

# Androgen Receptor Corepressors: An Overview

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Androgens play pivotal roles in sex differentiation and development, in reproductive functions, and sexual behavior. The actions of androgens are mediated through the intracellular androgen receptor (AR), a member of the nuclear receptor (NR) superfamily, which regulates a wide range of target gene expression. Recent studies indicate that the proper transcriptional activity of AR is modulated by AR coregulators, including coactivators that can enhance AR transactivation and corepressors that can suppress AR transactivation. Here, we summarize the recent discoveries relating to AR corepressor function with the following different mechanisms: (1) corepressors that inhibit the DNA binding or nuclear translocation of AR; (2) corepressors that recruit histone deacetylases; (3) corepressors that interrupt the interaction between AR and its coactivators; (4) corepressors that interrupt the interaction between the N-terminus and C-terminus of AR; (5) corepressors that function as scaffolds for other AR coregulators; (6) corepressors that target the basal transcriptional machinery; (7) other mechanisms. The potential impact and future directions of AR corepressors are also discussed.

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## INTRODUCTION

Androgens are indispensable for the development, regulation, and maintenance of male phenotype and reproductive physiology [1–5]. In addition, androgens play a crucial role in the normal development and function of female reproductive tissues. The effects of androgen are mediated by the androgen receptor (AR), a ligand-dependent transcriptional factor and member of the nuclear receptor (NR) superfamily [4,6–8]. The binding of hydrophobic ligands, such as testosterone (T) and 5 $\alpha$ -dihydrotestosterone (DHT), changes the conformation of AR, alters the transcriptional activity of AR, and regulates expression of its target genes [3].

AR shares a characteristic structure with other NRs: the variable NH<sub>2</sub>-terminal transactivation domain (NTD) possessing activation function domain 1 (AF-1), the highly conserved zinc finger-type DNA-binding domain (DBD), the hinge region, and the ligand binding domain (LBD) containing activation function domain 2 (AF-2) [9,10]. AF-1 functions in a ligand-independent manner while the activity of AF-2 requires cognate ligand binding. Recent studies suggest that interaction between NTD and LBD is necessary for the activation of AR [11–13].

After binding to androgen, AR is able to recruit general transcription factors to its target gene promoters. In addition to its direct interaction with several factors of the general transcriptional machinery, it has become clear that the transcriptional activity of AR is regulated by coregulators, including both coactivators and corepressors, by various mechanisms [14–16]. Coactivators are factors that can directly interact with AR and enhance its transcriptional activity. On the other hand, corepressors are factors that associate with AR and repress its transcriptional activity. Both types of coregulators are necessary for efficient modulation of AR target gene transcription. Therefore, mutations or changes in expression of coregulators may lead to the alteration of AR activity and hence result in disorders of

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Received 26 July 2004; Accepted 9 August 2004

DOI 10.1002/pros.20170

AR target tissues. To date, yeast two-hybrid system, Far-Western assay, and other techniques have led scientists to identify many AR coregulators, many of which contain multiple, distinct enzyme activities, such as kinases, ATPases, acetylases, deacetylases, and proteases (reviewed in [17]). Though most of the AR coregulators identified are coactivators, it is conceivable that AR corepressors also play critical roles in regulating AR activity precisely and efficiently. This review will summarize our current knowledge of the cellular and molecular biology of AR corepressors as shown in Table I. However, it is important to note that neither the relative importance of many of the identified AR corepressors in intact animal models nor their binding to the promoters of AR target genes in vivo has yet been examined, and therefore, their true physiological relevance in normal and pathological conditions in AR signaling remains to be established.

### **CLASSIFICATION OF AR COREPRESSORS BASED ON THEIR SUPPRESSION MECHANISMS**

#### **Corepressors That Inhibit the DNA Binding or Nuclear Translocation of AR**

In the absence of androgen, AR is localized in the cytoplasm and associated with heat shock proteins. Upon stimulation by agonists, including T, DHT, and other agonists, AR disassociates from heat shock proteins, translocates into the nucleus, and binds to its target gene promoters. A number of AR corepressors have been shown to affect AR transactivation through influencing the above steps.

#### **Calreticulin**

Calreticulin is a calcium binding protein present primarily in the lumen of the endoplasmic reticulum [18]. However, it can also localize to the cytoplasm adjacent to the cell membrane, and to the nucleus. Calreticulin can bind to an amino-acid sequence motif, KXGFFKR, located in the cytoplasmic domains of all integrin alpha-subunits. The amino-acid sequence, KVFFKR, is also present in the AR DBD and mediates the binding of AR to calreticulin [19]. Calreticulin association with the AR DBD inhibits AR binding to its DNA response elements and therefore reduces AR transactivation, as well as that of glucocorticoid and retinoic acid receptors (RAR) [19,20]. The vitamin D receptor (VDR) DBD contains the related motif KGFFRR, and its activity is also suppressed by calreticulin in a similar manner [21]. Interestingly, calreticulin has been identified as an androgen-responsive gene in the prostate, suggesting a feedback loop control [22]. As calreticulin is a high capacity intracellular  $Ca^{2+}$  binding protein, the observation that androgen

regulates the sensitivity of LNCaP cells to  $Ca^{2+}$  ionophore A23187-induced apoptosis indicates that calreticulin might mediate androgen regulation of this sensitivity [23]. Further characterization of the roles of calreticulin in prostate cancers may shed more light on the physiological function of calreticulin as an AR corepressor.

#### **ARA67/PAT1 (Protein Interacting With Amyloid Precursor Protein Tail I)**

In a recent study by our group, a novel AR corepressor, ARA67/PAT1, was characterized to suppress the transcriptional activity of AR [24]. ARA67/PAT1 has a tissue-specific expression pattern, and it is highly expressed in heart, placenta, and skeletal muscle. Studies have demonstrated that ARA67/PAT1 can bind microtubules and is involved in amyloid precursor protein (APP) secretion [25]. Although ARA67/PAT1 was initially isolated out using the AR N-terminal region as bait, ARA67/PAT1 interacts with multiple regions of the AR [24]. The interaction is strong in the N-terminus, moderate in the LBD, and weak in the DBD of AR. Furthermore, overexpression of ARA67/PAT1 can enhance the interaction between the AR N-terminus and C terminus (N/C interaction). ARA67/PAT1 promotes a cytoplasmic retention of AR in the presence of androgen, indicating that it may play a role in AR trafficking, correlating with its function in APP trafficking/processing. However, the role of this AR corepressor in prostate cancers needs to be further explored.

#### **Dosage-Sensitive Sex Reversal Adrenal Hypoplasia Congenita Critical Region on the X Chromosome Gene I (DAX-1)**

DAX-1 lacks the zinc-finger DNA-binding domain typical of most nuclear receptors [26]. Thus, it is an atypical member of the nuclear receptor family that is predominantly expressed in mammalian reproductive tissues [27,28]. Direct interactions between DAX-1 and AR involve the N-terminal repeat domain of DAX-1 and the C-terminal LBD of AR [29]. Although DAX-1 may inhibit AR transactivation at multiple levels, it is interesting to note that DAX-1 can sequester AR in the cytoplasm, indicating a possible function of DAX-1 as a cytoplasmic retention factor [29]. Finally, DAX-1 expression is strongly reduced in benign prostatic hyperplasia, suggesting that DAX-1 plays a role in limiting AR activity in the prostate [30].

#### **p21-Activated Kinase 6 (PAK6)**

PAK6 is a 75 kDa protein that contains a putative N-terminal Cdc42/Rac interactive binding motif and a

TABLE I. AR Corepressors

Corepressors	Region	Comments	Selected references
AES	LBD	While AES has no effect on TR- or ER-dependent transcription, AES specifically inhibits AR-dependent gene expression in TSA-insensitive manner	[100]
Akt	<sup>a</sup>	Akt phosphorylates AR and represses the binding of AR coactivator ARA70	[66]
ARA67/PAT1	Strong in the N-terminus, moderate in the LBD, and weak in the DBD	ARA67/PAT1 promotes a cytoplasmic retention of AR in the presence of androgen and reduces AR transactivation	[24]
ARR19	DBD	ARR19 co-translocates into the nucleus with AR and suppresses AR activity through the recruitment of histone deacetylase 4 (HDAC4)	[49]
Calreticulin	DBD	Calreticulin association with the AR DBD inhibits AR binding to its DNA response elements and reduces AR transactivation, as well as that of glucocorticoid, vitamin D, and retinoic acid receptors	[19–21]
Cyclin D1	The hinge region of AR	Although cyclin D1 enhances estrogen-responsive transcription, cyclin D1 suppresses the transactivation of AR, STAT3, and TR, cyclin D1 inhibition of AR may result from its capacity to inhibit the association between coactivator P/CAF and the AR	[72–74]
DAX-1	LBD	DAX-1 can sequester AR in the cytoplasm, indicating a possible function of DAX-1 expression is strongly reduced in benign prostatic hyperplasia	[29,30]
DJBP	DBD	The repression of AR activity by DJBP is mediated by recruitment of the corepressor complex containing HDAC1 and mSin3A	[50]
FLNa	The hinge region of AR	While FLNa has no effect on ER, PR, or GR, it exerts an inhibitory effect on AR through disruption of the AR N/C interaction and attenuation of interaction between AR and TIF2 by its calpaincleavage fragment	[84]
GSK3 $\beta$	N-terminus	GSK3 $\beta$ phosphorylates the AR N-terminus and suppresses AR transactivation	[90]
HBO1	DBD and LBD	HBO1 specifically inhibits AR transactivation, but not ER or TR transcriptional activity	[102]
HDAC1	DBD-LBD	HDAC1 binds to the AR in vivo and specifically down-regulates AR transcriptional activity without effecting AR protein levels	[45]
hRad9	LBD	The FXXLF motif within the C-terminus of hRad9 interrupts the DHT-induced interaction between the N-terminus and C-terminus of AR and reduces AR transactivation	[85]
NCoR	LBD	NCoR binds to the AR in either the presence or absence of DHT	[59]
PAK6	LBD or hinge region of AR	PAK6 inhibits both AR and estrogen receptor transactivation, active PAK6 inhibited nuclear translocation of the ligand-bound AR	[31–33]
PATZ	<sup>a</sup>	PATZ alone does not influence AR function, but suppresses AR activity in the presence of RNF4, a known AR coactivator	[93]

(Continued)

TABLE I. (Continued)

Corepressors	Region	Comments	Selected references
PTEN	DBD	PTEN suppresses AR activity via a PI3K/Akt-independent pathway in the early passage number of prostate cancer LNCaP cells	[106]
RACK1	LBD	RACK1 binds to the LBD of AR, through its sixth WD40 repeat, in the presence of androgen, promotes the PKC-mediated inhibition of AR	[92]
SHP	N-terminus and COOH-terminus	LXXLL motifs in SHP mediate its interaction with the AR LBD, and the AR N-terminal domain interacts with SHP, stabilizing the SHP-AR interaction, SHP interacts with and represses AR transcriptional activity	[61,64]
Smad4	DBD and LBD	Smad4 decreases AR-Smad3 interaction and inhibits Smad3-enhanced AR transactivation	[79–81]
SMAT	NH <sub>2</sub> -term LBD	SMRT can associate with AR in the absence or presence of agonist/antagonist, in addition to the recruitment of HDACs, SMRT may repress AR transactivation through other mechanism, such as inhibition of AR N/C interaction or competition with AR coactivators	[56–58]
SRY	DBD	SRY interacts directly with the AR DBD in vivo and in vitro and inhibits AR activity	[111]
TGIF	DBD	The transcriptional repression of AR by TGIF is mainly mediated through the HDAC pathway	[48]

<sup>a</sup>Although interaction with AR has been demonstrated, the precise domain of AR that interacts with the coregulator has not yet been determined.

carboxyl-terminal kinase domain [31]. It was first cloned as an AR-interacting protein and is highly expressed in testis and prostate tissues [31]. Interestingly, PAK6 is not stimulated by Cdc42 or Rac, but is stimulated by AR binding [32]. Controversial results have been reported on whether PAK6 binds to the hinge region or LBD of AR, and if it binds in a ligand dependent manner [31,32]. PAK6 inhibits both AR and estrogen receptor (ER) transactivation [31,32]. Interestingly, a recent report showed that active PAK6 inhibited nuclear translocation of the ligand-bound AR providing a possible mechanism for repression of AR activity by PAK6 [33]. The observation that activated PAK6 protein is differentially expressed among different prostate cancer cell lines suggests it may be an important factor in regulation of AR signaling in various forms of prostate cancer [33].

#### Corepressors That Recruit Histone Deacetylases

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play critical roles in altering the acetylation state of core histones, thereby regulating AR-mediated transcription as well as other nuclear

receptor activities [34–36]. Many AR coregulators, such as SRC-1, TIF2, Tip60, and CBP [37–40], possess HAT activities. Moreover, the finding that several transcription factors, such as p53, MyoD, and AR [39,41–43], are targets for direct acetylation and deacetylation suggests that HATs or HDACs may play an active role in regulating transcription factor function.

#### HDAC1

The demonstration that HDACs down-regulate the transcriptional activity of numerous transcription factors implicate deacetylation as a mechanism of transcriptional regulation [44]. It was revealed recently that HDAC1 binds to the AR in vivo and specifically down-regulates AR transcriptional activity without effecting AR protein levels [45]. In contrast, AR activity was not affected in the presence of HDAC5 or HDAC6, whereas co-transfection of HDAC3 enhanced AR activity. Importantly, the demonstration that HDAC1 interacts with and inhibits the inherent activities of the acetylation motif-containing DBD and LBD domains of AR suggests that HDAC1 inhibits both AR and p53 activity in a similar manner [45].

### **TGIF (5'TG3' Interacting Factor)**

TGIF is a homeodomain transcriptional repressor that inhibits retinoid X receptor (RXR) and Smad2 transactivation [46,47]. TGIF represses RXR-mediated transcription by competing with RXR for binding to its target elements. TGIF also interacts with the DBD of AR and selectively represses AR-mediated transcription on the androgen-induced promoters [48]. Although the DBD of AR is responsible for the interaction with TGIF, the transcriptional repression on AR by TGIF is mainly mediated through the HDAC pathway, since this repression is blocked by a HDAC inhibitor trichostatin A (TSA). HDAC-associated Sin3A corepressor directly binds to TGIF and mediates the interaction between HDAC1 and TGIF proteins [48].

### **ARR19 (Androgen Receptor Corepressor, 19 kDa)**

A novel AR corepressor, ARR19, is highly expressed in the murine testis and moderately expressed in other male reproductive organs. ARR19 associates with AR through its N-terminal and leucine zipper-containing regions, and the DBD of AR [49]. Furthermore, ARR19 co-translocates into the nucleus with AR upon androgen exposure. Interestingly, the repressive effect of ARR19 on AR transactivation is shown to occur through the recruitment of HDAC4 by ARR19. The HDAC inhibitor TSA blocked the repression of AR transactivation by ARR19 and the *in vivo* ChIP assay further showed that HDAC4 binds to an androgen regulated-promoter through ARR19. The role of this novel corepressor in cancers of the testis and other male reproductive tissues needs further exploration.

### **DJ-1-Binding Protein (DJBP)**

DJBP was recently characterized as a new AR corepressor specifically expressed in human testis [50]. DJBP binds to the DBD of the AR in a dependent, but not an LXXLL-dependent manner both *in vitro* and *in vivo*. The repression of AR activity by DJBP is mediated by recruitment of the corepressor complex containing HDAC1 and mSin3A. Furthermore, repression activity of DJBP toward the AR can be eliminated by addition of TSA, a specific inhibitor of HDAC, indicating that HDAC is involved in the repressive effect of DJBP on AR.

### **SMRT/NCoR (The Silencing Mediator for Retinoid and Thyroid Hormone Receptors/Nuclear Receptor Corepressor)**

SMRT and the related NCoR are well-characterized corepressors of numerous nuclear receptors [51,52]. In the absence of agonist, SMRT or NCoR interacts with

thyroid hormone receptor (TR) and all-trans retinoic acid receptor (RAR $\alpha$ ). Mutational analysis of the TR LBD has identified an NCoR interacting domain, termed the NCoR box. The evidence that loss of the NCoR box decreases repression by the unliganded TR indicates that interaction with NCoR is indispensable for TR- and RAR $\alpha$ -mediated transcriptional repression. Both NCoR and SMRT recruit HDACs to exert their actions in transcriptional repression. Recent studies reveal SMRT and NCoR interact with antagonist-bound progesterone receptor (PR) and ER to repress transcription [53,54]. Furthermore, SMRT and NCoR can counteract the coactivator effect of L7/SPA and suppress the partial agonist activity of antagonists [55].

Although it is widely speculated that HDACs may be involved in SMRT or NCoR mediated repression of AR transactivation, recent studies have provided new potential mechanisms for these two proteins to regulate AR activity. In contrast to the binding of SMRT to the LBD of TR or RAR $\alpha$ , SMRT has recently been identified as an AR NH<sub>2</sub>-terminal interacting protein in the presence of the androgen R1881, or the antagonist cyproterone acetate (CPA) [56]. However, there is a functional difference between the corepressor SMRT agonist bound AR versus antagonist-bound AR. For example, overexpression of SMRT leads to dramatically enhanced antagonism by CPA, while SMRT only weakly affects the agonist-bound AR. However, in a recent study by Liao et al. [57] SMRT was characterized as a corepressor binding to the LBD of AR. Although the AR LBD domain is sufficient to bind SMRT, the presence of the AR DBD/hinge region plays a critical role in enhancing SMRT binding. These results regarding the exact domain(s) of AR that mediates the SMRT-AR interaction are somewhat controversial. Both studies demonstrate that SMRT can associate with AR in the absence or in the presence of agonist/antagonist. Moreover, similar studies suggest that, in addition to the recruitment of HDACs, SMRT may repress AR transactivation through other mechanisms, such as inhibition of AR N/C interaction or competition with AR coactivators [56–58].

It was demonstrated that NCoR interacts directly with AR, thus repressing AR transactivation [59]. Previously NCoR was found to bind ER $\alpha$ , but only in the presence of antagonists such as 4 hydroxytamoxifen and raloxifene. However, NCoR binds to the AR in either the presence or absence of DHT, a physiological agonist of AR. Similar to SMRT, the mechanism of NCoR-mediated repression of AR activity is somewhat complex. The NCoR C terminus mediates the NCoR-AR interaction and is necessary for repression [59]. In contrast, the association of N-terminal NCoR with HDACs is not necessary for the repression of AR. Thus, NCoR may exert its repressive effect through

preventing coactivator protein binding and/or interfering with the critical AR N/C interaction. However, no reports have ruled out the possibility that the N-terminal repressor domains of NCoR may contribute to repression of AR transactivation of endogenous genes via recruitment of HDACs.

#### **Corepressors That Interrupt the Interaction Between AR and Its Coactivators**

Since androgen levels do not fluctuate dramatically in adult males, the relative levels of coactivators versus corepressors binding to AR may play a critical role in modulating AR function. Several AR corepressors were found to regulate AR activity via inhibiting association of coactivators with AR. Interestingly, the binding of agonist ligands to most steroid receptors causes a conformation change, which provides a high affinity binding site for coactivator proteins containing the LXXLL motif. In contrast, the AR LBD has minimal independent transcriptional activity, and this can be attributed to very weak binding by coactivators, such as SRCs. In addition to the above-mentioned SMRT and NCoR, several AR corepressors have been shown to attenuate AR transcriptional activity through competing for interaction between AR and coactivators.

#### **Short Heterodimer Partner (SHP)**

SHP was first described for its interaction with, and inhibition of, NRs, including RXR and ER [60–63]. Later, SHP was shown to be expressed in androgen target tissues, suggesting a role in the regulation of AR function [61]. Indeed, it was demonstrated that SHP interacts with and represses AR transcriptional activity [61,64]. LXXLL motifs in SHP mediate its interaction with the AR LBD, and the AR N-terminal domain interacts with SHP, stabilizing the SHP–AR interaction. Although SHP interacts with both the N-terminal and C-terminal domains of AR, it inhibits both AR-LBD and N-terminal-dependent transactivation of AR suggesting it does not enhance the AR N/C interaction. Moreover, SHP reverses AR coactivator-mediated activation while overexpression of AR coactivators, including FHL2 and TIF2, counters SHP-mediated inhibition of AR. Therefore, SHP competes with AR coactivators for binding to AR and affects AR activation level. Further characterization of the roles of SHP in AR-signaling pathways might provide new insights into androgen-dependent diseases.

#### **Akt**

Akt, an oncoprotein, is a serine/threonine kinase that plays critical roles in the phosphatidylinositol 3-kinase mediated pathways. Recent reports of Akt/AR

studies have yielded interesting yet controversial results [65–67]. Although the detailed mechanism by which Akt affects AR activity remains unclear, differential effects of Akt on AR may arise from different cell lines or cell conditions [67–69]. Akt can bind to AR and regulate receptor activity as an AR coregulator. It has been reported that Akt phosphorylates AR and represses the binding of AR coactivator ARA70 [66].

#### **Cyclin D1**

Recently, rapid progress has been made in the studies of effects of cyclin D1 on AR function [70–73]. Although cyclin D1 forms a trimeric complex with ER and SRC-1 through an LXXLL motif in its C terminus, and enhances estrogen-responsive transcription, cyclin D1 suppresses the transactivation of AR, STAT3, and TR [73,74]. The C-terminal domain of cyclin D1 and the hinge region of AR mediate AR–cyclin D1 interaction. Furthermore, cyclin D1 inhibition of AR may result from its capacity to inhibit the association between coactivator P/CAF and AR [72]. Its ability to inhibit clinically relevant polymorphisms and mutations of AR suggest that cyclin D1 may play important roles in the development of prostate cancers [73,75].

#### **Smad3/Smad4**

Smad proteins are key components mediating transforming growth factor signaling [76]. Smad3 interacts with both ER and the VDR, enhancing their transactivation [77,78]. In addition, Smad3 has been reported to associate with AR in immunoprecipitation and GST pull-down assays. However, there is conflicting evidence as to whether Smad3 acts as an AR corepressor [79,80] or coactivator [81]. Recent studies suggested that Smad4 may contribute to the effect of Smad3 on AR transactivation [82]. Both Smad3 and Smad4 interact with the AR DBD and LBD [82]. Furthermore, Smad4 decreases AR-Smad3 interaction and inhibits Smad3-enhanced AR transactivation. Therefore, the effect of Smad3/Smad4 on AR transactivation may be influenced by the composition of the Smads-AR-coregulators complex that exists in target gene promoter regions.

#### **Corepressors That Interrupt the Interaction Between the N-Terminus and C-Terminus of AR**

Unlike other steroid receptors, the N-terminal domain of AR is the major transactivation site of AR. Thus, the N/C interaction (interaction between the N-terminus and C-terminus) of AR is critical to reach full AR activation. It is noteworthy that it is difficult to distinguish the blocking of AR N/C interaction from the

competition for AR coactivator binding, as the binding of many coactivators requires AR N/C interaction.

### Filamin A (FLNa)

The LBD activity of AR can be enhanced by deletion of the hinge region, suggesting that association of AR corepressors occurs in this region [40]. FLNa is an actin-binding cytoskeletal protein containing an N-terminal actin-binding domain followed by 24 Ig-like repeats, the last of which represents the self-dimerization domain of the protein [83]. A recent study indicates that FLNa specifically interacts with the hinge region of AR. While FLNa has no effect on ER, PR, or GR, it exerts an inhibitory effect on AR through disruption of the AR N/C interaction and attenuation of interaction between AR and TIF2 by its calpain-cleavage fragment, FLNa (fragment 16–24) [84].

### hRad9

Our group recently identified hRad9, a key member of the checkpoint Rad protein family, as a coregulator that suppresses androgen-AR transactivation in prostate cancer cells [85]. The LBD of AR can interact with the C-terminus of hRad9. The FXXLF motif within the C-terminus of hRad9 interrupts the DHT-induced AR N/C interaction. This interaction between AR and hRad9 then results in the suppression of AR transactivation. Further studies of hRad9 may provide novel insights into the physiological interaction between the DNA damage repair pathways and androgen-regulated pathways.

### Glycogen Synthase Kinase 3 $\beta$ (GSK3 $\beta$ )

GSK3 $\beta$  is a serine/threonine protein kinase that was first described in a metabolic pathway for glycogen synthase regulation [86]. GSK3 $\beta$  phosphorylates a broad range of substrates, including several transcription factors such as c-myc, c-Jun, and the rat GR [87–89]. Our recent data suggested that GSK3 $\beta$  is ubiquitously expressed in prostate cells and interacts with AR *in vivo* and *in vitro* [90]. GSK3 $\beta$  phosphorylates the AR N-terminus and suppresses AR transactivation. AR N/C interaction was reduced with the addition of GSK3 $\beta$ .

### Corepressors That Function as Scaffolds for Other AR Coregulators

Interestingly, recent studies have indicated that some corepressors of AR have no effect on the binding of AR coactivators to the AR. These corepressors can usually form complexes with AR and AR coactivators *in vivo*. It

has been proposed that they may influence AR activity acting as scaffolds for other AR coregulators.

### Receptor for Activated C Kinase-I (RACK1)

RACK1 was first characterized as a PKC-anchoring protein, which determines the localization of activated PKC $\beta$ II [91]. Additionally, RACK1 binds to the AR LBD, through its sixth WD-40 repeat, in the presence of androgen [92]. Although RACK1 is involved in ligand-independent AR nuclear localization, it promotes the PKC-mediated inhibition of AR. RACK1 may act as an adaptor protein to bring PKC in close proximity to AR, thus allowing PKC to modulate AR transcriptional activity. However, the exact roles of this factor in prostate tumorigenesis remain to be determined.

### PATZ

PATZ was recently demonstrated to be a novel coregulator of AR [93]. PATZ alone does not influence AR function, but suppresses AR activity in the presence of RNF4, a known AR coactivator. Its repressive effect on AR is not dependent on histone deacetylases, but is strictly dependent upon association with RNF4. Instead of replacement of RNF4 in the presence of androgen, PATZ forms a ternary complex with AR and RNF4, suggesting that it does not interfere with RNF4 interaction with AR. Therefore, PATZ functions as a novel AR coregulator that acts by modulating the effect of AR coactivator, RNF4. This could represent a more general mechanism to finely tune the androgen response. Further experiments will be necessary to establish the detailed mechanism by which PATZ represses AR-mediated transactivation, and to assess its role in androgen-dependent growth.

### Corepressor That Targets the Basal Transcriptional Machinery

After binding to its target gene promoters, AR is able to recruit the general initiation factors associated with RNA polymerase II. Direct interaction between AR and the transcriptional machinery was demonstrated to involve TFIIF, the TATA box-binding protein, and TFIIF [94,95]. Therefore, it is possible that AR corepressors might block the function of the basal transcriptional machinery to suppress AR transactivation.

### Amino-Terminal Enhancer of Split (AES)

AES is a member of the highly conserved Groucho/TLE (transducin-like enhancer of split) family of corepressors [96,97]. AES is the shortest family member, sharing only the first two N-terminal domains, Q and GP, and lacking the CcN, SP, and WD-40 domains

[98,99]. AES shows direct interaction with full-length AR as well as with the N-terminus of AR. While AES has no effect on TR- or ER-dependent transcription, AES specifically inhibits AR-dependent gene expression in a TSA-insensitive manner, suggesting that HDAC-containing complexes are not involved in repression by AES [100]. Interestingly, AES interacts specifically with the basal transcription factor (TFIIIE), which is not reported to be an AR interacting protein, thus suggesting that AES may act by directly targeting the basal transcription machinery.

### Other AR Corepressors

In addition to the above-mentioned corepressors, there are several other coregulators that have been identified as AR corepressors. However, at present, the mechanisms by which these corepressors inhibit AR transactivation are not clear.

### HBO1

HBO1 was first identified as a protein that shares sequence homology with the MYST subfamily and interacts with the human origin recognition complex [101]. It was later demonstrated that HBO1 is a nuclear protein that is highly expressed in the human testis. HBO1 specifically interacts with the AR DBD and LBD, through its N-terminal region, in a ligand-enhanced manner [102]. HBO1 specifically inhibits AR transactivation, but not ER or TR transcriptional activity. The mechanism by which HBO1 suppresses AR function, and its biological importance awaits further studies.

### Phosphatase and Tensin Homolog Deleted on Chromosome Ten (PTEN)

The tumor suppressor gene *PTEN* is one of the most frequently mutated genes, and is linked to a variety of human cancers [103]. Frequent inactivation of *PTEN/MMAC1* was found in primary prostate cancers [104,105]. Our group has found that *PTEN* interacts with the AR DBD in vitro and in vivo [106]. *PTEN* inhibits AR transactivation via a PI3K/Akt-independent pathway in early passage number prostate cancer LNCaP cells, while *PTEN* also suppresses AR activity through a PI3K/Akt-dependent pathway in high passage number prostate cancer LNCaP cells (unpublished data). Yet the mechanism of *PTEN*-mediated AR repression remains unclear.

### Sex-Determining Region Y (SRY)

Human SRY is a testis-expressed protein containing a central sequence-specific high mobility group box DNA binding domain [107–109]. Expression of SRY triggers a cascade of events that leads to the develop-

ment of the Sertoli cell, Leydig cells, and the testis [110]. SRY was shown to interact directly with the AR DBD in vivo and in vitro. Since human SRY has not been reported to possess autonomous repressor domains, the mechanism by which SRY overexpression inhibits AR transactivation remains unclear [111].

### Ebp1

Members of the ErbB/HER2 family of receptors have been demonstrated to be involved in regulation of AR activity. High levels of HER2/Neu cause cell growth in an androgen-independent manner or increased sensitivity to low levels of androgen. Furthermore, overexpression of HER2/Neu enhanced AR transactivation and stimulated LNCaP cell growth. Ebp1 (ErbB3 binding protein 1), which contains an LXXLL motif, was first isolated by the yeast two-hybrid method using ErbB3 as bait. Later, it was demonstrated to directly interact with AR, and ectopic expression of Ebp1 reduced AR transactivation in prostate cancer cell lines. Ebp1-AR association was increased by androgen treatment, although the N-terminal domain of AR was responsible for binding Ebp1. Ebp1-AR interaction was mediated through the C-terminal 79 amino acids of Ebp1, which contains an LXXLL motif that is necessary for binding to AR.

### Testicular Orphan Receptor-2/Testicular Orphan Receptor-4 (TR2/TR4)

The human *TR2* and *TR4* genes were originally isolated from human prostate and testis cDNA libraries [112,113]. *TR4* has been demonstrated to interact with AR in vivo and in vitro [114]. Furthermore, *TR4* and AR show mutual suppression of their transactivation. The LNCaP cells' expression of endogenous PSA, an androgen target that is widely used as a marker for prostate cancer progression, was reduced with the addition of *TR4*. A recent study has indicated that heterodimerization occurs between the *TR2* and AR, and consequently, inhibition of AR transactivation [115]. The identification of cross talk between the AR and *TR2/TR4* not only extends the function of these receptors but also contributes to the understanding of the complex gene network controlled by the nuclear receptor superfamily. However, the detailed molecular mechanisms controlling the mutual inhibition of AR and *TR2/TR4* need further investigation.

Other AR coregulators, especially some transcriptional corepressors including nuclear factor kappa B/RelA, TSG101, and PIASy have been reported to down-regulate AR transactivation through direct binding [93,116–119]. Additional studies are necessary to elucidate the detailed mechanisms of the repressive

effects of such corepressors on AR, and their physiological roles in androgen-mediated diseases.

### POTENTIAL IMPACT AND FUTURE DIRECTIONS

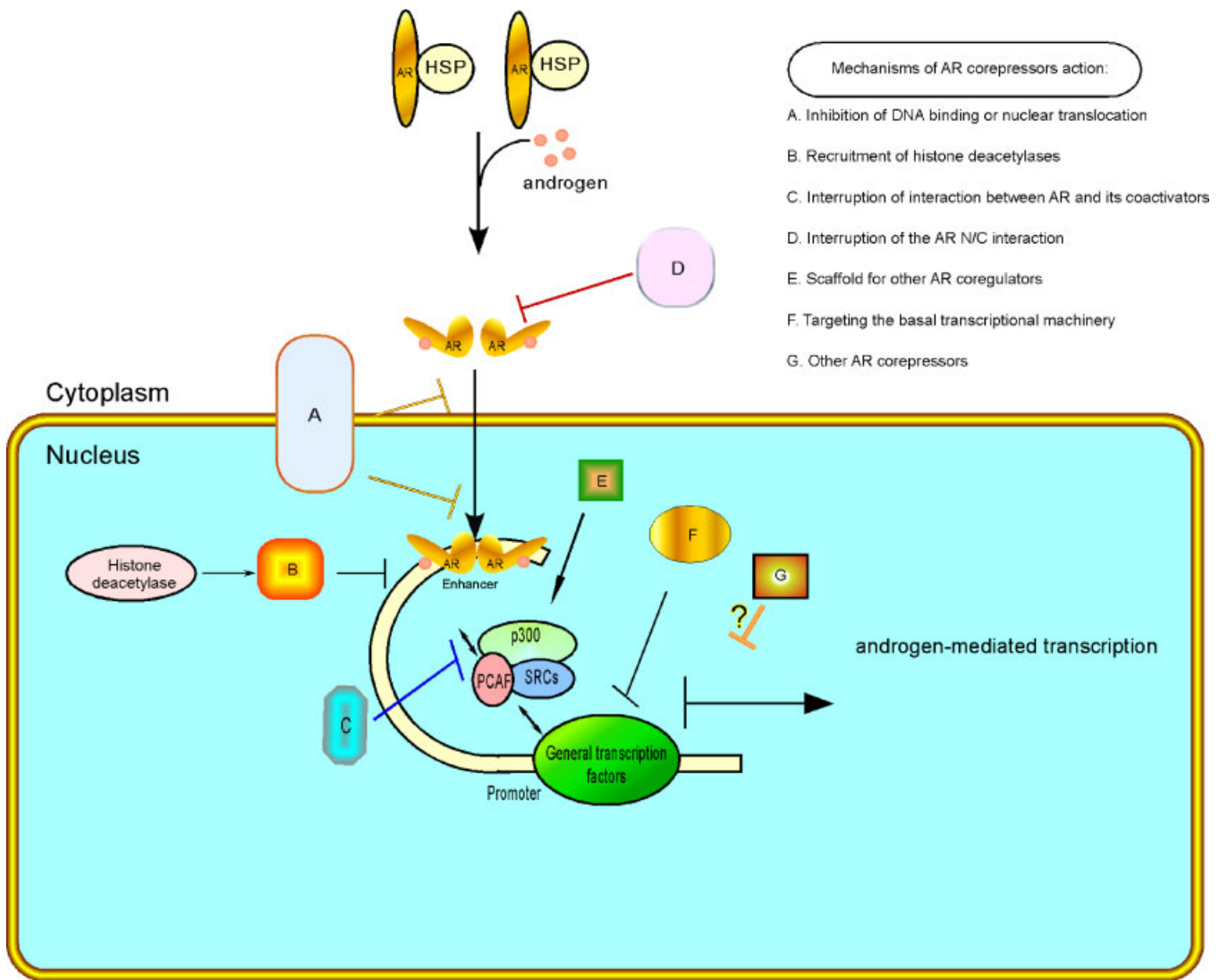
Mutations of the *AR* gene cause a wide range of abnormal phenotypes of male sexual development, including both complete and partial androgen insensitivity [120–123]. On the other hand, androgen is the major growth factor for normal prostate, and the AR signaling pathways play critical roles in the development and progression of prostate cancer [17,124]. Prostate cancer is the most common form of cancer and the second leading cause of cancer death in men in the United States [125]. Although prostate cancers are usually responsive to androgen ablation therapy initially, most tumors eventually relapse to an androgen-refractory state. Thus, androgen ablation therapy is not curative, no matter how complete the ablation is [126]. However, the AR is expressed in the majority of prostate cancers, both before and after androgen ablation therapy, suggesting that AR function may play a critical role in the proliferation of primary and metastatic prostate tumors [127]. A growing body of data has indicated the involvement of molecular changes leading to gain of function in the AR signaling pathway during the progression of prostate cancer. The gain of function changes in AR-mediated pathways provide growth advantages in prostate cancer cells due to the ability to activate AR signaling after androgen ablation therapy. Therefore, identification of the mechanisms underlying the regulation of AR signaling is critical to the design and development of novel therapies and pharmaceutical targets through which to treat prostate cancers or other AR related diseases.

Substantial evidence has suggested that the transcriptional activity of AR is regulated by its coregulators. Furthermore, recent studies indicate that mutations or altered expression of AR coregulators may contribute to the progression of prostate cancers. However, future studies are needed to further characterize the physiological roles of AR coregulators. First, the use of animal models, including those with targeted disruption of coregulators or induction of wild type or mutant coregulators will be helpful to determine biological roles of individual AR coregulators in different tissues or in pathological conditions. Importantly, the tissue-specific expression pattern of AR coregulators may help to increase understanding of their spatial and temporal specific roles *in vivo*, and provide detailed information on their roles in androgen-induced growth responses. Second, application of the oligonucleotide microarray or proteomics technologies in clinical studies may allow study of the status of

AR coregulators in cell signaling involved in mitogenic, apoptotic, and growth regulation pathways. Considering the progression from normal prostate epithelium to invasive prostate cancer, analysis of microarray data may be of interest to identify novel AR coregulators with expression changes that correlate with the progression of prostate cancer. Therefore, the further characterization of AR coregulators may provide insight into the signaling events occurring within human tumors, and may be critical for the development of individualized therapy. In addition, genome-wide analysis of coexpression of AR with other human genes in normal tissues, and in different developmental stages may lead to identification of novel AR coregulators that are difficult to isolate by using traditional methods, such as yeast two-hybrid method or immunoprecipitation. Third, given that multiple genetic changes are generally involved in advanced prostate cancers, it is necessary to develop molecular therapies targeting multiple steps of AR transactivation. Interestingly, some AR antagonists can function as agonist with alterations in the recruitment of coactivators and corepressors to the promoters of androgen receptor target genes. Therefore, the relative levels of AR corepressors versus coactivators may be particularly important in the regulation of AR transactivation. With the characterization of various mechanisms through which AR corepressors down-regulate AR function, the development of novel AR inhibitors that would promote corepressor binding and pure antagonistic action in prostate cancers becomes increasingly likely. Rapid, high-throughput screening of small molecules based on different mechanisms of inhibiting AR may be important in the development of such inhibitors using *in vitro* cell line models.

### CONCLUSIONS

Upon androgen stimulation, AR regulates transcription of its target genes, which is a process modulated by AR coregulators (both coactivators and corepressors). To exert their actions, many corepressors influence AR transactivation through various characterized mechanisms (as shown in Fig. 1). The diversity of coregulator function and their distribution pattern helps control AR transactivation in a sophisticated and complex manner. The increasing characterization of novel AR coregulators leads to the tantalizing suggestion that new pathways that participate in regulation of AR activity remain to be discovered. Interest in AR coregulator research has also been stimulated by the possibility that new therapeutic methods might be developed based on the association of particular AR corepressor mutations or altered expression with specific diseases. Therefore, coregulators have become good targets for potential



**Fig. 1.** A model of how AR corepressors regulate AR-mediated transcription.

drug treatment or diagnostic markers of prostate cancer. Increased understanding of the mechanisms of AR coregulator action offers exciting opportunities for the development of novel therapies.

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