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Molecular action of androgens

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Abstract

The androgen receptor (AR), which mediates androgen action in the cell, belongs to the superfamily of nuclear receptors, a large group of transcription factors. Recent studies have described how the AR acts on specific target genes. The receptor's specificity of action depends on its regulation at different levels: expression in the cells, ligand binding and DNA-specific sequence recognition by structurally conserved domains and regulation by transcriptional factors in an integrated response. We propose, here, an overview of recent works on the molecular regulation of androgen-dependent genes by AR.

Keywords: Androgen receptor; Transcription factor; Transactivation regulation; Structure; Cofactor

1. Introduction

Androgens play a crucial role in the development, maintenance, and regulation of male phenotype and reproductive physiology (Quigley et al., 1995). Androgen action is mediated by the androgen receptor (AR), a ligand-dependent transcriptional factor. AR belongs to the steroid/nuclear receptor (NR) superfamily (McKenna et al., 1999), a large group of transcription factors whose members share basic structural and functional homology. It mediates the effects of testosterone and dihydro-testosterone (DHT) in cells. The binding of these hydrophobic ligands induces AR to assume a configuration that leads to transcriptional activation (or inhibition) and allows transmission of extracellular signals into intracellular responses by targeting promoter response elements and recruiting cofactors.

AR shares a modular structure with other NRs and its structural organization shows a high degree of molecular identity with the other superfamily members. Clearly separated domains have been characterized (See Fig. 1). The variable N-terminal domain (NTD) is implicated in transcriptional activation and may participate in cofactor recruitment. NTD accounts for most of the AR-dependent transcription activation. It contains the transcriptional activation region AF-1 (aa 51–211), which is essential for transactivation activity in AR, and AF-5 (aa 370–494), which shows strong constitutive activity in the ligand-binding domain (LBD) deletion mutant AR (1–660) (Brinkmann et al., 1995). The highly conserved zinc finger-type DNA-binding domain (DBD) mediates the interaction with DNA response element. The LBD contains the strictly ligand-dependent transcriptional activation region, activation function domain 2 (AF-2) and functionally interacts with intermediary factors and nuclear cofactors (Berrevoets et al., 1998). Recent studies suggest that interaction between NTD and LBD results in the formation of new surfaces that facilitate interaction with NR cofactors (Müller et al., 2000).

2. The AR gene

The AR gene is localized on the X chromosome in position q11–q12 and is approximately 90 kb length. There are eight exons on the open reading frame. More

Abbreviations: AF-2, activation function domain 2; AR, androgen receptor; DBD, DNA-binding domain; DHT, dihydro-testosterone; FHL2, four-and-a-half lim-only protein 2; LBD, ligand-binding domain; NTD, N-terminal domain; SHP, short heterodimer partner.

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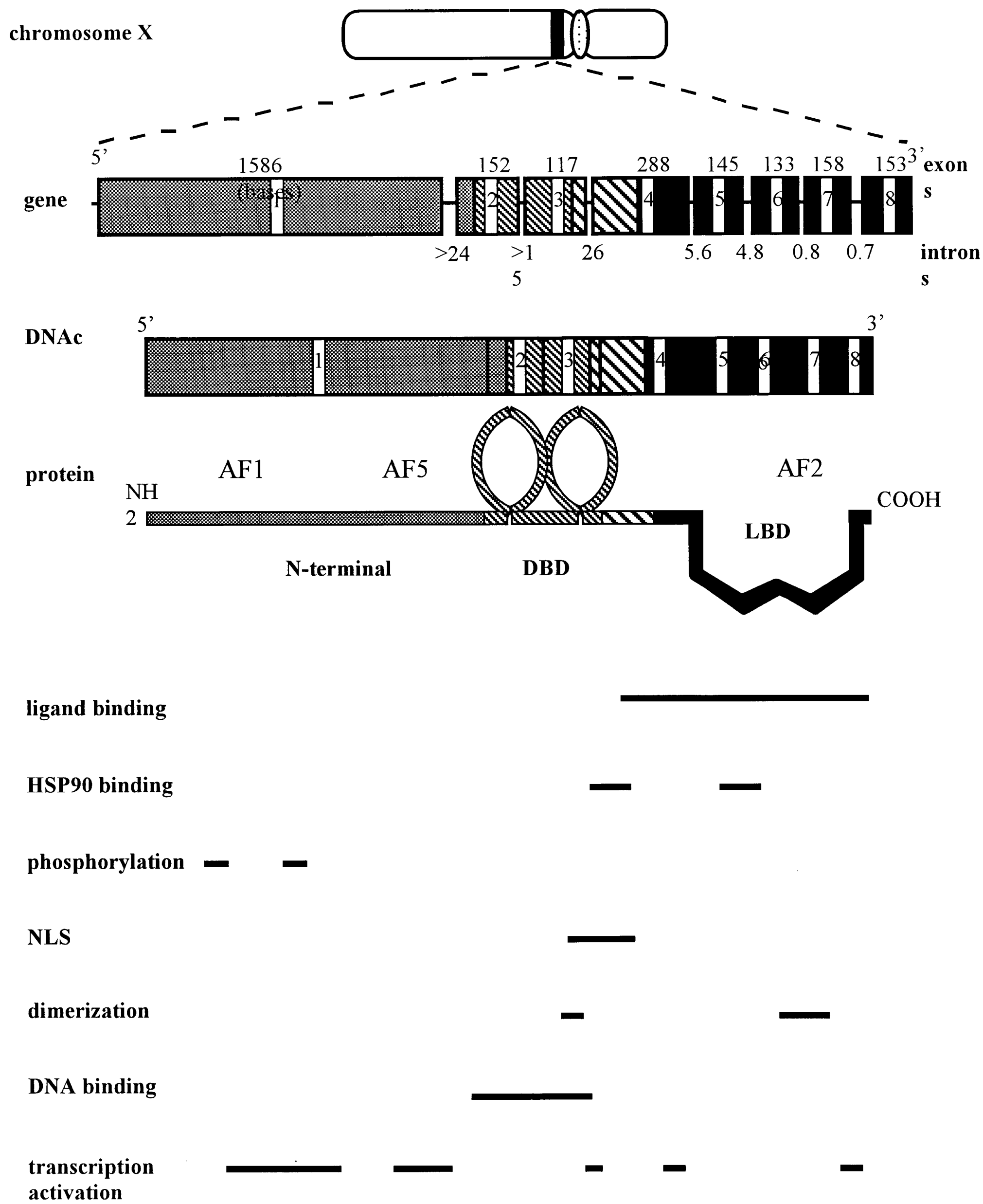

Fig. 1. Genetic and functional organization of AR.

than one RNA subtype has been described in eucaryote organisms (see Fig. 1). AR is expressed in the majority of human tissues and expression levels may differ by a factor of 2 or 3 between tissues. Development and aging can drastically influence AR expression levels in the same cell (Supakar et al., 1993). The AR promoter presents no consensus TATA and CAAT sequences, but it shows an AMPc response element and a large number of transcription factor response elements like SRY, SF1, estrogen receptor (ER), NFKB and AR itself. Moreover, two AR response elements (ARE) have been localized in the gene's fifth exon (Dai and Burnstein, 1996) and biphasic regulation of the AR mRNA by androgens has been described (Nirdé et al., 1998).

3. Ligand binding

Proteolysis assays first indicated the existence of NR sub-domains. The crystal structure of several nuclear receptor LBDs in unliganded and liganded (hormone or antihormone) forms have now been determined, revealing striking conservation despite modest sequence homology (Bourguet et al., 1995; Brzozowski et al., 1997; Egea et al., 2000; Matias et al., 2000; Renaud et al., 1995; Rochel et al., 2000; Sack et al., 2001; Tanenbaum et al., 1998; Uppenberg et al., 1998). This has contributed to a better understanding of NR activation. These crystal structures reveal a triple-layered antiparallel α-helical sandwich fold, composed of one β-Sheet (S1/S2) and 12 α-helixes (H1–H12), with the major difference between crystals being the folding back of H12 toward the LBD core. H4, 5, 6, 8, 9 are localized between H1, 2, 3 on one side and H7, 10, 11 on the other side.

These studies revealed a strong conservation of the ligand-binding pocket localized inside the protein, without easy access. It is principally composed of residues from H3, H5, loop L7–8, H8, H11, H12 and S1/S2. Two major types of contacts mediate ligand recognition: hydrogen binding for its extremities and hydrophobic van der Waals binding along its body. This hydrophobic content shows a clear complementation with the apolar properties of the ligands and the few polar residues ensure anchoring and selectivity. As shown on Fig. 2, the A-ring of DHT interacts with the side chains of Q711 and R752 in AR LBD and T 877 contributes to the discrimination of 17β substituents on the ligand D-ring together with N 705 (Matias et al., 2000; Poujol et al., 2000; Sack et al., 2001).

A particular motif of LBD, called AF2-AD, is clearly implicated in NR activation. This was first shown by directed mutagenesis. Comparison of the apo and holo structures of retinoid receptors (RARγ and RXRα) revealed that H12, which contains this motif, has a different position in the presence or absence of agonist ligand. Comparison of the LBD crystal structure of the human RARγ bound to all-*trans* retinoic acid and the RXRα apo-LBD revealed that they are similar except for the carboxy terminal part. It folds back toward the LBD core that contributes to the hydrophobic agonist ligand pocket. Renaud et al. proposed a 'mouse trap' mechanism whereby the agonist ligand binding induces a conformational transition of the amphipathic H12 and forms a transcriptionally active receptor (Renaud et al., 1995). This holo position of H12 allows the receptors to interact with nuclear cofactors.

The molecular basis for pure antagonism resides in the inability of H12 to adopt the holo position and, therefore, to contribute to the LBD–coactivator interface. Many crystals of NR-antagonist complexes have been isolated, ERa/RAL (Brzozowski et al., 1997); ERa/OHT (Shiau et al., 1998); ERb/RAL, ERb/genistein (Pike et al., 1999); and RARa/BMS614 (Bourguet et al., 2000). It is important to note that the position of H12 observed in the different crystallized NR-antagonist

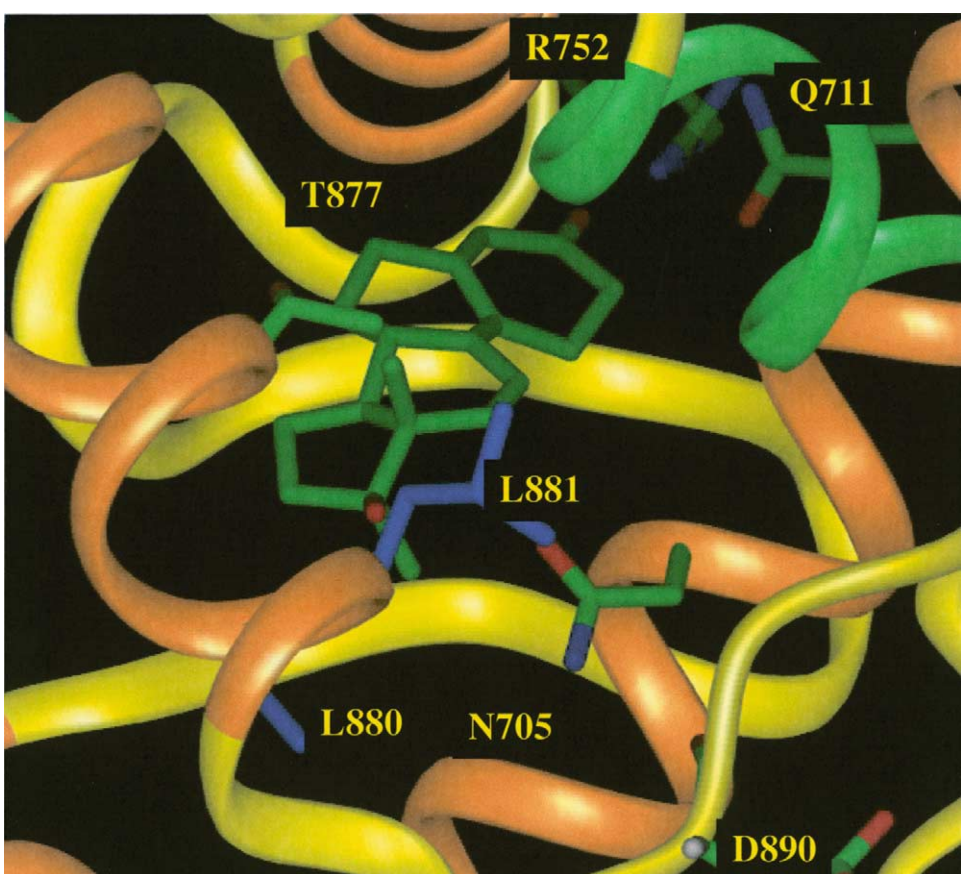

Fig. 2. 3D modeling LBD analysis of DHT bound in the AR ligand-binding pocket.

complexes need not be unique. The binding of other antagonists may surely result in different H12 apo-localizations.

For the partial antagonists, the equilibrium between the agonist holo position of H12 and its antagonist position may depend on the cellular context, particularly the local concentrations of cofactors. Thus, the ligand may act as either agonist or antagonist depending on this context. A pure antagonist would permanently preclude the holo conformation, thus locating H12 in an apo position, whereas a partial antagonist would still allow both apo and holo situations. In this case, coactivators could shift the equilibrium by stabilizing the active holo form with ligand action resulting in an agonist activation of the NR (Bourguet et al., 2000).

A recent study demonstrated the implication of the last 13 amino acids of AR in ligand binding. Mutagenesis and proteolysis provided evidence that these amino acids participate in the active conformation of the receptor, especially both hydrophobic and aromatic functions on position 916 (Tahiri et al., 2001).

4. Activation and translocation

Anchoring of ligand in the binding pocket is the first step in AR activation. This allows the cytoplasmic AR to release heat shock proteins (HSP) and become an active and nuclear transcription factor, capable of interaction with DNA on specific response elements. In the absence of ligand, analysis of cell extracts situates AR in a large heterocomplex having a molecular weight of 250–300 kDa and a sedimentation coefficient of approximately 10 S. In the presence of ligand, dissociation of this complex is observed. The AR sedimentation coefficient belongs to 4 or 5 S, and the receptor is able to bind with DNA. Many proteins of this complex have already been identified: HSP90, HSP70 and FKBP52 (Veldscholte et al., 1992).

HSP90 binds directly to AR LBD and maintains AR in a conformation able to bind hormone. Cytoplasmic HSP70 binds early to proteins when they are partially synthesized and participates in oligomer assembly. Monomeric HSP70 binds directly to AR LBD, participating in the 10 S complex. The FKBP52 protein does not interact directly with AR but with HSP90 in complexes with AR, ER, glucocorticoid receptor (GR) and progesterone receptor (PR).

After ligand binding, HSPs dissociate from AR, thereby allowing the receptor to change in a more compact conformation able to bind DNA, as demonstrated by proteolysis experiments. The HSP dissociation rate corresponds to the rate of AR appearance in compact form. HSP release unmasks the dimerization motifs and the nuclear location signal (NLS) site of the receptor that allows translocation to the nucleus. Moreover, proteolysis has demonstrated different conformations of AR in the presence of agonist or antagonist ligand (Kuil and Mulder, 1995).

Androgen binding promotes AR hyperphosphorylation by a process common to NRs and other transcription factors. When inactivated, AR is already phosphorylated, but new sites are targeted when AR is activated (Kuiper and Brinkmann, 1995). The role of these phosphorylations remains unclear. Studies have demonstrated serine-specific phosphorylations of the AF-1 activation domain of ERβ (Tremblay et al., 1999) and SF-1 (Hammer et al., 1999) by map kinases (MAPK), inducing coactivator recruitment and transcriptional activation. Moreover, a recent study demonstrated that phosphorylation on serine 260 on the RXR receptor could be implicated in hepatocarcinogenesis (Adachi et al., 2002). But the specificity and the physiological relevance of these phosphorylations are unclear and remain hypothetical.

AR translocates to the nucleus (Georget et al., 1997). The nuclear transport is selective, active and needs one or more NLSs. The first step, binding to nuclear pores, does not require energy. The second step, however, requires ATP to pass through the pores. This step is relayed by a receptor participating in a transport cycle between cytoplasm and nucleus (for review see Gorlich, 1998).

5. The DNA-binding domain

To mediate hormonal effects, the receptor needs to interact with short specific DNA sequences, called hormone responsive elements (HRE), that are localized in the promoter region of the genes. To identify an AR-specific sequence (ARE), Roche et al. used a bank of nucleotides and a protein corresponding to the AR DBD only (Roche et al., 1992). The ARE selected has the consensus sequence: GG(A/T)ACAnnnTGTTCT, which is very close to the GR consensus sequence. When an ARE is detected, a GRE is also detected nearby, suggesting that GR, AR, PR and MR interact with the same sequences. For some genes, however, like mouse vas deferens protein, secretory component, and others, AR efficiency is better than that of GR (Fabre et al., 1994; Haelens et al., 1999). Recent studies have shown that in response elements of the 'mouse sex-limited enhancer' and the 'human secretory component androgen response unit', specific point mutations in their left half-sites strongly enhance GR sensitivity. Moreover, they demonstrated that in some cases recognition of nonconventional ARE is important for androgen specificity (Schoenmakers et al., 2000; Verrijdt et al., 2000). The question of how cells expressing both receptors have specificity in gene expression and regulation has not yet been fully answered.

Most of our understanding of the NR DBD is a result of studies on GR, ER and RXR. Eight of the nine cysteines composing the DBD are implicated in the formation of zinc fingers, composed by tetraedric binding coordinated by zinc ions (Freedman, 1992). All these structures show conserved tridimensional organization in agreement with the primary sequence of the receptors. Using the data acquired on the GR DBD, we designed a model of the AR DBD. We observe two monomers bound to two half-sites in a head to head fashion. The P-box (G577SCKV581) presented to the half-site determines the orientation and the binding specificity. A recent study demonstrated that a point mutation of the AR P-box associated with partial androgen insensitivity syndrome (PAIS) resulted clearly in altered selectivity of DNA response element recognition (Nguyen et al., 2001).

Working both clinically, in silico, and in vitro helped us to determine the implication of residues V581 and R585 in DNA interaction and to specify the structural consequences of these residue substitutions (Lobaccaro et al., 1996). A similar approach to the residue substitutions R607Q and R608K, identified in PAIS and in male breast cancer, demonstrated a potential interaction surface at the top of the second zinc finger (Poujol et al., 1997).

It is widely acknowledged that steroid receptors bind DNA in a homodimeric manner. This is explained by the formation of a saline bridge between two residues strictly conserved in AR, PR, MR and GR. Heterodimerization in AR and GR has been described (Chen et al., 1997) without any functional relevance. There is no comparison with receptors (RAR, TR, VDR, PPAR) that heterodimerize functionally with RXR.

Moreover, the NR DBD seems to be implicated in NR shuttling. A recent study showed the implication of the DBD in nucleocytoplasmic shuttling of NRs. The authors demonstrated that the AR DBD and nine other NR DBDs contain a non-classical nuclear export signal (NES) sufficient and necessary for NR export. This phenomenon is saturable and prolonged nuclear localization of the GR increases transactivation. They proposed a model of NR cycle shuttling, in which NLS and NES or DBD-dependent export participate in the compartmentalization of the receptors (Black et al., 2001).

6. Transcription regulation

After binding to ARE, AR is able to recruit all the compounds of the transcriptional machinery, particularly RNA polymerase II (RNAP II) in mammalian cells. DNA is not always in its accessible uncondensed form and, in these cases, it is necessary to recruit nuclear transcriptional factors that interact with nuclear receptors. Their enzymatic activities (for example, acetylases) induce local chromatin decompaction that allows access to RNAP II and also allows the general transcriptional machinery to access to the target gene promoter (for review see Orphanides and Reinberg, 2002). These coregulators form a bridge between the transcriptional machinery and the receptors modulating transcription.

Direct recruitment of the transcriptional machinery by AR was shown to involve the interaction between the AR N-terminus, TFIIF compound and the TATA box-binding protein (TBP; McEwan and Gustafsson, 1997). In fact, indirect recruitment mediated by intermediary factors called cofactors was described.

Two-hybrid and far-western techniques led scientists to isolate NR-interacting nuclear factors. Many of them interact with AR and regulate its activity (cf. Table 1). In fact, a balance between tissue- and cell-dependent cofactors might allow a more specific response to a particular hormone. Multiprotein complexes containing these cofactors facilitate the access of NR and transcriptional machinery to their target genes (see Fig. 3). A large part of these cofactors interact with both NR and other transcription factors (Xu et al., 1999). Among these proteins, classes have been described, including the p160 family, CBP/p300, the PIAS family, the activator complexes SWI/SNF/BRG and TRAP/DRIP (Glass and

Table 1
Factors modulating AR transcriptional activity

Activators	Inhibitors
ARA70 (Yeh and Chang, 1996)	ARIP3 (Moilanen et al., 1999)
SRC1 (Ikonen et al., 1997)	TR4 (Lee et al., 1999)
TIF2 (Berrevoets et al., 1998)	HBO1 (Sharma et al., 2000)
CBP (Frønsdal et al., 1998)	Akt (Lin et al., 2001)
SNURF (Moilanen et al., 1998b)	SMAD3 (Hayes et al., 2001)
ANPK (Moilanen et al., 1998a)	AES (Yu et al., 2001)
Rb (Yeh et al., 1998)	PAK6 (Yang et al., 2001)
BAG-1L (Froesch et al., 1998)	SHP (Gobinet et al., 2001)
Tip60 (Brady et al., 1999)	PIASγ (Gross et al., 2001)
Ubc9 (Poukka et al., 1999)	Cycline D1 (Petre et al., 2002)
ARA54 (Kang et al., 1999)	DAX-1 (Holter et al., 2002)
ARA55 (Fujimoto et al., 1999)	
ARA24/Ran (Hsiao et al., 1999)	
ARA160 (Hsiao and Chang, 1999)	
CARM1 (Chen et al., 1999)	
Cycline E (Yamamoto et al., 2000)	
PIAS1 (Tan et al., 2000)	
FHL2 (Müller et al., 2000)	
TRAM-1 (Tan et al., 2000)	
BRCA1 (Park et al., 2000)	
CAK (Lee et al., 2000)	
Miz1 (Kotaja et al., 2000)	
Caveolin-1 (Lu et al., 2001)	
DJ-1 (Takahashi et al., 2001)	
ARA267-α (Wang et al., 2001)	
PIAS3 (Gross et al., 2001)	
GT198 (Ko et al., 2002)	
ART-27 (Markus et al., 2002)	

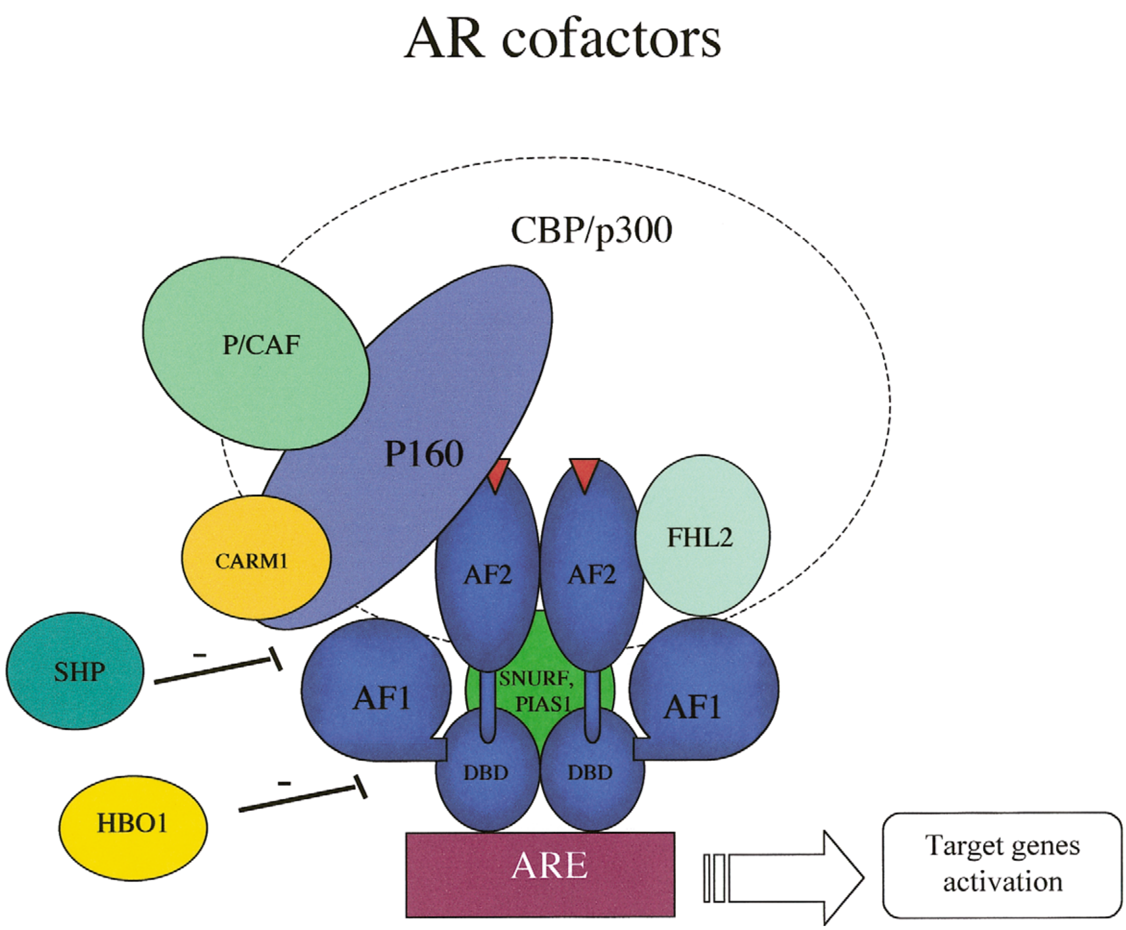

Fig. 3. Schematic organization of nuclear cofactor recruited by AR for gene activation.

Rosenfeld, 2000) and the inhibitor complexes NCoR/Sin3/RPD3 and NuRD (Ng and Bird, 2000). Each of these complexes contains proteins with various activities, acetylase/deacetylase; methylase; and kinase/phosphatase. Thus the transcription level in AR target genes could depend on the cell equipment in cofactor able to regulate AR activity. In fact, because of the large number of transcriptional cofactors already identified (Robyr et al., 2000), it is difficult to understand their true physiological relevance.

Among the coactivators identified for the AR, four-and-a-half lim-only protein 2 (FHL2) seems to be the unique AR-specific coactivator. It does not interact with other steroid receptors, and its expression pattern corresponds to androgen-regulated tissues (Müller et al., 2000). Coactivators like PIAS1 (protein inhibitor of activated STAT-1) and PIAS3 (protein inhibitor of activated STAT-3) seem to be good candidates for a physiological regulation of the AR-dependent transactivation because of their expression pattern, even though they do not show a particular specificity for AR (Gross et al., 2001; Tan et al., 2000).

Some NRs, like RXR, which are not sequestrated in the cytoplasm and bound to DNA in the absence of ligand, can recruit proteins and complexes with a strong inhibitory activity. They condense DNA by deacetylation, which leads to transcription repression. In the presence of agonist ligand, another complex is recruited, displacing the first inhibitory complex. This activator, multiproteic Core, has an acetylase activity that promotes DNA decompaction. AR bound to an agonist ligand, however, is able to recruit inhibitory proteins. These modulate AR activity in a different manner, by competing with coactivators, recruiting histone deacetylase complex, tethering AR in the cytoplasm or reducing AR DNA binding.

We worked on one of these coinhibitors, short heterodimer partner (SHP; Johansson et al., 1999; Seol et al., 1998). This orphan receptor (for review see Giguere, 1999) belongs to the NR superfamily, and its closest relative is DAX-1, another orphan receptor described as inhibiting AR by tethering it in the cytoplasm (Holter et al., 2002). We demonstrated that SHP inhibits AR transactivation and interacts directly with AR in a ligand-dependent manner. We hypothesized that SHP inhibits AR by both competing with coactivators and recruiting strong inhibitory proteins (Gobinet et al., 2001). A better understanding of the

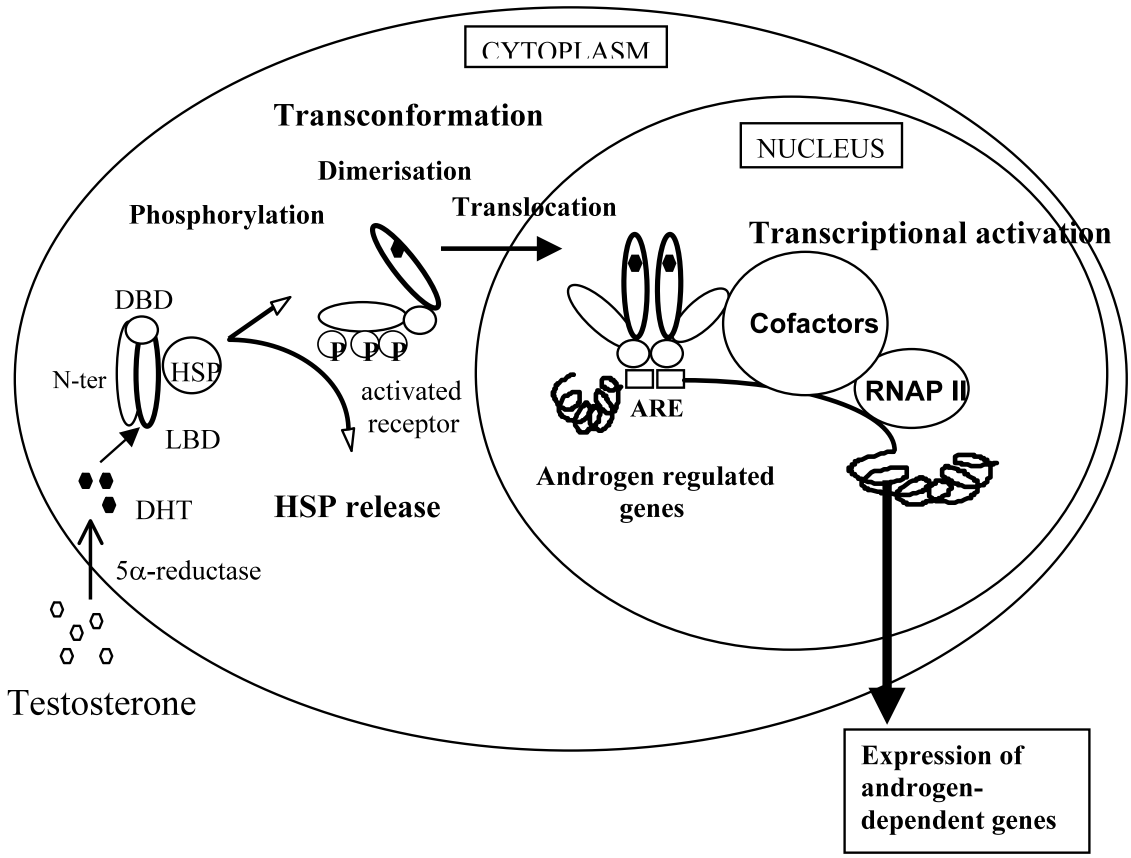

Fig. 4. General mechanism of androgen action in the cell, from the ligand to the genes.

inhibitory mechanisms of AR activity would improve our knowledge of AR misregulations.

Fig. 4 presents a synthetic scheme of AR activation mechanisms from ligand binding to gene regulation. Our understanding of the specificity and the physiological relevance of all these mechanisms remains incomplete. Numerous factors interact with NRs and are implicated in other transduction pathways of the cell. Integration of all these data into a group of inter-dependent mechanisms will undoubtedly improve our understanding of how genes are regulated. It is exciting to observe the specificity required at each step of gene expression. We expect that in the coming years new insight into molecular mechanisms will shed light on these events.

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