# Chapter 1

# Molecular Mechanisms of Androgen Action – A Historical Perspective

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## Abstract

Androgens and the androgen receptor (AR) are indispensable for expression of the male phenotype. The two most important androgens are testosterone and 5α-dihydrotestosterone. The elucidation of the mechanism of androgen action has a long history starting in the 19th century with the classical experiments by Brown-Séquard. In the 1960s the steroid hormone receptor concept was established and the AR was identified as a protein entity with a high affinity and specificity for testosterone and 5α-dihydrotestosterone. In addition, the enzyme 5α-reductase type 2 was discovered and found to catalyze the conversion of testosterone to the more active metabolite 5α-dihydrotestosterone. In the second half of the 1980s, the cDNA cloning of all steroid hormone receptors, including that of the AR, has been another milestone in the whole field of steroid hormone action. Despite two different ligands (testosterone and 5α-dihydrotestosterone), only one AR cDNA has been identified and cloned. The AR (NR3C4) is a ligand-dependent transcription factor and belongs to the family of nuclear hormone receptors which has 48 members in human. The current model for androgen action involves a multistep mechanism. Studies have provided insight into AR association with co-regulators involved in transcription initiation and on intramolecular interactions of the AR protein during activation. Knowledge about androgen action in the normal physiology and in disease states has increased tremendously after cloning of the AR cDNA. Several diseases, such as androgen insensitivity syndrome (AIS), prostate cancer and spinal bulbar muscular atrophy (SBMA), have been shown to be associated with alterations in AR function due to mutations in the AR gene or dysregulation of androgen signalling. A historical overview of androgen action and salient features of AR function in normal and disease states are provided herein.

**Key words:** Testosterone, transcription regulation, androgens, nuclear receptor, sexual differentiation, androgen insensitivity, functional domains, prostate cancer, mechanism of action.

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## 1. Introduction

Androgens are important hormones for expression of the male phenotype. They belong to the group of steroid hormones and have characteristic roles during male sexual differentiation, development and maintenance of secondary male characteristics, and initiation and maintenance of spermatogenesis (1). In this review, a short historical overview is presented with respect to the most important scientific achievements in uncovering the mechanism of androgen action starting from the experiment of Brown-Séquard in the late 19th century until the cloning of the androgen receptor (AR) cDNA in 1988. Subsequently the focus will be on the functional domain aspects of the AR and its crucial role in some pathological situations.

## 2. Historic Landmarks in Androgen Action

There is a long history on the elucidation of the nature, origin and mechanism of action of androgens. Already in 1889, the French physiologist and professor of medicine at the famous Collège de France in Paris, Charles Edward Brown-Séquard (1818–1894) (**Fig. 1.1**), communicated the first indirect evidence for androgen action via internal secretion after giving himself injections of a testicular extract. Brown-Séquard, at that time 72 years old, improved his health considerably from the injections as was established by himself and by a group of independent observers (2). Almost five decades later, in 1935 in the laboratory of professor Ernst Laqueur (**Fig. 1.2**) in the Netherlands, the active substance could be crystallized from an extract obtained from bull's testes. The crystalline compound appeared to fulfil all the criteria for a full androgenic hormone. It was chemically and physiologically characterized and was named by Laqueur and collaborators (2, 3) as "testosterone".

In the early 1950s the concept of receptors as mediators in the action of pharmacological compounds was postulated and established. It took another 15 years of research before this receptor concept could be successfully incorporated in the mechanism of action of steroid hormones. At the end of the 1950s, radioisotope labelling techniques for steroid hormones and technology for detection of tritiated compounds became available. These innovations facilitated the establishment of specific target tissue retention of hormones, and in particular steroid hormones.

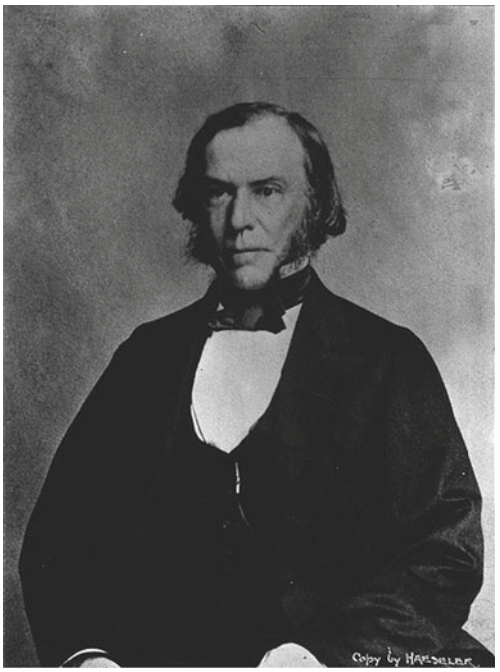

Fig. 1.1. Charles Edward Brown-Séquard (1818–1894).

Fig. 1.2. Ernst Laqueur (1880–1947).

The important breakthrough was made at the University of Chicago in the research group of Elwood Jensen (4, 5) (**Fig. 1.3**). The radiolabelled steroid hormone was oestradiol. Jensen and collaborators showed that unaltered radioactive oestradiol was

Fig. 1.3. Elwood Jensen.

retained specifically and for relatively longer time periods in uterine and breast tissues than in non-oestrogen target tissues after injection in immature animals. These were the first indications of specific steroid hormone-binding entities in target tissues: steroid receptors.

The sucrose density gradient approach published in 1966 by David Toft and Jack Gorski established further in an elegant and convincing way the protein nature of the binding entity (6). This approach has successfully stimulated in the next decade the search for other steroid hormone receptors, including the AR. Important findings in this respect were the presence of receptor dimers and heat-shock proteins in the complexes and the steroid hormone-induced conformational changes of the receptor complexes. All these steroid receptor proteins had a common functional property: upon hormone binding, they became active transcription regulatory proteins, either positively or negatively.

The first papers on the protein nature and on the isolation of ARs from androgen target tissues were published at the end of the 1960s by several research groups. Evidence for a specific androgen-binding protein isolated from prostate tissue cytosol, was published by the research groups of Ian Mainwaring from the ICRF in London, Shutsung Liao (**Fig. 1.4**) at the University of Chicago and Étienne-Émile Baulieu (**Fig. 1.5**) at the INSERM Institute in Kremlin-Bicêtre in Paris (7–9). Furthermore, a dynamic nature could be attributed to the size of the complex in which ARs and more generally the sex steroid nuclear receptors sedimented in sucrose density gradients. In cytosolic fractions the receptor complexes sedimented as 8–10S complexes, while in nuclear extracts the sizes were smaller (4–5S).

Fig. 1.4. Shutsung Liao.

Fig. 1.5. Étienne-Émile Baulieu.

This difference in size indicated a hormone-induced conformational change of the complex. This conformational change was accompanied by a temperature-dependent translocation of the receptor to the nucleus. A "two-step" mechanism for steroid hormone action was proposed by Elwood Jensen. First step: binding of the steroid to an unoccupied cytoplasmic receptor protein complex, second step: dissociation upon hormone binding

into a smaller complex and simultaneously translocation to the nucleus (10).

Another important finding in 1968 was the identification in the prostate of the role of 5α-dihydrotestosterone and the enzyme 5α-reductase by the groups of Jean Wilson in Dallas and Etienne-Emile Baulieu in Paris (11–13). The metabolism of testosterone to the more potent androgen 5α-dihydrotestosterone can be considered as another hallmark in the history of androgen action. Six years later in 1974 the specific role of 5α-dihydrotestosterone was established in the early differentiation of the urogenital sinus and tubercle (14).

During the 1970s and 1980s, much efforts were put in the purification and further characterization of ARs from different sources with the ultimate aim to generate antibodies as a tool for further studies (15–18). One of the main problems was the relatively low levels of AR protein in androgen target tissues as compared to other steroid receptors in their target tissues. A second complicating factor was the relatively high levels of proteolytic enzymes in some of the androgen target tissues (in particular prostatic tissue) (19). However, from these studies, a fairly true impression could still be obtained about the AR protein size despite the different sources. In addition, chemical- and photo-affinity labelling approaches have further contributed considerably to the characterization and the determination of the AR protein size (20–22).

In the second half of the1980s, the cloning of cDNAs of steroid receptors has been achieved by several research groups. The first paper was on the cDNA cloning of the human glucocorticoid receptor and was published by the group of Ron Evans in La Jolla, San Diego, in the "1985 Christmas issue of *Nature*" (23). The cloning of the glucocorticoid receptor cDNA was soon followed by the cloning of that of the human oestradiol receptor by the groups of Pierre Chambon in Strasbourg and of Geoffrey Greene of the University of Chicago in 1986 (24, 25). The information generated from these important steps in the characterization of steroid hormone receptors has stimulated the cDNA cloning of all steroid and nuclear receptors, including that of the human AR.

## 3. Testosterone Biosynthesis and Metabolism

The major circulating androgen is testosterone, which is synthesized from cholesterol in the Leydig cells of the testis. The biosynthetic conversion of cholesterol to testosterone involves several discrete steps, of which the first one includes the transfer

of cholesterol from the outer to the inner mitochondrial membrane by the steroidogenic acute regulatory (StAR) protein and the subsequent side chain cleavage of cholesterol by the enzyme P450scc (26). This conversion, resulting in the synthesis of pregnenolone, is the rate-limiting step in testosterone biosynthesis. Subsequent steps require several enzymes including 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase/C17-20-lyase and 17β-hydroxysteroid dehydrogenase type 3 (27).

Testosterone is metabolized in some target tissues to a more active metabolite: 5α-dihydrotestosterone. The irreversible conversion of testosterone to 5α-dihydrotestosterone is catalyzed by the microsomal enzyme 5α-reductase type 2 (SRD5A2) and is NADPH dependent (28). The cDNA of the gene for 5α-reductase type 2 codes for a protein of 254 amino acid residues with a predicted molecular mass of 28.4 kDa (29, 30). The $NH_2$-terminal part of the protein contains a subdomain suggested to be involved in testosterone binding, while the COOH-terminal region is involved in NADPH binding (31).

## 4. Physiological Effects of Androgens

As stated already before, the two most important androgens are testosterone and 5α-dihydrotestosterone. While acting through the same AR, each androgen has its own specific role during male sexual differentiation: testosterone is directly involved in development and differentiation of Wolffian duct-derived structures (epididymides, vasa deferentia, seminal vesicles and ejaculatory ducts), whereas 5α-dihydrotestosterone is the active ligand in a number of other androgen target tissues, such as the urogenital sinus and tubercle and their derived structures (prostate gland, scrotum, urethra and penis) (31, 32) (**Fig. 1.6**). The interaction of both androgens with the AR is different. The affinity of testosterone is twofold lower than that of 5α-dihydrotestosterone for the AR, while the dissociation rate of testosterone from the receptor is fivefold faster than that of 5α-dihydrotestosterone (33).

## 5. Androgen Action

### *5.1. The AR and the Nuclear Receptor Family*

Actions of androgens are mediated by the AR (NR3C4; nuclear receptor subfamily 3, group C, gene 4). This ligand-dependent transcription factor belongs to the superfamily of 48 known nuclear receptors (34). The nuclear receptor family includes

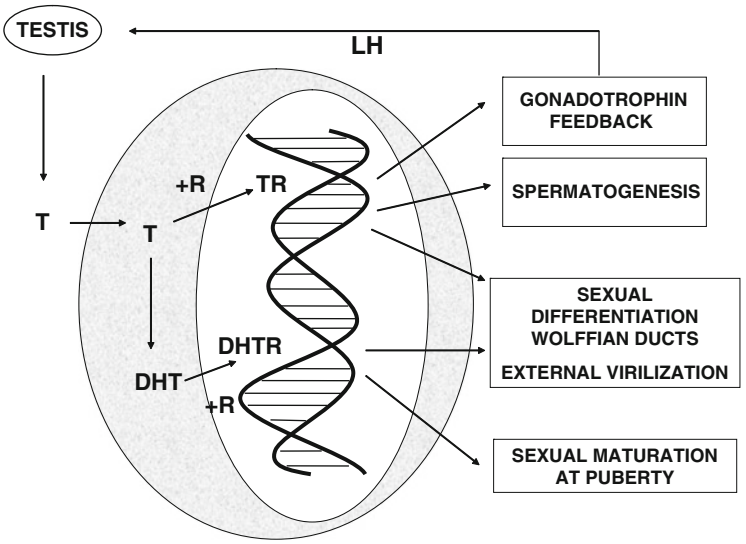


Fig. 1.6. Differential physiological actions of testosterone (T) and 5α-dihydrotestosterone (DHT) via the androgen receptor (R). LH, luteinizing hormone.

receptors for steroid hormones, thyroid hormones, all-*trans* and 9-*cis* retinoic acid, 1,25-dihydroxyvitamin D, ecdysone and peroxisome proliferator-activated receptors (35–37). Comparative structural and functional analysis of nuclear hormone receptors has revealed a common structural organization in four different functional domains.

The current model for androgen action involves a multistep mechanism (**Fig. 1.7**). Upon entry of testosterone into the androgen target cell, binding to the AR takes place either directly or after conversion to 5α-dihydrotestosterone. Binding to the receptor is followed by dissociation of heat-shock proteins in the cytoplasm, simultaneously accompanied by a conformational change of the receptor protein resulting in a transformation and a translocation to the nucleus. The receptor then dimerizes with a second molecule, binds to DNA and recruits further additional proteins (e.g. coactivators, general transcription factors and RNA polymerase II) resulting in specific activation or repression of transcription at discrete sites on the chromatin.

Interestingly, androgen signalling via the AR can also occur in a non-genomic, rapid and sex-nonspecific way by crosstalk with the Scr, Raf-1, Erk-2 pathway (38, 39).

### *5.2. Cloning and Structural Organization of AR Gene*

Since the cloning of the human AR cDNA, our insights into the mechanism of androgen action have increased tremendously. The cloning of the human AR cDNA was published in 1988 and 1989 by several groups just a few years after cloning of the cDNAs of the human glucocorticoid receptor, the human

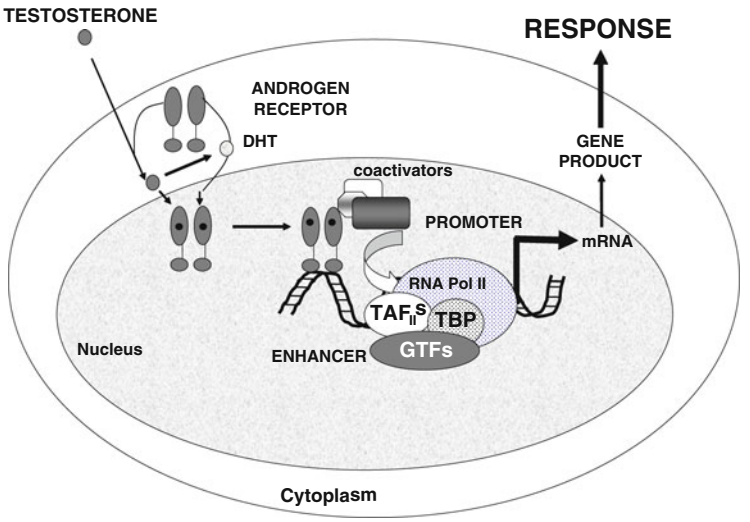


Fig. 1.7. Current model of androgen action. TAF$_{II}$s are TBP-associated factors; TBP is TATA-box-binding protein; GTFs are general transcription factors.

oestradiol receptor, the human mineralocorticoid receptor and the human progesterone receptor (40–43). Only one AR cDNA has been identified and cloned despite the two different ligands. The concept of two hormones and one receptor to explain the different actions of androgens has been generally accepted, and according to the information available from the human genome project, it is very unlikely that additional genes coding for a functional nuclear receptor with AR-like properties exist (37).

The *AR* gene is located on the human X chromosome at Xq11.2-q12 and consists of eight exons. The gene spans 186 kb in total (44, 45; www.genecards.org). The structural organization of the coding exons is essentially identical to that of the genes coding for the other steroid hormone receptors (i.e. exon/intron boundaries are highly conserved) (45, 46). As a result of differential splicing in the 3′-untranslated region, two AR mRNA species (8.5 and 11 kb, respectively) have been identified in several cell lines and both contain a 1.1-kb 5′-untranslated region (UTR) and a 2.7-kb open reading frame (ORF) (42, 47, 48) (**Fig. 1.8**).

The number of amino acid residues in the AR protein varies between individuals due to the polymorphic polyglutamine stretch and the less variable polyglycine stretch in the $NH_2$-terminal domain (NTD) (49, 50). Throughout this chapter, the numbering of the AR is based on the 919 amino acid residues according to the AR database (www.mcgill.ca/androgendb; 51). On SDS-PAGE, the AR appears as a 110–112-kDa doublet (52). However, in the presence of androgens, a 114-kDa band also

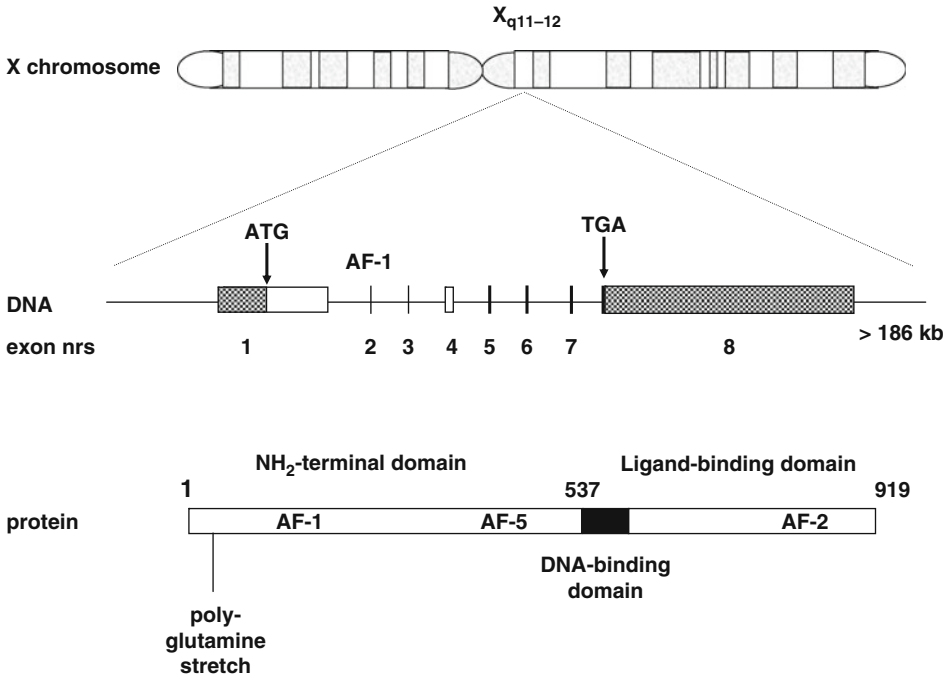


Fig. 1.8. Structural organization of the androgen receptor gene at the Xq11.2-q12 locus on the X chromosome and the derived protein size and structural organization.

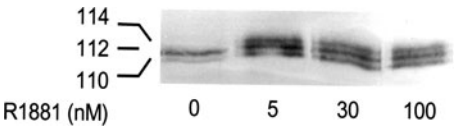


Fig. 1.9. Molecular size of androgen receptor isoforms on SDS-PAGE. The doublet in the absence of hormone (R1881, methyltrienolone) becomes a triplet upon hormone binding due to extra phosphorylation.

appears. These three bands represent different phosphorylated isoforms (53–56) (**Fig. 1.9**).

### *5.3. AR Polymorphisms*

The AR DNA- and ligand-binding domains have a high homology with the corresponding domains of the other members in the steroid receptor subfamily. In contrast, there is a remarkably low homology between the AR $NH_2$-terminal domain and that of the other steroid receptors (23, 24, 50, 57–59). A polyglutamine stretch, encoded by a polymorphic $(CAG)_nCAA$ repeat, is present in the $NH_2$-terminal domain (60). The length of the repeat has been used for identification of X chromosomes for carrier detection in pedigree analyses (61, 62).

Variation in length (9–38 glutamine residues) is observed in the normal population and has been suggested to be associated with a very mild modulation of AR activity (63). This assumption

is based on in vitro experiments after transient ectopic expression of AR cDNA-containing $(CAG)_nCAA$ repeats of different lengths (53, 64). Translating this finding to the in vivo situation, it can be envisaged that either shorter or longer repeat lengths can result in a relevant biological effect during the lifetime of the individual.

## 6. AR: Functional Domain Structure

### 6.1. The $NH_2$-Terminal Domain

The AR $NH_2$-terminal domain harbours the major transcription activation functions and several structural subdomains. Within its 538 amino acids, two independent activation domains have been identified: activation function 1 (AF-1) (located between residues 101 and 370) that is essential for transactivation potential of full-length AR and AF-5 (located between residues 360 and 485) that is required for transactivation potential of a constitutively active AR which lacks the LBD (65). Evidence is now available that the AF-5 region interacts with a glutamine-rich domain in p160 cofactors like SRC-1 and TIF2/GRIP1 and not with their LxxLL-like protein-interacting motifs (66). The $NH_2$-terminal domain is highly flexible: it has a structure between a fully unfolded state and a structured folded conformation, a molten-globule conformation (67).

Another function of the AR $NH_2$-terminal domain is its binding to the COOH-terminal LBD (N/C interaction) (68, 69). The $NH_2$-terminal regions required for the binding of the LBD have been mapped to two essential units – the first 36 amino acids and residues 370–494 (70). The hormone-dependent interaction of the $NH_2$-terminal domain with the ligand-binding domain can play a role in stabilization of the AR dimer complex and in stabilization of the ligand receptor complex by slowing down the rate of ligand dissociation and consequently decreasing receptor degradation (71, 72).

### 6.2. The DNA-Binding Domain

The DNA-binding domain is the best conserved among the members of the nuclear receptor superfamily. It is characterized by a high content of basic amino acids and by nine conserved cysteine residues. Detailed structural information has been published on the crystal structure of the DNA-binding domain of the glucocorticoid receptor complexed with DNA (73). Subsequently, 3D information became available for AR–DNA interaction at different types of response elements (74).

Briefly, the DNA-binding domain has a compact, globular structure in which three substructures can be distinguished: two zinc clusters and a more loosely structured carboxy-terminal extension (CTE) (75). Both zinc substructures centrally contain

one zinc atom which interacts via coordination bonds with four cysteine residues. Both the zinc coordination centres are C-terminally flanked by an α-helix (73). The two zinc clusters are structurally and functionally different and are encoded by two different exons. The α-helix of the most N-terminally located zinc cluster interacts directly with nucleotides of the hormone response element in the major groove of the DNA. Three amino acid residues at the N terminus of this α-helix are responsible for the specific recognition of the DNA sequence of the responsive element. These three amino acid residues, the so-called P(roximal) box [Glycine-Serine-Valine], are identical in the androgen, progesterone, glucocorticoid and mineralocorticoid receptors, and differ from the residues at homologous positions in the oestradiol receptor. It is not surprising therefore that the androgen, progesterone, glucocorticoid and mineralocorticoid receptors can recognize the same response element.

### 6.3. The Hinge Region

Between the DNA-binding domain and the ligand-binding domain, a non-conserved hinge region is located, which is also variable in size in different steroid receptors. The hinge region can be considered as a flexible linker between the ligand-binding domain and the rest of the receptor molecule. The hinge region is important for nuclear localization and contains a bipartite nuclear localization signal. In some nuclear receptors, including the AR, acetylation can occur in the hinge region at a highly conserved acetylation consensus site [KLLKK] (76).

### 6.4. The Ligand-Binding Domain

Finally, the second-best conserved region in the AR is the hormone-binding domain. This domain is encoded by approximately 250 residues in the C-terminal end of the molecule (23, 24, 42, 57, 58, 77). The crystal structure of the human AR ligand binding in complex with the synthetic ligand methyltrienolone (R1881) and 5α-dihydrotestosterone has been determined (78, 79). The three-dimensional structure has the typical nuclear receptor ligand-binding domain fold. Interestingly, the ligand-binding pocket consists of 18 amino acid residues interacting more or less directly with the bound ligand (78). The ligand-binding pocket is somewhat flexible and can accommodate ligands with different structures. The structural data are being used in designing optimized selective AR modulators (SARMs) (80).

Crystallographic data on the ligand-binding domain complexed with agonist predict 11 helices (no helix 2) with two anti-parallel β-sheets arranged in the so-called helical sandwich pattern. In the agonist-bound conformation, the C-terminal helix 12 is positioned in an orientation allowing a closure of the ligand-binding pocket. The fold of the ligand-binding domain upon hormone binding results in a globular structure with an interaction

surface for binding of interacting proteins such as coactivators. In this way the AR selectively recruits a number of proteins and can communicate with other partners of the transcription initiation complex. Crystallization studies of wild-type AR ligand-binding domain with antagonists are up till now unsuccessful (81).

The AF2 function in the ligand-binding domain is strongly dependent on the presence of nuclear receptor coactivators. In vivo experiments favour a ligand-dependent functional interaction between the AF-2 region in the ligand-binding domain with the $NH_2$-terminal domain (68, 70).

Deletions in the ligand-binding domain abolish hormone binding completely (82). Deletions in the N-terminal domain and DNA-binding domain do not affect hormone binding. Deletion of the ligand-binding domain leads to a constitutively active AR protein with transactivation capacity comparable to the full-length AR (82). Thus it appears that the hormone-binding domain acts as a repressor of the transactivation function in the absence of hormone. This regulatory function of the AR ligand-binding domain in the absence of hormone has also been reported for the glucocorticoid receptor (83).

### 6.5. Anti-androgens

AR antagonists are compounds that interfere in some way with the biological effects of androgens and are frequently used in the treatment of androgen-based pathologies. It was demonstrated with a limited proteolytic protection assay that binding of androgens by the AR results in two consecutive conformational changes of the receptor molecule. Initially, a fragment of 35 kDa, spanning the complete ligand-binding domain and part of the hinge region, is protected from digestion by the ligand. After prolonged incubation times with the ligand, a second conformational change occurs resulting in the protection of a smaller fragment of 29 kDa (84, 85). In the presence of several anti-androgens, only the 35-kDa fragment is protected from proteolytic digestion, and no smaller fragments are detectable upon longer incubations. This suggests that the 35-kDa fragment can be associated with an inactive conformation, whereas the second conformational change, only inducible by agonists and considered as the necessary step for transcription activation, is lacking upon binding of anti-androgens.

Based on the conformational changes of the AR ligand-binding domain, induced by androgens or anti-androgens, it can be concluded that the different transcriptional activities displayed by either full agonists, partial agonists or full antagonists are the result of recruitment of a different repertoire of co-regulators (coactivators or corepressors) as a consequence of these conformational changes. The differential recruitment of co-regulators can be considered as a special form of ligand-selective modulation of the AR ligand-binding domain and can be applied in a broader

sense also to the tissue-selective modulation of androgen action, where levels of coactivators and corepressors may ultimately determine the final activity (86).

## 7. AR Disorders

### 7.1. Androgen Insensitivity Syndrome

It has been known for quite some time that defects in male sexual differentiation in 46,XY individuals have an X-linked pattern of inheritance. It was Reifenstein who reported in 1947 on families with severe hypospadias, infertility and gynecomastia (87). The end-organ resistance to androgens has been designated as androgen insensitivity syndrome (AIS) and is distinct from other XY disorders of sex development (XY, DSD; formerly named male pseudohermaphroditism) like 17β-hydroxysteroiddehydrogenase type 3 deficiency or 5α-reductase type 2 deficiency (31, 88–90). It is generally accepted that defects in the *AR* gene can prevent the normal development of both internal and external male structures in 46,XY individuals and information on the molecular structure of the human *AR* gene has facilitated the study of molecular defects associated with androgen insensitivity. Naturally occurring mutations in the *AR* gene are an interesting source for the investigation of receptor structure–function relationships. The variation in clinical phenotypes provides the opportunity to correlate a mutation in the AR structure with the impairment of a specific physiological function.

### 7.2. Genetics of Androgen Insensitivity Syndrome

Since the cloning of the AR cDNA in 1988 and the subsequent elucidation of the genomic organization of the *AR* gene, tools have become available for the molecular analysis of the *AR* gene in individuals with AIS (45, 46). In addition to endocrinological data, the most reliable approach is sequencing each individual AR exon and flanking intron sequences. In general, androgen insensitivity can be routinely analyzed and almost 400 different mutations in the *AR* gene have been reported. Differential diagnosis is now possible from entirely different syndromes presenting with similar phenotypes including testicular enzyme deficiencies, 5α-reductase type 2 deficiency and Leydig cell hypoplasia due to inactivating luteinizing hormone receptor mutations. Furthermore, in pedigree analysis, intragenic polymorphisms, such as the highly polymorphic $(CAG)_nCAA$ repeat encoding a polyglutamine stretch, the polymorphic GGN repeat encoding a polyglycine stretch, the *Hin*dIII polymorphism (44) and the *Stu*I polymorphism (90), can be used as X-chromosomal markers

(61, 91–93). Extensive general information can be obtained at the Internet site www.genecards.org on the *AR* (NR3C4) gene and on the 375 identified single-nucleotide polymorphisms (SNPs).

### 7.3. Mutations in the AR Gene

In the *AR* gene, four different types of mutations have been detected in 46,XY individuals with AIS: single point mutations resulting in amino acid substitutions or premature stop codons, nucleotide insertions or deletions most often leading to a frame shift and premature termination, complete or partial gene deletions (>10 nucleotides), and intronic mutations in either splice/donor or splice/acceptor sites which affect the splicing of AR RNA (51). In general, in 70% of the cases, *AR* gene mutations are transmitted in an X-linked recessive manner, but in 30% of the cases, the mutations arise de novo. When de novo mutations occur after the zygotic stage, they result in somatic mosaicisms (94). The most recent update on *AR* gene mutations is available at http://www.mcgill.ca/androgendb/ (51).

### 7.4. Male Infertility

Several investigations into male infertile patients found an association between a longer $(CAG)_nCAA$ repeat and the risk of defective spermatogenesis (95–97). This suggests that a less active AR, due to a moderate expanded repeat length, may be a factor in the aetiology of male infertility.

### 7.5. Spinal and Bulbar Muscular Atrophy

Kennedy's disease, also known as spinal and bulbar muscular atrophy (SBMA), is a slowly progressing degeneration of lower motor neurons, resulting in muscle weakness in adult males (98–100). La Spada and colleagues (101) were the first to demonstrate a direct correlation of SBMA with an extension of the $(CAG)_nCAA$ repeat in exon 1, which encodes the polymorphic polyglutamine stretch in the AR $NH_2$-terminal domain. In the normal human population the repeat length is 34 or fewer, depending on the ethnic background (60, 102, 103). In SBMA, full penetrance alleles have a repeat length of 38 or more (**Fig. 1.10**). Reduced penetrance has been suggested for alleles with repeat lengths of 36 and 37. There is no consensus as to the clinical significance of alleles with a repeat length of 35 (114).

SBMA is associated with nuclear accumulation of the AR protein with the expanded polyglutamine stretch in motor neurons. Clinical symptoms usually manifest in the third to fifth decade and result from severe depletion of lower motor nuclei in the spinal cord and brainstem (63, 98, 104). In addition, SBMA patients frequently exhibit endocrinological abnormalities including testicular atrophy, infertility, gynecomastia and elevated LH, FSH and oestradiol levels. Sex differentiation proceeds normally and characteristics of mild androgen insensitivity appear later in life.

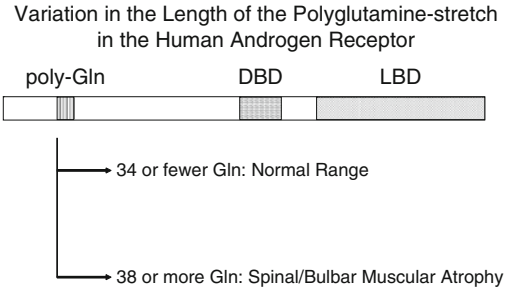


Fig. 1.10. Variation in the polyglutamine stretch encoded by the $(CAG)_nCAA$ repeat in exon 1. The normal range of this stretch is 34 or fewer glutamine residues, while in the motor neuron disease spinal/bulbar muscular atrophy (also called Kennedy's disease), the $(CAG)_nCAA$ repeat in exon 1 is expanded and full penetrance alleles have 38 or more glutamine residues (114).

### *7.6. Prostate Cancer*

Prostate cancer is the second leading cause of cancer deaths in men in Western countries (105). Most prostate cancers express relatively high levels of AR protein (106). Initially, prostate cancer is androgen dependent, because removal of androgens or blocking the AR by anti-androgens results in growth arrest of the tumour. However, tumour growth arrest is only temporary and most tumours undergo a transition to an androgen-unresponsive state.

Despite many suggestions for a possible mechanism for the development of the androgen-unresponsive state of prostate tumours, the exact mechanism underlying the transition to androgen independency is still unclear (107, 108). Since the AR is expressed in androgen-independent prostate tumours, it is assumed that the AR is still involved in some way in tumour growth. One mechanism may be higher AR protein expression caused by amplification of the *AR* gene (109–111). Under extremely low androgen levels (by hormone deprivation), the AR can still be activated. Also somatic mutations in the *AR* gene can result in a more active receptor protein (www.mcgill.ca/androgendb) or may broaden the ligand specificity towards anti-androgens or other steroid hormones, such as those found for the AR mutant T877A (112, 113). This mutation is frequently found in androgen-independent prostate tumours. Another mechanism that has been proposed is the increased expression of AR-specific cofactors, resulting in an enhanced AR activity and consequently in enhanced tumour growth. Finally, a mechanism involving ligand-independent activation of the AR has been suggested. This might be achieved by crosstalk with other activated signal transduction pathways.

## 8. Conclusions

Androgens (testosterone and 5α-dihydrotestosterone) are important steroid hormones for expression of the male phenotype. Their actions are mediated by the AR. The development of the field of androgen action over the last century has culminated in the cloning of the AR cDNA and its cofactors and their implications in both physiological and pathological conditions. As evidenced by the various tools that have been developed to study androgen action, presented in the ensuing chapters in this volume, the future is bright to further unravel the molecular mechanisms of androgen action as they relate to different normal and disease states. The specific roles of the various AR co-regulators and the manner in which their actions are coordinated on androgen target genes will be a predictable subject of future investigations. Recently developed quantitative microscopic interaction assays will allow the investigation of the dynamic behaviour of interacting molecules with the androgen receptor in time and space in single living cells. In this respect, the genome-wide analysis of potential AR-binding sites and their characterization is also a prerequisite for a functional correlation of these sites with an androgen-dependent gene expression profile. Structure–function determinants of the androgen receptor underlying androgen action can stimulate development of patient-tailored therapeutics. Although we have gained great insight into androgen action until now, much more work is needed to learn how androgens work, e.g. in regulating metabolism, their effects on the nervous system and in female physiology, as well as in disease states. It is thus an exciting time when the collaborative efforts of basic laboratory and clinical scientists, equipped with the plethora of techniques described in this book, are required which will result in the uncovering of this important body of knowledge.

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