give a consensus ARE, 5′-GGA/TACANNNTGTTCT-3′, similar to glucocorticoid-responsive element (GRE). Identical sequences have been found in many androgen-responsive genes[41]. The sequences neighbouring two 6 bp half-sites influence both the binding affinity of the receptor and the functional activity of the response element. The SRE for GR, PR and AR share similarities in nucleotide sequence. One copy of SRE is usually sufficient to bring a

promoter under moderate hormonal control and two or more copies often provide a synergistic response to the related hormone.

Recent evidence shows that glucocorticoid, progesterone, estrogen and androgen receptors bind to their SRE as dimers, one molecule to each half-site. This interaction appears to be cooperative. Receptor dimers bind with greater affinity and stability to their SRE. Interactions between receptor dimers at separate SRE allow a higher-order cooperative interaction that stabilizes the two dimers into a tetrameric structure with a 100-fold greater affinity for its SRE than does a single dimer. Such protein–protein interactions may occur among homologous or heterologous receptor complexes or receptor–promoter/TATA box complexes. These interactions stabilize TF at the promoters of target genes and thereby induce the formation of a stable preinitiation complex near the transcription start site. Receptors appear to enhance transcription by stimulating the assembly of the preinitiation complex or by stabilizing general TF like TF IIA, IIB, IID, IIE/F, RNA polymerase, etc., at the TATA box directly[42]. Subsequently, the synthesis of new RNA and proteins is stimulated or repressed (Table 1)[43–64] and this ultimately leads to alteration in the functional activity of the cells (Figure 3).

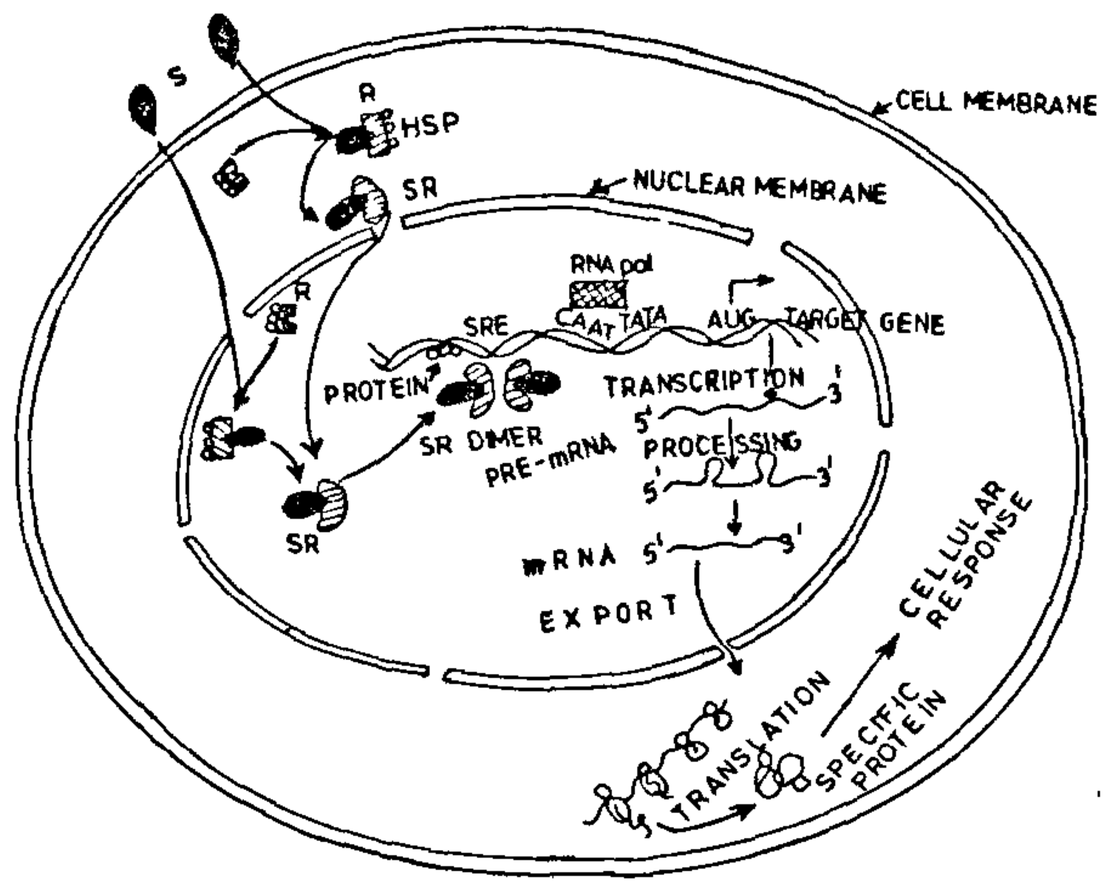

Figure 3. Molecular pathway for steroid hormone action S = steroid hormone, R = receptor, HSP = heat shock protein; SRE = steroid-responsive element

Involvement of chromatin

High-affinity steroid-binding sites are tightly associated with the nuclear matrix of hormone-responsive tissues and these binding sites are diminished after the withdrawal of hormone[65]. However, following the hormone treatment, DNaseI hypersensitive regions appear in the vicinity of the regulated promoters[66]. Also, the binding of SR to SRE results in removal or reorganization of nucleosomes[67, 68] and exposure of adjacent binding sites for TF[69, 70]. These observations suggest that steroid hormones act by altering the chromatin structure of target genes. However, little is known on this aspect, primarily because of the lack of experimental tools to manipulate the complex structure of chromatin and test potential functions.

Table 1. Some androgen-responsive genes

Responsive gene	Target tissue	Reference
1 Genes whose transcription is stimulated by androgen		
Major urinary protein (MUP)	Mouse liver	43
Kidney–androgen regulated protein (KAP)	Mouse kidney	44, 45
β-Glucuronidase (GUS)	Mouse kidney	46
Mouse vas deferens protein (MVDP)	Mouse vas deferens	47
Androgen-induced growth factor (AIGF)	Mouse carcinoma cell line (SC-3)	48
Angiotensinogen	Rat kidney	49
Androgen-binding protein (ABP)	Rat brain	50
Prostatic steroid-binding protein (PSBP)	Rat prostate	51
Acidic epididymal protein (AEG)	Rat epididymis	52
Androgen receptor (AR)	Rat penis	53
Kallikrein (KLK)	Human prostate	54
Prostate-specific antigen (PSA)	Human prostate	55
2. Genes whose transcription is repressed by androgen		
Senescence marker protein (SMP)-2	Rat liver	56
Cytokeratin 8	Rat prostate	57
c-*myc* Proto-oncogene	Rat prostate	58
Sulphated glycoprotein-2	Rat prostate	60
Androgen receptor (AR)	Rat prostate, testis and epididymis	61
Androgen receptor (AR)	Rat seminal vesicle	62
Androgen receptor (AR)	Human prostate cancer cell line (LNCaP)	63
Prostatic acid phosphatase (PAP)	Human prostate	55
Gonadotropin α-subunit	Human prostate	64

Mutations in AR and the associated abnormalities

Approximately 80 different mutations have been reported in the AR gene. These mutations are responsible for either complete or partial androgen-insensitivity syndrome (AIS)[71, 72]. Most of these mutations are scattered over HBD and DBD, and some are found in ATD. So far no mutation has been detected in the AR promoter or 5′- and 3′-UTR. Studies on mutated AR help in the identification of key amino acids in different domains of AR and their specific physiological functions. In addition, mutation analysis allows early detection of the defect, which has implications for potential treatment.

Three cases of naturally occurring mutations have been reported with partial or complete deletion of the AR gene resulting in complete AIS[73]. In one person, the gene was completely deleted and in two other individuals E5–E8 and E3–E8 were deleted. Each of these mutations inactivates AR function, even though some of the mutant AR binds to androgen. In a family with androgen resistance associated with hypospadias, 16–32 residues long polyglutamine stretch of ATD is reduced to 12 (ref. 74). The shortened glutamine stretch increases the thermolability of AR. On the other hand, in Kennedy syndrome the length of the stretch increases[75] to 40–60. This increase seems to correlate with the severity of the disease[76]. The Kennedy syndrome is a rare X-linked neurodegenerative disorder prevalent in men aged 30–40 years and is characterized by a progressive spinal and bulbar muscular atrophy associated with signs of androgen insensitivity and infertility[77, 78].

Majority of point mutations occurring in DBD and HBD are missense mutations resulting in the synthesis of an AR which is unable to bind to its ligand. Mutation in E2 changes *cys* 550 to *tyr*, *gly* 559 to *val* and *cys* 567 to *phe*[79]. *Cys* 567 is one of the four conserved cys residues which form coordination bonds with Zn atom. The mutated receptor displays normal binding to the ligand but has impaired biological activity. Sequence analysis has further identified the substitution of *val* 581 by *phe* in the first zinc finger and highly conserved *arg* 614 by *his* in the second zinc finger[80, 81]. These mutations render AR nonfunctional and result in complete AIS. Using single-strand conformation polymorphism (SSCP) assay, a useful screening method for rapid detection of nucleotide sequence alterations, and direct DNA sequencing, a G→T nucleotide substitution changing *gly* 743 to *val* was found[82] in E5 at nucleotide 2590. A single amino acid change from *met* (ATG) 786 to *val* (GTG) leads to complete AIS[83]. The mutation in E6 from thymine to guanine changes *met* 807 to *arg*. The mutated receptor binds to DNA *in vitro* but fails to transactivate[84]. In prostate cancer cell line, *thr* 868 is mutated to *ala*, leading to increased affinity of the mutant receptor for progesterone, estrogen and antiandrogens[85]. This shows that *thr* 868 is essential for androgen-binding specificity and functional activity. Further investigations on mutations in different AR-associated forms of androgen resistance will help to unravel the molecular basis of androgen action in male physiology and pathology.

Following the cloning of cDNAs for SR, a remarkable progress has been made during the past decade towards the understanding of the structure–function relationship of SR. However, a number of additional important questions remain to be answered. For instance, it is not known how receptor molecules distinguish between positive and negative response elements and act accordingly to induce or repress the expression of hormone-responsive genes. Furthermore, it is essential to elucidate the role that chromatin plays in the modulation of the expression of target genes. Intensive investigation in this field may lead to a deeper understanding of growth, maturity, reproduction and fertility, and development of new rational therapeutic and preventive approaches for genetic diseases.

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