The Prostate 63:117–130 (2005)

Androgen Receptor Corepressors: An Overview

Liang Wang,[1] Cheng-Lung Hsu,[1,2] and Chawnshang Chang[1]*

[1]George H. Whipple Laboratory for Cancer Research, Departments of Pathology, Urology, Radiation Oncology, and the Cancer Center, University of Rochester Medical Center, Rochester, New York

[2]Division of Hematology-Oncology, Department of Internal Medicine, Chang Gung Memorial Hospital, Taipei, Taiwan

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. *Prostate 63: 117–130, 2005.* © 2004 Wiley-Liss, Inc.

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 5α-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_2-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

*Correspondence to: Chawnshang Chang, George H. Whipple Laboratory for Cancer Research, Departments of Pathology, Urology, Radiation Oncology, and the Cancer Center, University of Rochester Medical Center, Rochester, New York 14642.
E-mail: chang@urmc.rochester.edu
Received 26 July 2004; Accepted 9 August 2004
DOI 10.1002/pros.20170
Published online 14 October 2004 in Wiley InterScience (www.interscience.wiley.com).

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 ProteinTail 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-I*)

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 transcrtption, AES specifically inhibits AR-dependent gene expression in TSA-insensitive manner	[100]
Akt	[a]	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]
GSK3β	N-terminus	GSK3β 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 avtivity 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	[a]	PATZ alone does not influence AR function, but suppresses AR activity in the presence of RNF4, a known AR coactivatior	[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_2-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]

[a]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 (RARα). 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 RARα-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 RARα, SMRT has recently been identified as an AR NH2-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 ERα, 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 3β (GSK3β)

GSK3β is a serine/threonine protein kinase that was first described in a metabolic pathway for glycogen synthase regulation [86]. GSK3β 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 GSK3β is ubiquitously expressed in prostate cells and interacts with AR in vivo and in vitro [90]. GSK3β phosphorylates the AR N-terminus and suppresses AR transactivation. AR N/C interaction was reduced with the addition of GSK3β.

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-1 (RACK1)

RACK1 was first characterized as a PKC-anchoring protein, which determines the localization of activated PKCβII [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 TFIIH [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 (TFIIE), 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 ChromosomeTen (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 RegionY (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 development 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 downregulate 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

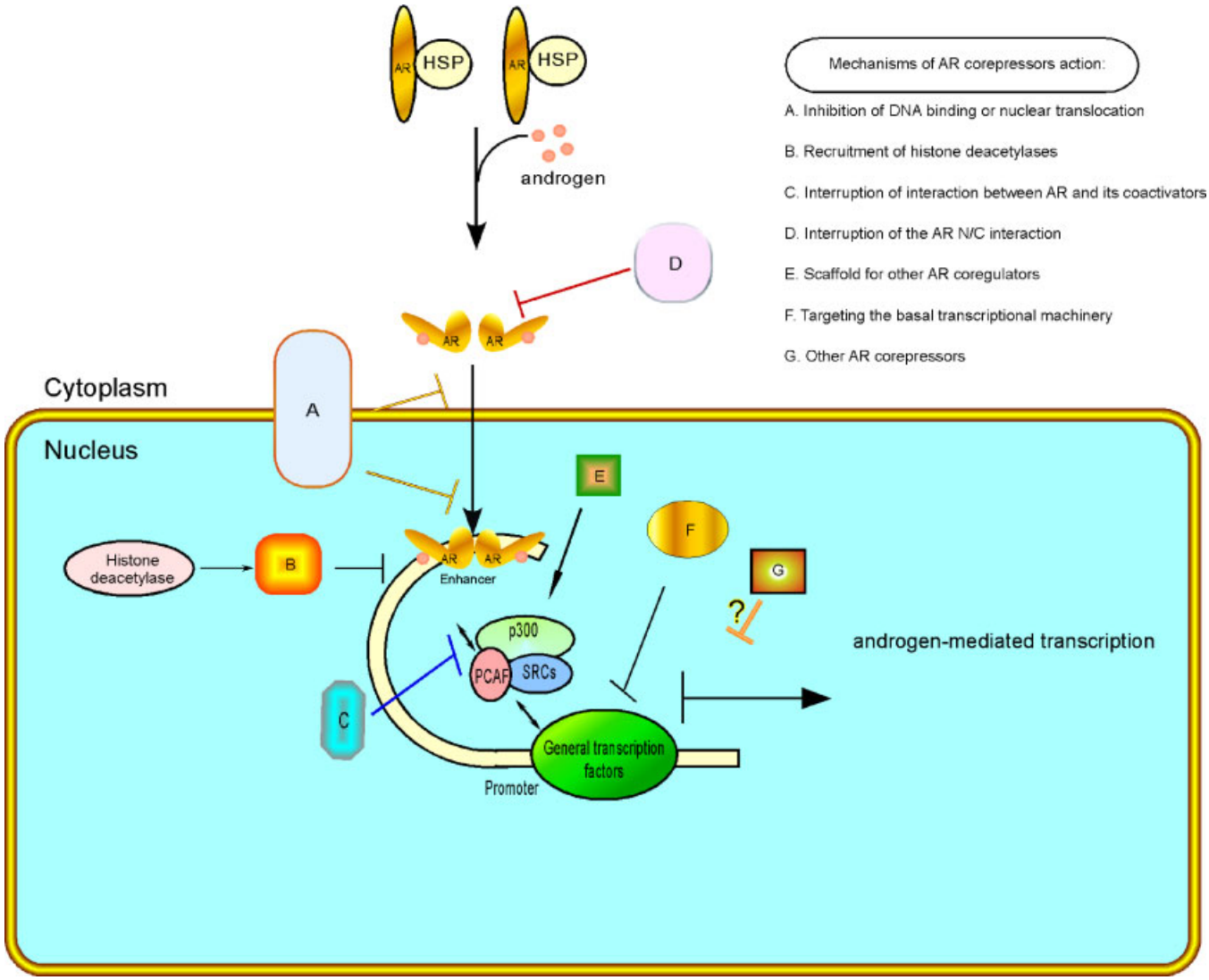

Fig. 1. A model of how AR corepressors regulate AR-mediated transcription. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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.

REFERENCES

1. Sperling LC, Heimer WL II. Androgen biology as a basis for the diagnosis and treatment of androgenic disorders in women. I. J Am Acad Dermatol 1993;28(5 Pt 1):669–683.
2. Eder IE, Culig Z, Putz T, Nessler-Menardi C, Bartsch G, Klocker H. Molecular biology of the androgen receptor: From molecular understanding to the clinic. Eur Urol 2001;40(3):241–251.
3. Chang C, Saltzman A, Yeh S, Young W, Keller E, Lee HJ, Wang C, Mizokami A. Androgen receptor: An overview. Crit Rev Eukaryot Gene Expr 1995;5(2):97–125.
4. Roy AK, Lavrovsky Y, Song CS, Chen S, Jung MH, Velu NK, Bi BY, Chatterjee B. Regulation of androgen action. Vitam Horm 1999;55:309–352.
5. Lyon MF, Glenister PH, Lamoreux ML. Normal spermatozoa from androgen-resistant germ cells of chimaeric mice and the role of androgen in spermatogenesis. Nature 1975;258(5536): 620–622.
6. Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol 2002;16(10):2181–2187.
7. Chang CS, Kokontis J, Liao ST. Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 1988;240(4850):324–326.
8. Chang CS, Kokontis J, Liao ST. Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc Natl Acad Sci USA 1988; 85(19):7211–7215.
9. Kumar R, Thompson EB. The structure of the nuclear hormone receptors. Steroids 1999;64(5):310–319.
10. Roy AK, Tyagi RK, Song CS, Lavrovsky Y, Ahn SC, Oh TS, Chatterjee B. Androgen receptor: Structural domains and functional dynamics after ligand-receptor interaction. Ann NY Acad Sci 2001;949:44–57.

11. He B, Kemppainen JA, Wilson EM. FXXLF and WXXLF sequences mediate the NH2-terminal interaction with the ligand binding domain of the androgen receptor. J Biol Chem 2000;275(30):22986–22994.
12. Langley E, Zhou ZX, Wilson EM. Evidence for an anti-parallel orientation of the ligand-activated human androgen receptor dimer. J Biol Chem 1995;270(50):29983–29990.
13. Chang C, Norris JD, Gron H, Paige LA, Hamilton PT, Kenan DJ, Fowlkes D, McDonnell DP. Dissection of the LXXLL nuclear receptor-coactivator interaction motif using combinatorial peptide libraries: Discovery of peptide antagonists of estrogen receptors alpha and beta. Mol Cell Biol 1999;19(12):8226–8239.
14. Yeh S, Chang C. Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 1996;93(11):5517–5521.
15. Kang HY, Yeh S, Fujimoto N, Chang C. Cloning and characterization of human prostate coactivator ARA54, a novel protein that associates with the androgen receptor. J Biol Chem 1999; 274(13):8570–8576.
16. He B, Minges JT, Lee LW, Wilson EM. The FXXLF motif mediates androgen receptor-specific interactions with coregulators. J Biol Chem 2002;277(12):10226–10235.
17. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: An overview. Endocr Rev 2002;23(2):175–200.
18. Michalak M, Corbett EF, Mesaeli N, Nakamura K, Opas M. Calreticulin: One protein, one gene, many functions. Biochem J 1999;344 (Pt 2):281–292.
19. Dedhar S, Rennie PS, Shago M, Hagesteijn CY, Yang H, Filmus J, Hawley RG, Bruchovsky N, Cheng H, Matusik RJ, Giguère V. Inhibition of nuclear hormone receptor activity by calreticulin. Nature 1994;367(6462):480–483.
20. Burns K, Duggan B, Atkinson EA, Famulski KS, Nemer M, Bleackley RC, Michalak M. Modulation of gene expression by calreticulin binding to the glucocorticoid receptor. Nature 1994; 367(6462):476–480.
21. Wheeler DG, Horsford J, Michalak M, White JH, Hendy GN. Calreticulin inhibits vitamin D3 signal transduction. Nucleic Acids Res 1995;23(16):3268–3274.
22. Zhu N, Pewitt EB, Cai X, Cohn EB, Lang S, Chen R, Wang Z. Calreticulin: an intracellular Ca^{++}-binding protein abundantly expressed and regulated by androgen in prostatic epithelial cells. Endocrinology 1998;139(10):4337–4344.
23. Zhu N, Wang Z. Calreticulin expression is associated with androgen regulation of the sensitivity to calcium ionophore-induced apoptosis in LNCaP prostate cancer cells. Cancer Res 1999;59(8):1896–1902.
24. Zhang Y, Yang Y, Chang C. ARA67 functions as a repressor to suppress androgen receptor transactivation. Mol Cell Biol 2004; 24(3):1044–1057.
25. Zheng P, Eastman J, Vande Pol S, Pimplikar SW. PAT1, a microtubule-interacting protein, recognizes the basolateral sorting signal of amyloid precursor protein. Proc Natl Acad Sci USA 1998;95(25):14745–14750.
26. Burris TP, Guo W, McCabe ER. The gene responsible for adrenal hypoplasia congenita, *DAX-1*, encodes a nuclear hormone receptor that defines a new class within the superfamily. Recent Prog Horm Res 1996;51:241–259; discussion 259–260.
27. Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, Lalli E, Moser C, Walker AP, McCabe ER, Meitinger T, Monaco A, Sassone-Corsi P, Camerino G. An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature 1994;372(6507): 635–641.
28. Tamai KT, Monaco L, Alastalo TP, Lalli E, Parvinen M, Sassone-Corsi P. Hormonal and developmental regulation of DAX-1 expression in Sertoli cells. Mol Endocrinol 1996;10(12):1561–1569.
29. Holter E, Kotaja N, Makela S, Strauss L, Kietz S, Janne OA, Gustafsson JA, Palvimo JJ, Treuter E. Inhibition of androgen receptor (AR) function by the reproductive orphan nuclear receptor DAX-1. Mol Endocrinol 2002;16(3): 515–528.
30. Agoulnik IU, Krause WC, Bingman WE III, Rahman HT, Amrikachi M, Ayala GE, Weigel NL. Repressors of androgen and progesterone receptor action. J Biol Chem 2003;278(33): 31136–31148.
31. Yang F, Li X, Sharma M, Zarnegar M, Lim B, Sun Z. Androgen receptor specifically interacts with a novel p21-activated kinase, PAK6. J Biol Chem 2001;276(18):15345–15353.
32. Lee SR, Ramos SM, Ko A, Masiello D, Swanson KD, Lu ML, Balk SP. AR and ER interaction with a p21-activated kinase (PAK6). Mol Endocrinol 2002;16(1):85–99.
33. Schrantz N, Da Silva Correia J, Fowler B, Ge Q, Sun Z, Bokoch GM. Mechanism of p21-activated kinase 6 (PAK6)-mediated inhibition of androgen receptor signaling. J Biol Chem 2004; 279(3):1922–1931.
34. Mathis DJ, Oudet P, Wasylyk B, Chambon P. Effect of histone acetylation on structure and in vitro transcription of chromatin. Nucleic Acids Res 1978;5(10):3523–3547.
35. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature 1997;389(6649):349–352.
36. Xu L, Glass CK, Rosenfeld MG. Coactivator and corepressor complexes in nuclear receptor function. Curr Opin Genet Dev 1999;9(2):140–147.
37. Brady ME, Ozanne DM, Gaughan L, Waite I, Cook S, Neal DE, Robson CN. Tip60 is a nuclear hormone receptor coactivator. J Biol Chem 1999;274(25):17599–17604.
38. Ma H, Hong H, Huang SM, Irvine RA, Webb P, Kushner PJ, Coetzee GA, Stallcup MR. Multiple signal input and output domains of the 160-kilodalton nuclear receptor coactivator proteins. Mol Cell Biol 1999;19(9):6164–6173.
39. Fu M, Wang C, Reutens AT, Wang J, Angeletti RH, Siconolfi-Baez L, Ogryzko V, Avantaggiati ML, Pestell RG. p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. J Biol Chem 2000;275(27):20853–20860.
40. Wang Q, Lu J, Yong EL. Ligand- and coactivator-mediated transactivation function (AF2) of the androgen receptor ligand-binding domain is inhibited by the cognate hinge region. J Biol Chem 2001;276(10):7493–7499.
41. Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell 1997; 90(4):595–606.
42. Sartorelli V, Puri PL, Hamamori Y, Ogryzko V, Chung G, Nakatani Y, Wang JY, Kedes L. Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program. Mol Cell 1999;4(5):725–734.
43. Mal A, Sturniolo M, Schiltz RL, Ghosh MK, Harter ML. A role for histone deacetylase HDAC1 in modulating the transcriptional activity of MyoD: Inhibition of the myogenic program. EMBO J 2001;20(7):1739–1753.

44. Luo J, Su F, Chen D, Shiloh A, Gu W. Deacetylation of p53 modulates its effect on cell growth and apoptosis. Nature 2000; 408(6810):377–381.
45. Gaughan L, Logan IR, Cook S, Neal DE, Robson CN. Tip60 and histone deacetylase 1 regulate androgen receptor activity through changes to the acetylation status of the receptor. J Biol Chem 2002;277(29):25904–25913.
46. Wotton D, Lo RS, Lee S, Massague J. A Smad transcriptional corepressor. Cell 1999;97(1):29–39.
47. Bertolino E, Reimund B, Wildt-Perinic D, Clerc RG. A novel homeobox protein which recognizes a TGT core and functionally interferes with a retinoid-responsive motif. J Biol Chem 1995;270(52):31178–31188.
48. Sharma M, Sun Z. 5′TG3′ interacting factor interacts with Sin3A and represses AR-mediated transcription. Mol Endocrinol 2001;15(11):1918–1928.
49. Jeong BC, Hong CY, Chattopadhyay S, Park JH, Gong EY, Kim HJ, Chun SY, Lee K. Androgen receptor corepressor-19 kDa (ARR19), a leucine-rich protein that represses the transcriptional activity of androgen receptor through recruitment of histone deacetylase. Mol Endocrinol 2004;18(1): 13–25.
50. Niki T, Takahashi-Niki K, Taira T, Iguchi-Ariga SM, Ariga H. DJBP: A novel DJ-1-binding protein, negatively regulates the androgen receptor by recruiting histone deacetylase complex, and DJ-1 antagonizes this inhibition by abrogation of this complex. Mol Cancer Res 2003;1(4):247–261.
51. Privalsky ML. Regulation of SMRT and N-CoR corepressor function. Curr Top Microbiol Immunol 2001;254:117–136.
52. Ordentlich P, Downes M, Evans RM. Corepressors and nuclear hormone receptor function. Curr Top Microbiol Immunol 2001; 254:101–116.
53. Wagner BL, Norris JD, Knotts TA, Weigel NL, McDonnell DP. The nuclear corepressors NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor. Mol Cell Biol 1998;18(3):1369–1378.
54. Huang HJ, Norris JD, McDonnell DP. Identification of a negative regulatory surface within estrogen receptor alpha provides evidence in support of a role for corepressors in regulating cellular responses to agonists and antagonists. Mol Endocrinol 2002;16(8):1778–1792.
55. Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L, Horwitz KB. The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. Mol Endocrinol 1997;11(6):693–705.
56. Dotzlaw H, Moehren U, Mink S, Cato AC, Iniguez Lluhi JA, Baniahmad A. The amino terminus of the human AR is target for corepressor action and antihormone agonism. Mol Endocrinol 2002;16(4):661–673.
57. Liao G, Chen LY, Zhang A, Godavarthy A, Xia F, Ghosh JC, Li H, Chen JD. Regulation of androgen receptor activity by the nuclear receptor corepressor SMRT. J Biol Chem 2003;278(7): 5052–5061.
58. Huang ZQ, Li J, Wong J. AR possesses an intrinsic hormone-independent transcriptional activity. Mol Endocrinol 2002; 16(5):924–937.
59. Cheng S, Brzostek S, Lee SR, Hollenberg AN, Balk SP. Inhibition of the dihydrotestosterone-activated androgen receptor by nuclear receptor corepressor. Mol Endocrinol 2002;16(7):1492–1501.
60. Seol W, Chung M, Moore DD. Novel receptor interaction and repression domains in the orphan receptor SHP. Mol Cell Biol 1997;17(12):7126–7131.
61. Johansson L, Thomsen JS, Damdimopoulos AE, Spyrou G, Gustafsson JA, Treuter E. The orphan nuclear receptor SHP inhibits agonist-dependent transcriptional activity of estrogen receptors ERalpha and ERbeta. J Biol Chem 1999;274(1):345–353.
62. Seol W, Hanstein B, Brown M, Moore DD. Inhibition of estrogen receptor action by the orphan receptor SHP (short heterodimer partner). Mol Endocrinol 1998;12(10):1551–1557.
63. Johansson L, Bavner A, Thomsen JS, Farnegardh M, Gustafsson JA, Treuter E. The orphan nuclear receptor SHP utilizes conserved LXXLL-related motifs for interactions with ligand-activated estrogen receptors. Mol Cell Biol 2000;20(4):1124–1133.
64. Gobinet J, Auzou G, Nicolas JC, Sultan C, Jalaguier S. Characterization of the interaction between androgen receptor and a new transcriptional inhibitor, SHP. Biochemistry 2001;40(50): 15369–15377.
65. Wen Y, Hu MC, Makino K, Spohn B, Bartholomeusz G, Yan DH, Hung MC. HER-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the Akt pathway. Cancer Res 2000;60(24):6841–6845.
66. Lin HK, Yeh S, Kang HY, Chang C. Akt suppresses androgen-induced apoptosis by phosphorylating and inhibiting androgen receptor. Proc Natl Acad Sci USA 2001;98(13):7200–7205.
67. Li P, Nicosia SV, Bai W. Antagonism between PTEN/MMAC1/TEP-1 and androgen receptor in growth and apoptosis of prostatic cancer cells. J Biol Chem 2001;276(23):20444–20450.
68. Lin HK, Hu YC, Yang L, Altuwaijri S, Chen YT, Kang HY, Chang C. Suppression versus induction of androgen receptor functions by the phosphatidylinositol 3-kinase/Akt pathway in prostate cancer LNCaP cells with different passage numbers. J Biol Chem 2003;278(51):50902–50907.
69. Lin HK, Wang L, Hu YC, Altuwaijri S, Chang C. Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. EMBO J 2002;21(15): 4037–4048.
70. Petre-Draviam CE, Cook SL, Burd CJ, Marshall TW, Wetherill YB, Knudsen KE. Specificity of cyclin D1 for androgen receptor regulation. Cancer Res 2003;63(16):4903–4913.
71. Petre CE, Wetherill YB, Danielsen M, Knudsen KE. Cyclin D1: Mechanism and consequence of androgen receptor co-repressor activity. J Biol Chem 2002;277(3):2207–2215.
72. Reutens AT, Fu M, Wang C, Albanese C, McPhaul MJ, Sun Z, Balk SP, Janne OA, Palvimo JJ, Pestell RG. Cyclin D1 binds the androgen receptor and regulates hormone-dependent signaling in a p300/CBP-associated factor (P/CAF)-dependent manner. Mol Endocrinol 2001;15(5):797–811.
73. Knudsen KE, Cavenee WK, Arden KC. D-type cyclins complex with the androgen receptor and inhibit its transcriptional transactivation ability. Cancer Res 1999;59(10):2297–2301.
74. Coqueret O. Linking cyclins to transcriptional control. Gene 2002;299(1–2):35–55.
75. Drobnjak M, Osman I, Scher HI, Fazzari M, Cordon-Cardo C. Overexpression of cyclin D1 is associated with metastatic prostate cancer to bone. Clin Cancer Res 2000;6(5):1891–1895.
76. Derynck R, Zhang Y, Feng XH. Smads: Transcriptional activators of TGF-beta responses. Cell 1998;95(6):737–740.
77. Matsuda T, Yamamoto T, Muraguchi A, Saatcioglu F. Cross-talk between transforming growth factor-beta and estrogen

receptor signaling through Smad3. J Biol Chem 2001;276(46): 42908–42914.

78. Subramaniam N, Leong GM, Cock TA, Flanagan JL, Fong C, Eisman JA, Kouzmenko AP. Cross-talk between 1,25-dihydroxyvitamin D3 and transforming growth factor-beta signaling requires binding of VDR and Smad3 proteins to their cognate DNA recognition elements. J Biol Chem 2001;276(19): 15741–15746.
79. Hayes SA, Zarnegar M, Sharma M, Yang F, Peehl DM, ten Dijke P, Sun Z. SMAD3 represses androgen receptor-mediated transcription. Cancer Res 2001;61(5):2112–2118.
80. Chipuk JE, Cornelius SC, Pultz NJ, Jorgensen JS, Bonham MJ, Kim SJ, Danielpour D. The androgen receptor represses transforming growth factor-beta signaling through interaction with Smad3. J Biol Chem 2002;277(2):1240–1248.
81. Kang HY, Lin HK, Hu YC, Yeh S, Huang KE, Chang C. From transforming growth factor-beta signaling to androgen action: Identification of Smad3 as an androgen receptor coregulator in prostate cancer cells. Proc Natl Acad Sci USA 2001;98(6): 3018–3023.
82. Kang HY, Huang KE, Chang SY, Ma WL, Lin WJ, Chang C. Differential modulation of androgen receptor-mediated transactivation by Smad3 and tumor suppressor Smad4. J Biol Chem 2002;277(46):43749–43756.
83. Stossel TP, Condeelis J, Cooley L, Hartwig JH, Noegel A, Schleicher M, Shapiro SS. Filamins as integrators of cell mechanics and signalling. Nat Rev Mol Cell Biol 2001;2(2):138–145.
84. Loy CJ, Sim KS, Yong EL. Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions. Proc Natl Acad Sci USA 2003;100(8):4562–4567.
85. Wang L, Hsu CL, Ni J, Wand PH, Yeh S, Keng P, Chang C. Human checkpoint protein hRad9 functions as a negative coregulator to repress androgen receptor transactivation in prostate cancer cells. Mol Cell Biol 2004;24(5):2202–2213.
86. Cohen P, Nimmo HG, Proud CG. How does insulin stimulate glycogen synthesis? Biochem Soc Symp 1978;43:69–95.
87. Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. Genes Dev 2000;14(19):2501–2514.
88. de Groot RP, Auwerx J, Bourouis M, Sassone-Corsi P. Negative regulation of Jun/AP-1: Conserved function of glycogen synthase kinase 3 and the *Drosophila* kinase shaggy. Oncogene 1993;8(4):841–847.
89. Rogatsky I, Waase CL, Garabedian MJ. Phosphorylation and inhibition of rat glucocorticoid receptor transcriptional activation by glycogen synthase kinase-3 (GSK-3). Species-specific differences between human and rat glucocorticoid receptor signaling as revealed through GSK-3 phosphorylation. J Biol Chem 1998;273(23):14315–14321.
90. Wang L, Lin HK, Hu YC, Xie S, Yang L, Chang C. Suppression of androgen receptor-mediated transactivation and cell growth by the glycogen synthase kinase 3b in prostate cells. J Biol Chem 2004;279(31):32444–32452.
91. Ron D, Jiang Z, Yao L, Vagts A, Diamond I, Gordon A. Coordinated movement of RACK1 with activated betaIIPKC. J Biol Chem 1999;274(38):27039–27046.
92. Rigas AC, Ozanne DM, Neal DE, Robson CN. The scaffolding protein RACK1 interacts with androgen receptor and promotes cross-talk through a protein kinase C signaling pathway. J Biol Chem 2003;278(46):46087–46093.
93. Gross M, Liu B, Tan J, French FS, Carey M, Shuai K. Distinct effects of PIAS proteins on androgen-mediated gene activation in prostate cancer cells. Oncogene 2001;20(29):3880–3887.
94. McEwan IJ, Gustafsson J. Interaction of the human androgen receptor transactivation function with the general transcription factor TFIIF. Proc Natl Acad Sci USA 1997;94(16):8485–8490.
95. Lee DK, Duan HO, Chang C. From androgen receptor to the general transcription factor TFIIH. Identification of cdk activating kinase (CAK) as an androgen receptor NH(2)-terminal associated coactivator. J Biol Chem 2000;275(13):9308–9313.
96. Chen G, Courey AJ. Groucho/TLE family proteins and transcriptional repression. Gene 2000;249(1–2):1–16.
97. Javed A, Guo B, Hiebert S, Choi JY, Green J, Zhao SC, Osborne MA, Stifani S, Stein JL, Lian JB, van Wijnen AJ, Stein GS. Groucho/TLE/R-esp proteins associate with the nuclear matrix and repress RUNX (CBF(alpha)/AML/PEBP2(alpha)) dependent activation of tissue-specific gene transcription. J Cell Sci 2000;113(Pt 12):2221–2231.
98. Tetsuka T, Uranishi H, Imai H, Ono T, Sonta S, Takahashi N, Asamitsu K, Okamoto T. Inhibition of nuclear factor-kappaB-mediated transcription by association with the amino-terminal enhancer of split, a Groucho-related protein lacking WD40 repeats. J Biol Chem 2000;275(6):4383–4390.
99. Choudhury BK, Kim J, Kung HF, Li SS. Cloning and developmental expression of *Xenopus* cDNAs encoding the enhancer of split groucho and related proteins. Gene 1997;195(1):41–48.
100. Yu X, Li P, Roeder RG, Wang Z. Inhibition of androgen receptor-mediated transcription by amino-terminal enhancer of split. Mol Cell Biol 2001;21(14):4614–4625.
101. Iizuka M, Stillman B. Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. J Biol Chem 1999;274(33):23027–23034.
102. Sharma M, Zarnegar M, Li X, Lim B, Sun Z. Androgen receptor interacts with a novel MYST protein, HBO1. J Biol Chem 2000; 275(45):35200–35208.
103. Cantley LC, Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc Natl Acad Sci USA 1999;96(8):4240–4245.
104. Vlietstra RJ, van Alewijk DC, Hermans KG, van Steenbrugge GJ, Trapman J. Frequent inactivation of PTEN in prostate cancer cell lines and xenografts. Cancer Res 1998;58(13):2720–2723.
105. Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova GS, Sidransky D. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. Cancer Res 1997;57(22):4997–5000.
106. Lin HK, Hu YC, Lee DK, Chang C. Regulation of androgen receptor signaling by PTEN tumor suppressor through distinct mechanisms in prostate cancer cells. Mol Endocrinol 2004; (Epub ahead of print).
107. Peters R, King CY, Ukiyama E, Falsafi S, Donahoe PK, Weiss MA. An SRY mutation causing human sex reversal resolves a general mechanism of structure-specific DNA recognition: Application to the four-way DNA junction. Biochemistry 1995; 34(14):4569–4576.
108. Harley VR, Lovell-Badge R, Goodfellow PN. Definition of a consensus DNA binding site for SRY. Nucleic Acids Res 1994; 22(8):1500–1501.
109. Poulat F, Girard F, Chevron MP, Goze C, Rebillard X, Calas B, Lamb N, Berta P. Nuclear localization of the testis determining gene product SRY. J Cell Biol 1995;128(5):737–748.
110. Lamb DJ. Genes involved in testicular development and function. World J Urol 1995;13(5):277–284.

111. Yuan X, Lu ML, Li T, Balk SP. SRY interacts with and negatively regulates androgen receptor transcriptional activity. J Biol Chem 2001;276(49):46647–46654.

112. Chang C, Da Silva SL, Ideta R, Lee Y, Yeh S, Burbach JP. Human and rat TR4 orphan receptors specify a subclass of the steroid receptor superfamily. Proc Natl Acad Sci USA 1994;91(13): 6040–6044.

113. Chang C, Kokontis J, Acakpo-Satchivi L, Liao S, Takeda H, Chang Y. Molecular cloning of new human TR2 receptors: A class of steroid receptor with multiple ligand-binding domains. Biochem Biophys Res Commun 1989;165(2):735–741.

114. Lee YF, Shyr CR, Thin TH, Lin WJ, Chang C. Convergence of two repressors through heterodimer formation of androgen receptor and testicular orphan receptor-4: A unique signaling pathway in the steroid receptor superfamily. Proc Natl Acad Sci USA 1999;96(26):14724–14729.

115. Mu X, Chang C. TR2 orphan receptor functions as negative modulator for androgen receptor in prostate cancer cells PC-3. Prostate 2003;57(2):129–133.

116. Sun Z, Pan J, Hope WX, Cohen SN, Balk SP. Tumor susceptibility gene 101 protein represses androgen receptor transactivation and interacts with p300. Cancer 1999;86(4):689–696.

117. McKay LI, Cidlowski JA. Cross-talk between nuclear factor-kappa B and the steroid hormone receptors: Mechanisms of mutual antagonism. Mol Endocrinol 1998;12(1):45–56.

118. Supakar PC, Jung MH, Song CS, Chatterjee B, Roy AK. Nuclear factor kappa B functions as a negative regulator for the rat androgen receptor gene and NF-kappa B activity increases during the age-dependent desensitization of the liver. J Biol Chem 1995;270(2):837–842.

119. McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: Interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. Endocr Rev 1999;20(4):435–459.

120. Griffin JE. Androgen resistance—The clinical and molecular spectrum. N Engl J Med 1992;326(9):611–618.

121. Warner CL, Griffin JE, Wilson JD, Jacobs LD, Murray KR, Fischbeck KH, Dickoff D, Griggs RC. X-linked spinomuscular atrophy: A kindred with associated abnormal androgen receptor binding. Neurology 1992;42(11):2181–2184.

122. Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS. Androgen receptor defects: Historical, clinical, and molecular perspectives. Endocr Rev 1995;16(3): 271–321.

123. Grino PB, Griffin JE, Cushard WG Jr., Wilson JD. A mutation of the androgen receptor associated with partial androgen resistance, familial gynecomastia, and fertility. J Clin Endocrinol Metab 1988;66(4):754–761.

124. Isaacs JT. Role of androgens in prostatic cancer. Vitam Horm 1994;49:433–502.

125. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics, 2000. CA Cancer J Clin 2000;50(1):7–33.

126. Maximum androgen blockade in advanced prostate cancer: An overview of 22 randomised trials with 3,283 deaths in 5,710 patients. Prostate Cancer Trialists' Collaborative Group. Lancet 1995;346(8970):265–269.

127. Thalmann GN, Anezinis PE, Chang SM, Zhau HE, Kim EE, Hopwood VL, Pathak S, von Eschenbach AC, Chung LW. Androgen-independent cancer progression and bone metastasis in the LNCaP model of human prostate cancer. Cancer Res 1994;54(10):2577–2581.