Androgen Receptor

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Abstract

Androgen receptor (AR) belongs to the steroid hormone receptor family of the nuclear receptor superfamily and acts as a hormone-controlled transcription factor that conveys the messages of both natural and synthetic androgens to the genes and gene programs. The androgen-regulated genes have a central role in the development and maintenance of the male phenotype and reproductive physiology. AR gene resides on the X chromosome, and mutations in the gene lead to a wide array of androgen insensitivity disorders in males. AR-mediated gene regulation is a rigorously regulated process that involves a coordinated interaction of AR with other DNA sequence-specific transcription factors, such as pioneer factor forkhead box1 and coregulator proteins, including PIAS1. AR signaling starts in the cytosol where hormone binding releases the receptor from a chaperone complex, leading to receptor homodimerization and nuclear translocation and binding to androgen response elements in the regulatory regions of AR target genes. Classic models of AR action view the AR chromatin-binding sites as upstream regulatory elements in gene promoters, but recent genome-wide methods have revealed that the AR regulates transcription mostly from the distal chromatin-binding sites, enhancers. This highlights the importance of chromatin structure and long-range chromatin interactions in the regulation of transcription by AR. In addition to androgens, the AR activity is regulated by several posttranslational modifications, such as SUMOylation, which influence the chromatin binding, protein stability, and interaction of the receptor with other proteins.

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Androgen Receptor Gene and Protein

The androgen receptor (AR), also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4), is a ligand-dependent transcription factor (TF) belonging to the steroid hormone receptor (SR) group of the nuclear receptor (NR) superfamily. In humans, this superfamily contains 48 NRs. The human AR gene is located on the X chromosome. It consists of eight exons which are separated by relatively long introns (Fig. 1). The AR promoter region lacks a TATA box and a CCAAT box, but contains binding sites for Sp1, NFκB, and c-MYC (Burnstein 2005; Shiota et al. 2011). The retinoblastoma/E2F pathway that controls genes that are essential for DNA synthesis and cell cycle progression inhibits the AR expression via the AR gene promoter (Davis et al. 2006; Valdez et al. 2011). Expression of the AR gene is regulated by androgens, but response to androgens varies in a tissue- and cell-type-specific fashion. Interestingly, loss of an enhancer in the human AR locus is associated with anatomical loss of androgen-dependent sensory vibrissae and penile spines in the human lineage compared to closely related species, including chimpanzees (McLean et al. 2011).

Androgens control male sexual development during embryogenesis and sexual maturation at puberty and maintain reproductive functions and behavior in the adult. AR mRNAs are detectable in male and female mouse embryos from E11 to E12.5 onward, shortly before embryonic hormone synthesis starts (Crocoll et al. 1998; Young and Chang 1998). The male reproductive tissues, such as epididymis and prostate, show the highest AR expression. Both basal and luminal epithelium of human prostate express the AR, with the luminal cells showing higher levels than the basal cells (Abate-Shen and Shen 2000). Rodent prostate gland has a multilobular structure, and expression of AR varies with age and lobe (Banerjee et al. 2001; Sugimura et al. 1986). Interestingly, increased expression of AR has been shown to correlate with development of age-dependent, spontaneous hyperplasia of rat
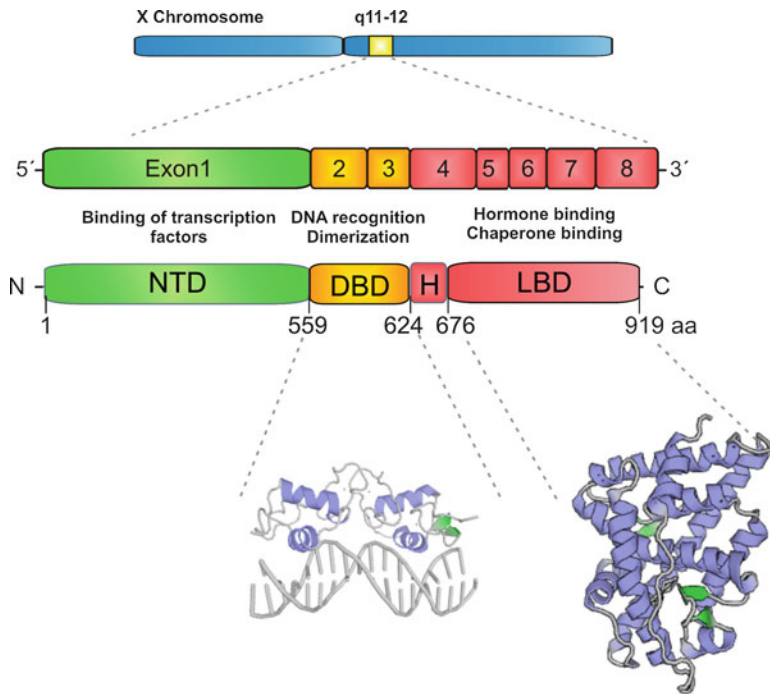
Fig. 1 Genomic organization of the androgen receptor (AR) gene and functional domain structure of the AR protein. The AR gene contains eight exons and it encodes for a 919-amino acid-long protein consisting of four major functional domains: N-terminal domain (NTD), DNA-binding domain (DBD), hinge region (H), and ligand-binding domain (LBD). The principal functions of the domains are also shown. Bottom, crystal structure of the AR DBD (PDB: 1R4l) and the AR LBD (PDB: 1E3G)

prostate (Banerjee et al. 2001; Prins and Putz 2008). Whether such changes in the expression of AR occur in human prostate is not known.

In addition to the reproductive tissues, AR is expressed at low level in almost all tissues, including the brain. In mouse, hypothalamic expression of AR is sex, age, and region dependent, with the expression levels increasing toward adulthood and adult males showing higher levels than the females (Brock et al. 2015). In aged male rats, hypothalamic expression of the AR has been found to rise, which may be a compensatory consequence of reduced testosterone concentrations (Munetomo et al. 2015; Wu and Gore 2010). Analogous information of the hypothalamic expression of AR in humans is not available (Harman et al. 2001). Androgens seem to also directly regulate the development of cortex and hippocampus, both of them showing a robust increase in the AR expression during the first 3 weeks after birth of mouse (Tsai et al. 2015).

The AR plays an important role in female reproduction as well, being expressed in various ovarian cell types. In addition to the patients suffering from the androgen

insensitivity syndrome (AIS), the AR's fundamental role for male sexual differentiation and reproductive function has been demonstrated with AR knockout mouse models (Jääskeläinen 2012; Wang et al. 2005). Global AR knockout male mice, similar to complete AIS-suffering patients, have female external genitalia and are infertile, lacking normal spermatogenesis (Yeh et al. 2002). In female mice, global AR knockout results in changes in ovarian function with compromised fertility as well as retarded development of mammary glands (Hu et al. 2004; Walters 2015). Moreover, cell-selective AR knockout models with Cre/loxP technology have revealed tissue- and cell-specific functions of AR in both male and female animals (De Gendt and Verhoeven 2012; Zhou 2010). The AR has important biological actions also in the immune, bone, cardiovascular, hematopoietic systems, and skeletal muscle. The AR is expressed widely in different types of muscle cells, including in myoblasts, myofibers, and satellite cells. Androgen-induced increase in muscle mass is due to hypertrophy of both slow- and fast-twitch muscle fibers (Finkelstein et al. 2013; Kadi et al. 2000; Sinha-Hikim et al. 2004). However, the molecular mechanisms underlying the anabolic effects of androgens in muscle hypertrophy are not completely solved. They seem to be diverse and mediated via several cellular targets and biochemical pathways.

The AR gene encodes a 110-kDa protein composed of 919 amino acid residues, although the number of amino acid residues can vary between individuals due to the presence of polymorphic polyglutamine and polyglycine stretches (Palazzolo et al. 2008). Two AR isoforms, the full-length receptor and an amino-terminally truncated form, have been identified in normal human tissues, but the functional significance of the smaller AR is not established (Ahrens-Fath et al. 2005). The AR has also several splice variants that have been detected in cell- and animal-based models and tissues from prostate cancer patients. The variants commonly lack the C-terminal region of the receptor, which leads to constitutive activity due to the absence of ligand-binding domain and therefore also resistance to antiandrogens (Daniel and Dehm 2016).

The AR is structurally and functionally related to other SRs, glucocorticoid receptor (GR), progesterone receptor (PR), mineralocorticoid receptor (MR), and two estrogen receptors (ER) α and β, from which all but the ERs share in principle the same DNA-binding sites, although specific sites are also found (Huang et al. 2010). SRs' structures can be roughly divided into four distinct domains: N-terminal-activating domain (NTD), DNA-binding domain (DBD), hinge region, and C-terminal ligand-binding domain (LBD) (Fig. 1).

The AR NTD includes ∼60% of the protein's amino acids (558 residues), and it contains the polyglutamine (poly-Q) and polyglycine repeat sequences. Normally, the number of glutamines in the poly-Q repeats ranges from 8 to 31, and repeats with lengths over 40 can cause spinal and bulbar muscular atrophy (Kennedy's disease) (La Spada et al. 1991). The shorter poly-Q repeats commonly impose a higher AR transactivation activity, while longer repeats reduce the activity. The NTD is the most variable domain within the SRs, and the AR NTD shows only limited amino

acid sequence homology with the other SRs. For example, the sequence similarity between AR NTD and PR NTD is only 20%. The NTD is therefore likely to markedly contribute to the specificity of the AR. The NTD that on its own is lacking a well-defined three-dimensional structure interacts in an androgen-dependent manner with the C-terminal LBD (N/C interaction), which stabilizes the transcriptionally active AR dimer complex. This interaction is required for the full transcriptional potential of AR. The NTD is also a site for the interaction between the AR and many structurally diverse coregulators (Davey and Grossmann 2016). The NTD contains a ligand-independent activation function (AF)-1 that is required for the maximal activity of the AR, and in the absence of the LBD, the AF-1 becomes constitutively active. The AF-1 also mediates direct interdomain interactions between the NTD and the LBD, which also stabilizes the AR dimer and reduces the rate of hormone dissociation (McEwan 2004).

The DBD that is a relatively small (∼100 amino acids) protein domain is the best conserved region among the NRs. The amino acid sequence of the AR DBD is 76–79% identical with those of its closest relatives, GR, MR, and PR. The AR DBD consists of two α-helices that are arranged into two structurally and functionally different zinc finger domains (Helsen et al. 2012). The helices are located at the C-terminal ends of the zinc fingers. They are oriented vertically to each other and form the base of a hydrophobic core. Both zinc fingers contain four cysteine residues which coordinate one zinc ion. The N-terminal zinc finger is making direct contacts with the DNA, and the C-terminal one creates a dimerization interface by forming salt bridges with the corresponding region of another AR molecule, resulting in stabilization of DNA binding (Jakob et al. 2007; Shaffer et al. 2004). The amino acids in the first zinc finger's DNA recognition helix, the so-called P box, responsible for the sequence-specific DNA contacts are identical in the corresponding positions of the GR, the MR, and the PR. The hinge region located between the DBD and the LBD contains the main nuclear localization signal for the import of the receptor into the nucleus as well as a nuclear export signal that mediates the export of the AR to the cytoplasm upon ligand removal. The hinge region also mediates interaction with heat shock chaperone proteins when androgen is not bound to receptor (Huang et al. 2010).

The AR LBD consists of 11 α-helices and two antiparallel β-sheets which form a ligand-binding pocket (LBP) as in other members of the SR family. Ligand binding induces a conformational change in the LBD, causing rearrangement of α-helix 12 harboring ligand-dependent AF-2. The AF-2 acts as a lid to close the LBP upon ligand binding. There are 18 amino acid residues critical for the receptor's interaction with androgen (Matias et al. 2000; Sack et al. 2001). The AF-2 is also involved in the recruitment of coactivators and corepressors. Deletion of LBD leads to a constitutively active AR (Jenster et al. 1991). Several amino acid substitutions in or around AR LBD α-helices 3, 4, 5, and 11 of AIS patients have been found to disrupt the interaction of the LBD with the NTD, even though the mutations have no severe effects on hormone binding (Thompson et al. 2001).

Androgen Receptor-Mediated Regulation of Transcription

Androgen binding initiates a conformational change in the AR and a subsequent transfer of the receptor dimer to the nucleus where it binds to specific androgen response elements (AREs) on the regulatory regions of its target genes. In this way, the AR conveys the message of androgens directly to the level of genetic programs (Gao et al. 2005; Green et al. 2012; Sampson et al. 2013). In the absence of ligand, AR exists mainly in the cytosol in a multiprotein chaperone complex with heat shock proteins, such as heat shock protein 90, immunophilins, p23, FKBP51, FKBP52 and Cyp40, and serine/threonine phosphatase 5 (Fig. 2) (Echeverria and Picard 2010). The androgen-induced conformational change in the AR releases the receptor from its chaperone protein complex, leading to receptor phosphorylation, homo-dimerization, and nuclear translocation (Fig. 2) (McEwan 2004; Palvimo 2012). The AR moves into the nucleus very rapidly; it can be detected on chromatin within a few minutes after androgen exposure with maximal binding present at 2 h (Massie et al. 2011). In the nucleus, the AR dimer binds to AREs in the regulatory regions of its target genes (Pihlajamaa et al. 2014; Sahu et al. 2011). This initiates the formation of a multiprotein complex which exerts the activation or repression of AR target genes. If the AR binds to promoter regions, the receptor may interact directly with general transcription machinery components TFIIB and TFIIH associated with RNA polymerase II (Pol II) (Lavery and McEwan 2008; Lee and Chang 2003; Roy and Singer 2015). The multiprotein complexes residing on distal regulatory regions, enhancers, are likely to consist of both directly DNA-binding collaborating TFs and non-DNA-binding coregulators, such as steroid receptor coactivators (SRC) 1, 2, and 3 and cAMP response element-binding protein (CBP)/300 as well as Mediator complex bridging interactions with the Pol II transcription apparatus. (Shang et al. 2002; Wang et al. 2007). In this way, androgen signaling alters gene transcription and ultimately leads to specific biological responses (Fig. 2).

Recently, chromatin immunoprecipitation (ChIP) combined with massively parallel DNA sequencing (ChIP-seq) has enabled investigation and mapping of a given TF's targets at the level of the whole genome. ChIP-seq studies that thus far have mostly been carried out in a few prostate cancer cell lines have typically revealed tens of thousands of androgen-binding sites (ARBs) across the human genome (Massie et al. 2011; Sahu et al. 2011; Toropainen et al. 2015). The collection of ARBs in a given cell line or tissue is termed AR cistrome. Interestingly, the majority of ARBs reside in distal intronic and intergenic regions relative far away from the promoter regions of the target genes. Chromosome conformation capture assays have further demonstrated that the distally bound AR can communicate with promoters through chromatin looping (Wang et al. 2007). Therefore, the classic model of AR binding to the proximal promoter of target genes has been revised. The AR cistromes exhibit cell-type-specific features. ChIP-seq profiling of ARBs in the prostate, epididymis, and kidney has found evidence for only a minor overlap, highlighting tissue specificity of the AR cistromes (Pihlajamaa et al. 2014).

ChIP-seq studies have also uncovered more details about the AREs. Motif analyses of the AR-bound DNA sequences have confirmed that the AR binds mainly
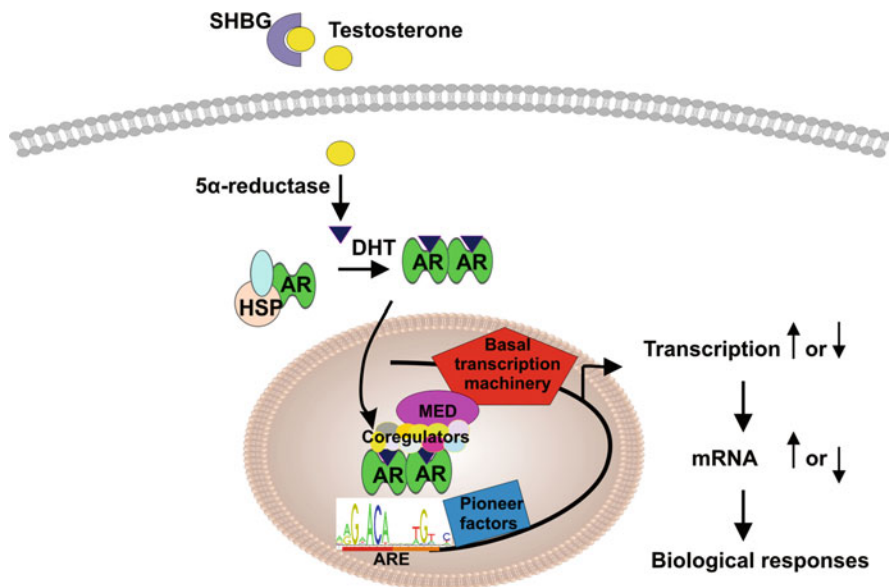
Fig. 2 Androgen receptor (AR)-mediated signaling. After synthesis, testosterone bound to serum sex hormone-binding globulin (SHBG) is transported to target tissues, such as prostate. In prostate cells, it is converted to 5α-dihydrotestosterone (DHT) by 5α-reductase. Binding of DHT to the AR induces a conformational change in the receptor, resulting in release of chaperone heat shock proteins (HSP), dimerization, and translocation of the AR to the nucleus. There, the receptor binds to the androgen-response elements (ARE) under the guidance of pioneer factors and it interacts with coregulators and Mediator complex (MED), which alters, induces, or represses, target gene transcription

to two types of elements. The "classic," canonical AREs containing two 5′-AGAACA-3′ inverted half-sites, or hexamer repeats, with a three-nucleotide spacer (Mangelsdorf et al. 1995; Roche et al. 1992) are found from ∼60 % of the ARBs (Massie et al. 2011; Toropainen et al. 2015). In keeping with the sequence similarity between the closely related SRs, the classic AREs can be recognized also by the GR, the PR, and the MR, and AR and GR cistromes overlap significantly (Sahu et al. 2011). Notably, the best characterized androgen-regulated genes are not generally regulated by perfect inverted repeat-containing AREs. The "selective" AREs in turn resemble more the direct repeats of the same hexamer, and they are not recognized by the GR or the MR (Denayer et al. 2010; Sahu et al. 2014). Interestingly, the selective chromatin binding is achieved through a less stringent sequence requirement for the 3′-hexamer with its second zinc finger of the AR being important for determining the selectivity (Sahu et al. 2014). Thus, the AR seems to use surprisingly relaxed response element stringency for selective chromatin binding and transcriptional regulation in vivo.

The role of AREs in the transcriptional repression by the AR is poorly defined in comparison to the transcriptional activation. Previous single target gene- and

reporter gene-based assays have indicated that the AR can repress transcription without directly binding to DNA, but by binding and inhibiting the activity of activator protein 1 (AP-1) and nuclear factor κB (NFκB) subunit RelA. Competition for the CBP can also contribute to the transcriptional interference between the AR and the AP-1 and the NFκB (Foradori et al. 2008; Kallio et al. 1995; Lu et al. 2000; Palvimo et al. 1996).

Nongenomic Androgen Action

Some effects of androgens are too rapid (occur in seconds to minutes) to be mediated via regulation of gene transcription by the AR. In the literature actions which require neither AR nuclear translocation nor AR DNA binding have been commonly referred to as "nongenomic," "nonclassical," or "noncanonical" AR signaling. Cytoplasmic AR may facilitate activation of various kinase-signaling cascades, including the Src family kinases, Ras-Raf-1, phosphatidylinositol 3-kinase/Akt, and protein kinase C, leading the activation of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) (Zarif and Miranti 2016). The activated ERK may in turn phosphorylate the AR, which may enhance AR's genomic activity (Foradori et al. 2008; Liao et al. 2013). Activation of the Src-Raf-1 signal-transducing pathway by nongenomic AR signaling has been reported to lead to S-phase entry and cell proliferation. Moreover, androgens, possibly through membrane-associated AR, have been shown to rapidly increase intracellular calcium which functions as a ubiquitous secondary messenger. However, more studies are needed to establish and complete the mechanistic picture of nongenomic AR action.

Androgen Receptor Target Genes

The AR is capable of regulating thousands of genes whose products are involved in many important cellular functions (Lamont and Tindall 2010). AR target gene classification simply based on AR chromatin-binding events is demanding, because the AR-binding enhancers may reside many hundreds of kilobases or even mega-bases away from the transcription start sites of the AR target genes. Although AR-regulated gene programs are in a cell-, tissue-, and developmental stage-specific fashion involved in several biological processes and pathways, they seem to share "core" target genes. Many genes of the latter group are involved in the regulation of cell cycle and apoptosis, increasing cell proliferation and inhibiting apoptosis. Many of them also play a role in cellular differentiation. The AR also regulates anabolic gene programs in glucose consumption, lipid turnover, and muscle biomass production. Genome-wide data from murine prostate, kidney, and epididymis confirm that the AR-regulated transcription programs in vivo are tissue specific, regulating distinct biological pathways (Pihlajamaa et al. 2014).

The testis is not only the main source of androgens but also numerous genes in the tissue are regulated by androgens. Spermatogenesis is controlled by a hierarchical network of regulatory systems, and androgens play a critical role in it. The cell junction dynamics and cytoskeletal elements are among the AR targets. The genes of this group include claudin-11, occludin, gelsolin, and vimentin (Wang et al. 2006). Biological functions, such as signal transduction, MAPK activity, cell adhesion, calcium binding, and insulin-like growth factor binding, are also regulated by the AR in mouse testis (Verhoeven et al. 2010). Reproductive homeobox X-linked gene 5 (Rhox5) that in addition to the testis is expressed in the epididymis and ovary is induced by androgens through several AREs (Hu et al. 2007). Rhox5 encodes for a TF critical for germ cell development, sperm production, sperm motility, and fertility (Maclean et al. 2005). Moreover, the expression of testis-specific X-linked gene (Tsx) and dopamine receptor 4 (Drd4) are upregulated by the AR (Schauwaers et al. 2007).

Genome-wide identification of AR-regulated genes in mouse epididymis has confirmed several AR target genes which are, for instance, involved in metabolism, signal transduction, biological regulation, localization, development, and transport (Hu et al. 2010; Sipilä et al. 2006). Among those genes are glutathione peroxidase 5, an antioxidant scavenger that protects spermatozoa from damaging effects (Chabory et al. 2009); sperm-associated antigen 1 gene involved in fertilization; and cysteine-rich secretory protein 1 that is needed for the sperm-egg fusion (Roberts et al. 2008). Many of beta-defensing family members' genes that have important roles in sperm maturation and fertility are also AR targets (Hu et al. 2014).

The prostate gland is secreting semen fluid factors which enhance sperm viability. The AR is needed for the embryonic development, growth, and maturation of the prostate. In normal adult prostate, the primary function of the AR is to induce expression of genes required to promote differentiation, suppress proliferation, and promote secretion (Sensibar 1995; Yadav and Heemers 2012). In prostate cancer cells, AR upregulates the expression of cell cycle regulators, including cell division cycling 25A, cyclin-dependent kinase 6, and E2F transcription factor 1. In addition, the AR regulates anabolic programs through genes of enzymes involved in glycolytic flux, such as hexokinase II, and utilization of glycolysis metabolites in the production of lipids, e.g., fatty acid synthase, and nucleotides, e.g., ADP-ribose pyrophosphatase (Massie et al. 2011; Sharma et al. 2013).

Androgens increase skeletal muscle mass. Insulin-like growth factor 1 (IGF-1) gene is one of the important AR targets in skeletal muscle. IGF-1 is upregulated by androgens and promotes myogenesis and maintenance growth of muscle fiber (Chen et al. 2005; Gentile et al. 2010; Serra et al. 2011). In the skeletal muscle, androgens also enhance the expression of follistatin and hepatocyte growth factor, but decrease the expression of GR, myogenin, calcineurin, and c-Myc (MacKrell et al. 2015; Rana et al. 2014). Myostatin that is a negative regulator of skeletal muscle growth is surprisingly also upregulated by androgens (Dubois et al. 2014). This may be a mechanism by which androgens restrain their own anabolic effects in the skeletal muscle.

Androgen Receptor Collaborating Transcription Factors

The function of AR in transcriptional regulation is tightly associated with that of other TFs. Recent genome-wide ChIP-seq studies have highlighted the role of other TFs, especially that of so-called AR-collaborating TFs, in the regulation of AR target gene transcription. It seems that the AR cistromes differ to a much lesser extent between cell lines and tissues than the AR-regulated transcriptomes. This strongly suggests that other TFs and coregulators are critically involved in determining the specificity of AR-mediated gene regulation. Several TFs, including AP-1, ERG, HOXB13, FOXA1, GATA2, OCT-1, RXR, ETS1, and HNF-4α, have been shown to interact physically and functionally with the AR (Heemers and Tindall 2007). ChIP-seq studies have confirmed the predicted de novo co-occupancy between the AR and several collaborating TFs (http://genome.ucsc.edu/ENCODE/).

Pioneer factors are TFs that are able to access their own DNA recognition motifs even in transcriptionally silent, compact chromatin (Zaret and Carroll 2011). They are thought to displace nucleosomes to loosen chromatin and bind to the genome for a period prior to the binding of other factors. Thus, they enable the binding of other TFs that cannot on their own access to their target sequences in compacted chromatin. The binding of pioneer factors can also lead to formation of compact chromatin with binding of corepressors. Forkhead box A1 (FOXA1) and GATA-binding protein 4 (GATA4) were the first pioneer factors to be identified during development of the liver and heart, respectively, where they have especially crucial roles (Holtzinger and Evans 2005; Lee et al. 2005). In addition to the FOXA family members and GATA2, 3, and 4, several TFs, including TLE factors, PU.1, AP-2α, and -γ, have been shown to possess similar pioneer factor properties.

Pioneer factors significantly contribute to recognition of SR targets on chromatin (Carroll et al. 2006; Hurtado et al. 2011; Laganiere et al. 2005; Yu et al. 2005). In particular, FOX and GATA motifs are enriched within AR- and ER-binding sites. The FOXA1 shows tissue specificity in its function (Pihlajamaa et al. 2014). FOXA1 is a proven key pioneer factor for the AR. It can both facilitate AR-mediated gene activation and function in AR-mediated gene repression (Gao et al. 2003; Lupien et al. 2008). The role of the FOXA1 in AR regulation is versatile, but the function of the FOXA1 not only depends on target genes but also on the equilibrium between the two TFs (Jin et al. 2014). Interestingly, silencing of the FOXA1 in prostate cancer cells led to a global redistribution of ARBs, creating new ARBs and initiating new transcriptional programs (Sahu et al. 2011). Thus, FOXA1 can facilitate binding of AR to some chromatin regions and block the binding to other regions, and there is a class of AR-binding sites that is independent of FOXA1. In the absence of FOXA1, AR binds to more "specific" ARE motifs devoid of the forkhead-binding sites in their close proximity (Jin et al. 2014). These FOXA1 data are derived almost exclusively from prostate cancer cells. Notably, data from murine prostate, kidney, and epididymis indicate that tissue-specific pioneer TFs indeed associate with AR cistromes and transcription programs: hepatocyte nuclear factor 4α (Hnf4α) in the kidney and activating enhancer-binding protein 2α (AP-2α) in the epididymis define tissue-specific AR recruitment, whereas in the mouse prostate as in the prostate

cancer cells, FOXA1 serves for the same purpose. These pioneering factors are constitutively bound to chromatin and guide AR to specific genomic loci upon hormone exposure. These data indicate that hormone-bound AR and AREs are required but not sufficient for tissue-specific AR gene programs (Hu et al. 2010; Pihlajamaa et al. 2014).

AR Coregulators

In addition to the pioneer TFs and general Pol II transcription apparatus, AR-interacting coregulators are required for the AR to enable regulation of target genes. Hormone-bound AR interacts with the coregulator proteins, coactivators, and corepressors, either prior to its chromatin binding or on the chromatin to enhance or repress AR target gene transcription. Whether a given coregulator functions as an activator or a repressor can depend on the target gene and cell context. In contrast to collaborating TFs, coregulator levels are not thought to significantly alter the basal rate of the AR target gene transcription. In addition to binding directly to the AR, coregulators in many cases interact and communicate with other transcription regulatory proteins without interacting directly with the DNA. AR has been reported to interact with more than 200 putative coregulators (Heemers and Tindall 2007). The majority of these coregulators are postulated to associate directly with the AR. This could be due to sequential binding and their interaction with different domains of the AR. However, the biological significance of a relatively small number of the coregulators has been validated. It is also likely that there is a lot of redundancy among the coregulators. Coregulators are often, probably in most cases, components of multi-subunit coregulator complexes possessing a multitude of enzymatic activities. As most of the putative AR coregulators are also able to modulate transcription mediated by several NRs plus other types of TFs, the number of truly AR-specific coregulators is likely to be very small. The AR coregulators have versatile functions, but they can be divided into two families: those which facilitate AR's DNA occupancy, chromatin remodeling, and recruitment of basal transcription machinery and those which modulate the AR protein itself.

Chromatin remodeling complexes alter chromatin structure and unwrap the histone-DNA complexes to render them more permissive for transcription, or, conversely, they can condense the chromatin structure and promote gene repression (Li et al. 2007). Many of the AR coregulators are components of the chromatin-remodeling complexes. AR-interacting protein (ARIP) 4 is a member of the SNF2-like family, which is known for its chromatin-remodeling properties. ARIP4 contains the SNF2 domain and is an active DNA-dependent ATPase able to generate super-helical torsion on linear DNA fragments (Rouleau et al. 2002). In addition, coregulators can covalently modify, acetylate, phosphorylate, methylate, ubiquitinate, and SUMOylate histone residues, i.e., they write histone marks. Specific combinations of covalent histone modifications can loosen or tighten the DNA-histone interactions and create binding surfaces for other chromatin and transcription regulatory proteins that read histone marks (Li et al. 2007). Together

these histone marks have been suggested to form a histone code for the regulation of gene transcription. Many NR coregulators possess histone acetylase, deacetylase, methylase, or demethylase activity, i.e., they erase histone marks. Increased histone N-terminal tail acetylation and histone acetyltransferase (HAT) activity are generally associated with transcriptional activation (Barth and Imhof 2010; Fullgrabe et al. 2011). Steroid receptor coactivator 1, 2, and 3 (members of p160/SRC gene family) and CBP/p300 are recognized AR-interacting HATs (Wang et al. 2011). The role of histone methyl marks is more complex than that of acetyl marks. Active enhancer regions, for example, show high levels of H3K4me2 and H3K4me3 (H3 lysine 4 di- and trimethylation) as well as H3K36me2 and H3K36me3, but are devoid of H3K27me3 and H4K20me3 (Barth and Imhof 2010). Demethylases, such as lysine-specific demethylase 1 (LSD1/KDM1A), are also known to interact with AR (Yamane et al. 2006). The coactivator activity of LSD1 may derive from its ability to demethylate repressive H3K9me2 and H3K9me3, but it may also demethylate H3K4, leading to repression of genes. Mediator complex bridging interactions to Pol II transcription apparatus are also shown to be important in AR-dependent transcription, as, e.g., MED1 (TRAP220) coactivates AR activity (Taatjes 2010).

Other coregulators may affect AR-dependent transcription by modulating appropriate folding, hormone binding, N/C interaction, stability, or correct subcellular localization of the AR. These coregulators are often recruited to the hinge region or the AR NTD, especially in AF-1 (Heemers and Tindall 2007). For example, filamin can facilitate the translocation of the ligand-bound receptor to the nucleus. AF-1 can bind to an LxxLL motif (where L is leucine and x any amino acid), which is commonly present in coregulators (van de Wijngaart et al. 2012). The AR-associated protein of 70 kDa is an example of this category of coregulators. It can stabilize the ligand-bound receptor (Heinlein and Chang 2002). Some coregulators, such as PIAS proteins, can promote posttranslational modifications in AR, which regulate AR's transcriptional activity. Recent genome-wide studies suggest that protein inhibitor of activate STAT 1 (PIAS1) functions as a genuine and chromatin-bound AR coregulator, interacting also with FOXA1 and regulating AR target genes in prostate cancer cells (Toropainen et al. 2015).

Similarly, corepressors utilize various mechanisms for repressing AR-dependent transcription. Histone deacetylases (HDAC) remove acetyl group, which leads to the formation of a condensed nucleosomal structure and silencing of transcriptional activity. For example 5′TG3′-interacting factor can repress AR-mediated transcription by forming a complex with Sin3A and HDAC1 (Sharma and Sun 2001). Corepressors, such as RAD9, may regulate AR N/C interaction, inhibiting ligand binding and competing for coactivator recruitment (Hsu et al. 2005). Nuclear receptor corepressor (NCOR) 1 and 2 may also interfere with the AR N/C interaction, but their effect on the repression of AR-dependent transcription is likely to be more important through corepressor complexes harboring HDACs. Proteins, such as PTEN, may prevent AR's nuclear translocation by sequestering the AR in the cytoplasm (Burd et al. 2006).

Taken together, it seems that AR-collaborating pioneer TFs and coregulators ultimately determine the AR's binding site selection on chromatin and

transcriptional activity in a cell- and tissue-specific manner. Different tissues and cells display different patterns of pioneer TFs and coregulators, which are thought to significantly contribute to distinct androgen-regulated gene programs in different tissues.

Enhancer RNAs in AR-Regulated Transcription

High-throughput sequencing techniques have recently uncovered a widespread transcription from enhancers (Lam et al. 2014). These enhancer RNAs (eRNAs) produced by Pol II are typically bidirectional from enhancer regions, and the majority of them are not spliced or polyadenylated. The enhancer-associated transcript levels correlate positively with the expression of the nearest genes (De Santa et al. 2010; Li et al. 2013). Transcription of the eRNAs can be dynamically regulated by NRs, including the AR. The eRNAs may participate in enhancer-promoter loop formation and chromatin remodeling. Chromatin looping in transcriptional activation by the AR can involve interactions with the Mediator complex, offering scaffolds for AR-associated protein complexes for further contributing specific regulation of AR transcription programs (De Santa et al. 2010; Kaikkonen et al. 2013a). Interestingly, topoisomerase I was recently found to be modulating eRNA synthesis and AR-driven enhancer activation through its DNA-nicking activity (Puc et al. 2015).

Posttranslational Modifications of the AR

In addition to the androgenic hormones, the activity of AR is regulated by posttranslational modifications (PTMs), phosphorylation and lysine modifications, acetylation, methylation, ubiquitination, and SUMOylation. The PTMs are likely to cross talk with each other, thus providing a complex regulatory code for the AR action (Gioeli and Paschal 2012).

Altogether 17 amino acid residues have been reported to be phosphorylated in the AR. As several phosphorylation sites may influence each other, the complexity of phosphorylation-based regulation of the AR is potentially huge. Most of the AR phosphorylation sites reside in the NTD (Koryakina et al. 2014). Depending on phosphorylation sites, the phosphorylation can occur either in the absence or presence of androgen. The AR is phosphorylated within 15 min of its synthesis, which is thought to allow ligand binding, and binding of androgen induces further phosphorylation events. For example, several cyclin-dependent kinases (CDKs) are able to phosphorylate the AR NTD. Similarly, growth factors can induce the AR phosphorylation (Gioeli and Paschal 2012). MAPK phosphorylates many sites of AR, including S650 in the hinge domain, which regulates the nuclear transport of the receptor (Gioeli et al. 2006; Koryakina et al. 2014). Phosphorylation of the AR is a reversible process. Two protein phosphatases, PP1 and PP2, have been found to dephosphorylate the AR (Chen et al. 2009; Ikonen et al. 1994). Despite intense research on AR

phosphorylation, there is scanty information of the role of the phosphorylation in the regulation of AR target genes. However, for example, phosphorylation of AR S308 by CDK5 has been recently demonstrated to result in differential expression of AR target genes, including several growth-priming TF genes (Lindqvist et al. 2015).

The AR has been reported to be acetylated in three lysines located in the hinge region in the motif 630-KLKK-633 partly overlapping with the major nuclear localization signal of the receptor. Acetyltransferases p300 and p300/CBP, Tat-interacting protein 60 kDa, and N-acetyltransferase arrest-defect 1 protein can directly acetylate AR and thereby increase its activity (Coffey and Robson 2012). Conversely, HDAC1 is able to deacetylate AR and suppress its activity. The acetylation may modulate the AR's transcriptional activity by favoring its nuclear translocation and by shifting the balance between coactivator and corepressor binding. The modification may affect the capability of the AR to regulate cellular growth and apoptosis and is thus potentially physiologically important. Interestingly, the AR hinge region lysines K630 and K632 are also prone to methylation by SET domain-containing protein 9 (SET9) (Ko et al. 2011). SET9 seems to also coactivate the AR by facilitating the AR N/C interaction. The demethylase(s) targeting the AR have not yet been reported.

Ubiquitination, conjugation of ubiquitin to lysine residues, is an essential mechanism to control the turnover of proteins, but the versatile modification also affects signaling pathways without promoting proteins to degradation. AR, like other SRs, seems to be degraded via the ubiquitin-proteasome system. All ubiquitinated lysines in the AR have not yet been definitely established, but at least K845 and K847 in the LBD represent two conserved ubiquitin acceptors. Ubiquitin E3 ligases mouse double minute 2 homolog (MDM2), C-terminus of HSP70-interacting protein (CHIP), and ring finger protein (RNF6) have been reported to promote the ubiquitination of the AR (Chymkowitch et al. 2011; Xu et al. 2009). Interestingly, RNF6-induced AR K6/27 ubiquitination enhances the transcriptional activity of the receptor, while MDM2- or CHIP-mediated ubiquitination promotes the AR degradation without influencing its transcriptional activity (Xu et al. 2009).

The AR was the first NR shown to be modified by small ubiquitin-related modifier (SUMO), i.e., SUMOylated (Knutson et al. 2012; Poukka et al. 2000; Tian et al. 2002; Treuter and Venteclef 2011). The SUMOylation pathway is analogous to ubiquitination, but it does not generally promote protein degradation but regulates protein-protein interactions, and cells possess distinct machinery for the modification. AR SUMOylation sites, K386 and K520, are located in the NTD which is the domain harboring the SUMOylation sites also in GR and PR (Tian et al. 2002; Treuter and Venteclef 2011; Knutson et al. 2012). Agonist-induced conformation of the AR favors receptor SUMOylation (Kaikkonen et al. 2009; Rytinki et al. 2012). Due to the rapid turnover and highly dynamic nature of SUMOylation, the steady-state level of endogenous AR SUMO conjugates is very low, but cell stress, such as heat shock or heavy metal exposure, dramatically augments AR SUMOylation (Kaikkonen et al. 2013b; Rytinki et al. 2012). The modification modulates the transcriptional activity of AR in a target gene- and pathway-selective fashion (Poukka et al. 2000; Sutinen et al. 2014a; Treuter and Venteclef 2011).

SUMOylation is likely to affect the AR target gene selection via regulating the receptor's interactions with other TFs, including FOXA1. Interestingly, SUMOylation also regulates the transcriptional activity and chromatin occupancy of FOXA1 (Sutinen et al. 2014b), and components of the SUMOylation machinery function as coregulators for AR (Kotaja et al. 2000; Kaikkonen et al. 2009). Notably, PIAS1 acts as a chromatin-bound AR coregulator that also interacts with the FOXA1, functioning either as a coactivator or a corepressor in an AR target gene-selective fashion (Toropainen et al. 2015).

Summary

The AR is mandatory for the development and maintenance of male reproductive tissues and secondary sexual characteristics. The receptor is also expressed outside the reproductive tissues, and it has important biological actions also the immune, neural, cardiovascular, and hemopoietic systems both in health and disease. Testosterone and 5α-dihydrotestosterone bind to the AR with a strong affinity at the low nanomolar range, which drives the androgen-AR dimer complex into the nucleus to the regulatory regions of genes, mostly to distal enhancers, to modulate, either activate or repress, transcription. Recent genome-wide methods have revealed a coordinated network of transcriptional changes orchestrated by the AR together with coregulator proteins and other sequence-specific TFs in prostate cancer cells. However, there is still scanty genome-wide information of AR's chromatin occupancy and target gene programs in normal androgen target tissues in vivo. It is therefore essential to expand the systematic genome-wide analyses to male reproductive tissues. In this way, it will be possible to reveal the true, in vivo "coregulator and pioneer TF code" that defines the AR function in a cell- and tissue-specific fashion. A better understanding of the tissue-specific mechanisms of AR function should also contribute to the development of improved treatments for androgen-linked diseases.

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