0163-769X/02/$20.00/0
Printed in U.S.A.

Endocrine Reviews 23(2):175–200

Androgen Receptor (AR) Coregulators: An Overview

CYNTHIA A. HEINLEIN AND CHAWNSHANG CHANG

George Whipple Laboratory for Cancer Research, Departments of Pathology, Urology, and Radiation Oncology, University of Rochester, Rochester, New York 14642

The biological action of androgens is mediated through the androgen receptor (AR). Androgen-bound AR functions as a transcription factor to regulate genes involved in an array of physiological processes, most notably male sexual differentiation and maturation, and the maintenance of spermatogenesis. The transcriptional activity of AR is affected by coregulators that influence a number of functional properties of AR, including ligand selectivity and DNA binding capacity. As the promoter of target genes, coregulators participate in DNA modification, either directly through modification of histones or indirectly by the recruitment of chromatin-modifying complexes, as well as functioning in the recruitment of the basal transcriptional machinery. Aberrant coregulator activity due to mutation or altered expression levels may be a contributing factor in the progression of diseases related to AR activity, such as prostate cancer. AR demonstrates distinct differences in its interaction with coregulators from other steroid receptors due to differences in the functional interaction between AR domains, possibly resulting in alterations in the dynamic interactions between coregulator complexes. (*Endocrine Reviews* 23: 175–200, 2002)

Abbreviations: AF, Activation function; ANPK, androgen receptor-interacting nuclear kinase; CAK, cdk-activating kinase; CBP, CREB-binding protein; cdk, cyclin-dependent kinase; CREB, cAMP response element-binding protein; DBD, DNA-binding domain; DHT, dihydrotestosterone; DRIP, VDR interacting protein; ECM, extracellular matrix; f-actin, filamentous actin; FAK, focal adhesion kinase; GSK3, glycogen synthase kinase-3; GST, glutathione-*S*-transferase; GTF, general transcription factor; HAT, histone acetyltransferase; HMG, high-mobility group; LBD, ligand-binding domain; NCoR, nuclear receptor corepressor; NFκB, nuclear factor κB; NLS, nuclear localization signal; PBP, PPARγ binding protein; p/CAF, p300/CBP-associated factor; PTEN, phosphatase and tensin homologue deleted from chromosome 10; PIAS, protein inhibitor of activated STAT; pol II, polymerase II; RTS, Rubinstein-Taybi Syndrome; SET, Su(var)3–9, Enhancer of Zeste, and Trithorax; Rb, retinoblastoma gene product; SMRT, silencing mediator of retinoid and thyroid hormone receptor; SRC, steroid receptor coactivator; STAT, signal transducer and activator of transcription; TAF, TBP-associated factor; TBP, TATA-binding protein; TCF/LEF, T cell factor and lymphoid enhancer factor; TFIID, transcription factor IID; TIF, transcriptional intermediary factor; TRAP, TR-associated protein; TR2 and TR4, testicular orphan receptors 2 and 4.

I. Introduction

ANDROGENS MEDIATE A wide range of developmental and physiological responses and are especially important in male sexual differentiation and pubertal sexual maturation, the maintenance of spermatogenesis, and male gonadotropin regulation (1–4). The effects of androgens are mediated through the androgen receptor (AR), a 110-kDa ligand-inducible nuclear receptor that regulates the expression of target genes through binding to an androgen response element (5–9). Mutations of the AR that alter its ability to bind androgens, or alter its transcriptional activity after ligand binding, may result in male infertility or complete or partial androgen insensitivity (10–14). Somatic AR mutations have also been found in some prostate tumors (15).

It has become clear that the transcriptional activity of AR, as well as other members of the nuclear receptor superfamily, is modulated by coregulatory proteins. Coregulators are broadly defined as proteins that interact with nuclear receptors to enhance transactivation (coactivators) or reduce transactivation (corepressors) of target genes but do not significantly alter the basal transcription rate (16). Steroid receptors have been shown to interact with other DNA-binding proteins, resulting in modulation of steroid receptor transcriptional activity. AR has been found to interact with a number of transcription factors including AP-1 (17), Smad3 (18, 19), nuclear factor κB (NFκB) (20, 21), sex-determining region Y (SRY) (22), and the Ets family of transcription factors (23). Although AR is normally thought to function as a homodimer, it has been found to heterodimerize with other nuclear receptors including the estrogen receptor (ER) (24), glucocorticoid receptor (GR) (25), and testicular orphan receptor 4 (TR4) (26). While the interaction between AR and other transcription factors or nuclear receptors has been shown to alter AR transcriptional activity, these interacting proteins are not considered to be either type I or type II coregulators (as defined below). Coregulators are not generally considered to possess specific DNA binding affinity (27, 28).

Coregulators are now known to use multiple mechanisms to influence nuclear receptor transcription and can be categorized based on their functional characteristics. Coregulators can be divided into two major types. Type I coregulators function primarily with the nuclear receptor at the target gene promoter to facilitate DNA occupancy, chromatin remodeling, or the recruitment of general transcription factors associated with the RNA polymerase II holocomplex. The functional characteristics of this type of coregulator have recently been reviewed (29). Examples of this type of coregulator are cAMP response element binding protein (CREB)-binding protein (CBP)/p300 and SRC-1, which both possess histone acetyltransferase (HAT) activity (30, 31) and interact with the basal transcriptional machinery (32, 33). Other type I coregulators include the tissue- and transcription factor-restricted TATA-binding protein (TBP)-associated factors (TAFs) (34, 35), the DRIP/TRAP/Mediator complex (36–38), and the SWI/SNF chromatin remodeling complex (reviewed in Ref. 29). While these factors have been found to function as coregulators of some nuclear receptors, they have not been characterized as AR coregulators. It remains to be established whether this represents a genuine difference in the control of AR transcription. The type II coregulators function primarily to enable the nuclear receptor to be competent to direct target gene expression by modulating the appropriate folding of AR and ligand binding or facilitating NH_2/COOH-terminal interaction. These actions may contribute to AR protein stability in the presence of agonistic ligands or influence the subcellular distribution of AR, resulting in an overall influence on AR transcriptional activity. This category includes coregulators that stabilize the ligand-bound receptor, such as ARA70 (39–41), and coregulators such as filamin (42) that facilitate the translocation of the ligand-bound receptor to the nucleus. However, it is important to note that the relative importance of many of the identified AR coregulators has not yet been examined in intact animal models, and therefore their true physiological relevance in normal and pathological conditions remains to be established.

II. The Androgen Receptor (AR)

The AR is a member of the nuclear receptor superfamily, members of which function as ligand-inducible transcription factors that mediate the expression of target genes in response to ligands specific to each receptor, including steroids, retinoids, vitamin D, thyroid hormone, hydrocholesterol metabolites, and xenobiotic agents. Nuclear receptors can be subdivided into three general types (16, 43, 44). The classical steroid receptors such as AR, the ER, progesterone receptor (PR), GR, and mineralocorticoid receptor (MR) are grouped as type 1 receptors. These nuclear receptors typically form ligand-induced homodimers, binding to inverted repeat DNA response elements. The type 2 nuclear receptors dimerize with the 9-*cis* retinoic acid receptor (RXR) and include the receptors for vitamin D3 (VDR), thyroid hormone (TR), all-*trans* retinoic acid (RAR), and the peroxisome proliferator-activated receptors (PPAR). The DNA response elements of this group of nuclear receptors are characteristically direct repeats. The third type of nuclear receptors are the orphan receptors, such as TR2, TR4, and chicken ovalbumin upstream promoter transcription factor (COUP-TF) (45–47), the ligands for which remain unclear. Although AR is normally thought to function as a homodimer, heterodimers between AR and TR4 (26), or ERα (24), have been reported and in both cases result in a decrease in AR transcriptional activity. Phosphorylation has been shown to modify the ligand-induced activity of steroid receptors, notably AR (48–52) and ER (53), as well as other members of the nuclear receptor superfamily (54). However, it has become apparent recently that at least some nuclear receptors may also become transcriptionally active independently of their cognate ligand through phosphorylation (28, 55, 56), although the physiological impact of ligand-independent activation has yet to be established.

AR, in common with other members of the nuclear receptor superfamily, can be subdivided into four functional domains: the NH_2-terminal transactivation domain (or A/B domain), the DNA-binding domain (DBD), hinge region, and ligand-binding domain (LBD). Using deletion and mutational analyses of nuclear receptors in transfection experiments, two transcriptional activation functions have been identified. An NH_2-terminal activation function (AF-1) functions in a ligand-independent manner when artificially separated from the LBD, creating a constitutively active receptor (57, 58). A ligand-dependent AF-2 function is located in the LBD, and mutation or deletion of the AF-2 domain dramatically reduces transcriptional activation in response to ligand (58–63).

The NH_2-terminal domain is the most variable between nuclear receptors in terms of both length and sequence. In the case of AR, there are two discrete regions within the NH_2 terminus that contribute to transactivation. The full-length receptor requires a region primarily located between amino acids 141 and 338 for full ligand-inducible transcriptional activity (64, 65). This region contains a polymorphic polyglutamine repeat that ranges from 8 to 31 repeats in normal individuals, with a modal length of 20 (6, 66). Charged, glutamine-rich regions are found in a number of coactivators and transcription factors, including SRC-3, CBP, TAFII130, and Sp1, and are thought to modulate protein-protein interactions (67–69). Longer polyglutamine tract length results in decreased AR transcriptional activity *in vitro* (70, 71). Clinically, men whose AR has a polyglutamine tract length at the long end of the normal range ($\geq$28) have an increased incidence of impaired spermatogenesis and infertility (72). Expansion of the polyglutamine tract to more than 40 repeats causes the rare neuromuscular disorder, spinal and bulbar muscular atrophy (SBMA or Kennedy's disease), which is also associated with decreased virilization, testicular atrophy, reduced sperm production, and infertility (73). The polyglutamine tract forms part of the interaction surface for the AR coactivator ARA24 and expansion of the polyglutamine tract from 25 to 49 repeats results in a reduction of AR-ARA24 interaction, possibly because the expanded glutamine repeats result in an abnormal conformation of the AR NH_2-terminal (74). The ligand-independent AF-1 region of the AR NH_2-terminal is located from amino acids 360–494 (57). The transactivational activity of the AR AF-1 region is

only detected in AR fragments lacking the LBD and is thought to function in this context by recruiting coactivators and/or general transcription factors (GTFs). However, amino acid substitutions in the AF-1 domain have been identified in patients suffering from complete androgen insensitivity (75, 76) and in patients with oligospermia (77), indicating the importance of this region in function of the full-length AR. A motif within the AF-1 domain, 433(WXXLF)473, has been shown to interact with the AR LBD (78). The AR NH_2/COOH-terminal interaction has been shown to be facilitated by several coactivators and is important in stabilizing bound ligand (62, 79, 80). It is possible that in the full-length AR protein, the AF-1 domain functions to interact with coactivators and provides an interaction surface for the AR COOH terminus. Because AR has two separate NH_2-terminal transactivational domains, it is possible that each domain interacts with different coregulators or transcription factors, possibly in a promoter context-dependent manner (57).

The DBD of all members of the nuclear receptor superfamily consists of two zinc fingers that recognize specific DNA consensus sequences. AR binds as a dimer to the consensus inverted repeat androgen response element, GGTACAnnnTGTTCT, as well as to more complex response elements (81–85). Some coregulators exert their function on AR transcription by modulating the ability of AR to bind its recognition sequence, a function that is considered to be one of the characteristics of a type I coregulator (29). The AR corepressor calreticulin inhibits AR transactivation by interacting with the AR DBD to prevent DNA binding (86). Alternatively, the coactivator RAF binds to the NH_2-terminal domain of AR but exerts its effect by enhancing AR DNA binding (87).

As the name implies, the hinge region of hormone receptors links the DBD and LBD. AR, in common with other steroid receptors, has a ligand-dependent bipartite nuclear localization signal (NLS) located in the COOH terminus of the DBD and the hinge domain (88, 89). The hinge NLS of GR has been shown to interact with importin α to mediate nuclear trafficking (90). In AR, the NLS is located between amino acids 617 and 633 (88, 89). Clinically, an arginine-to-proline substitution at position 617 (R617P) of AR has been observed in two unrelated patients with partial or complete androgen insensitivity (91, 92) and as a somatic mutation in a metastatic prostate cancer specimen (93). This mutation does not alter the apparent dissociation constant (K_d) for dihydrotestosterone (DHT) or effect DNA binding, but abolishes transcriptional activation in response to DHT (91, 92). It is therefore possible that the R617P mutation inhibits the ability of AR to translocate to the nucleus.

The LBD of AR, in addition to forming the ligand-binding pocket, mediates the interaction between AR and heat shock proteins (94) and interacts with the AR NH_2 terminus to stabilize bound androgen (62). X-ray crystallographic studies indicate that the LBD has a similar structure between nuclear receptors, with the ligand-binding pocket formed by 11–13 α-helices (95–97). By convention, the LBD α-helices are numbered according to those of the RXRα crystal structure (98, 99). X-ray crystallographic studies demonstrate that AR, similar to PR, ERα, and ERβ, lacks a helix 2 (95, 97, 100, 101). Comparison of the crystal structures of receptors in the absence of ligand and in the ligand-bound state show that ligand binding induces a conformational change in which helix 12 and the AF-2 domain fold back across the ligand-binding pocket (97, 99). Crystallographic analysis of AR bound to the synthetic androgen R1881 demonstrates that it closely resembles the structure of PR (100). However, the AR helix 12 is split into two shorter helical segments in this structure, which is not observed in PR (100). It is unclear whether this conformation of the AR helix 12 is ligand specific, since the structure of AR bound to DHT showed a continuous helix 12 (101). In the case of some nuclear receptors, including PPARγ (96) and ERα (102), the conformation of helix 12 upon ligand binding generates a ligand-dependent interaction surface for coregulators (99). Although the crystal structure of AR suggests that ligand binding induced a LBD conformation similar to ER and potentially generates a similar coregulator interaction surface, functional analyses of the full-length receptors suggest that distinct differences exist between the coregulator interaction domains of AR and ER. This may be because the interaction between the AR NH_2 terminus and the LBD generates a potential coregulator interaction surface that differs from that of ER. Unfortunately, to date, the three-dimensional structure has not been determined for any full-length nuclear receptor.

Members of the SRC family of coactivators [SRC-1, transcriptional intermediary factor 2 (TIF-2), and SRC-3] typically interact with the LBD of nuclear receptors through LXXLL motifs (where L is leucine and X is any amino acid) that form amphipathic α-helices. The LXXLL domains of the coactivator interact with the nuclear receptor partly through the hydrophobic surface of the receptor AF-2 domain (103, 104). However, the AF-2 of AR is relatively weak compared with ER and GR. In transfection experiments, the NH_2 terminus of AR is able to mediate transcription of a reporter gene to the same extent as the full-length receptor in the presence of androgen (65). While SRC-1 and TIF-2 interact with the AR AF-2, this interaction is not essential for coactivation (62, 63). Instead, SRC-1 and TIF-2 primarily interact with the AR NH_2 terminus and possibly the DBD. This interaction, in contrast to several other nuclear receptors, does not require the coactivators to contain intact LXXLL motifs (62, 63) (Fig. 1). A SRC-1 mutant carrying no functional LXXLL motifs was able to potentiate AR transcriptional activity to the same extent as wild-type SRC-1 (63). However, the absence of LXXLL motifs abolishes the ability of SRC-1 to enhance ER transactivation (63, 105). Mammalian two-hybrid assays and glutathione-*S*-transferase (GST) pull-down interaction studies suggest that the AR NH_2 and COOH termini interact directly and that this interaction is mediated through LXXLL-like motifs present in the AR NH_2 terminus interacting with the AR AF-2 domain (62, 78, 106). It is possible that the AR NH_2 terminus competes with LXXLL-containing coactivators for binding to the AR AF-2 (78, 106). The type II coregulator ARA70 contains an LXXLL motif that forms part of the interaction surface with PPARγ and RXRα (107). However, mutation of the ARA70 LXXLL motif does not alter its ability to interact with AR (S. Yeh and C. Chang, manuscript in preparation). Point mutagenesis studies within the AR LBD suggest that the NH_2/COOH-

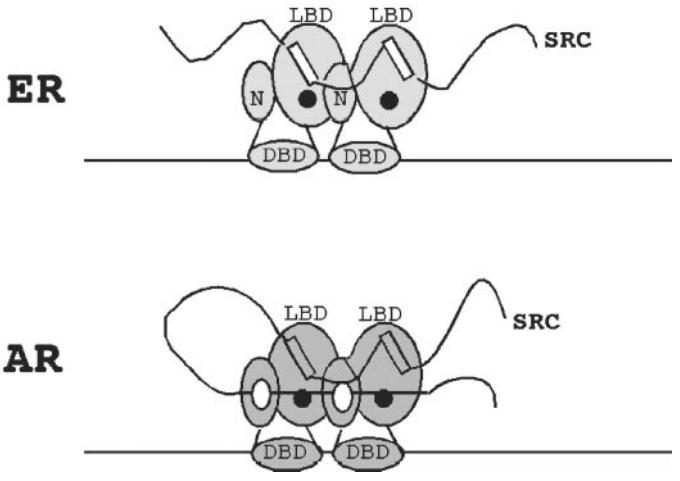

FIG. 1. Members of the SRC family of coactivators interact differently with AR and ER. The receptor LBD, DBD, and NH_2 terminus (N) are indicated. The ER dimer binds to SRC-1 through an interaction between the ER LBD and the LXXLL motifs of SRC-1 (ligand bound to the LBD is represented by a *black dot*; the contacting LXXLL motifs are represented by *open rectangles*) (108, 185). The LXXLL motifs of SRC-1 interact with the ligand-bound LBD of AR, but this interaction is not required for SRC-1 to enhance AR transcription (indicated by *solid rectangles*) (63). However, the interaction between SRC-1 and the AR NH_2 terminus is necessary for SRC-1 function. SRC-1 interacts with AR through a glutamine-rich region (indicated by *open circles*) located NH_2 terminal to the LXXLL motifs (63).

terminal interaction positions or stabilizes helix 12 across the ligand-binding pocket, resulting in a reduced dissociation rate of bound androgen (62). In the case of ER, SRC-1 interacts with the LBD to stabilize this interdomain interaction (108). In contrast, SRC-1 and TIF-2 do not stabilize the NH_2/COOH-terminal interaction of AR (62). Instead, this stabilization may be mediated by CBP (80).

While the AR NH_2/COOH-terminal interaction may reduce the importance of LXXLL motifs in AR coregulators, it is possible that similar short motifs may function in coregulator-AR interactions. Using a peptide library to screen for AR-interacting peptides, we have found that FXXLF motifs (where F is phenylalanine) strongly interact with AR. A FXXLF motif is present in the AR NH_2 terminus and is necessary for the NH_2/COOH-terminal interaction (78). This motif is also present in several AR coregulators including ARA70, ARA55, ARA54, and FHL2. Mutation of the FXXLF motif to FXXAA in ARA70 and ARA55 reduces their ability to enhance AR transcription. The functional interaction between coregulator FXXLF motifs and the AR NH_2 and COOH termini is currently under investigation (C.-L. Hsu and C. Chang, manuscript in preparation).

III. Interaction of AR with General Transcription Factors (GTFs)

Transcriptional activation by steroid receptors ultimately requires the recruitment of RNA polymerase II (pol II) to the promoter of target genes. Transcription initiation has been extensively reviewed elsewhere and will be summarized here only briefly (109). Pol II recruitment is mediated through the assembly of GTFs to form the preinitiation complex, the first step of which is the binding of TBP near the transcriptional start site. TBP is part of a multiprotein complex, transcription factor IID (TFIID), which also contains general and promoter-specific TBP-associated factors (the TAF_{II} proteins). TBP binding induces DNA bending, bringing sequences upstream of the TATA element in closer proximity, presumably enabling interaction between GTFs and steroid receptor-coregulator complexes. TFIIB binds directly to TBP and functions to recruit the TFIIF-pol II complex. TFIIF domains, in addition to interacting with TFIIB and pol II, apparently also serve in transcription initiation and elongation. The ATPase and kinase TFIIE and the helicase TFIIH are then recruited to pol II to facilitate DNA strand separation before transcription initiation.

While one mechanism of coregulator action is to facilitate or prevent communication between the nuclear receptor and the transcriptional machinery, nuclear receptors have been shown to directly interact with various GTFs (35, 110–112). GTFs themselves are not considered coregulatory proteins because they influence the basal transcription rate (16). We and others have shown that the AR NH_2 terminus is able to recruit TFIIF directly (113, 114). AR and RARα have both been demonstrated to interact directly with TFIIH through their NH_2-terminal domains (110, 114). TFIIH is a multisubunit factor consisting of six core subunits (p89, p80, p62, p52, p44, and p34) and a protein kinase moiety CAK [cyclin-dependent kinase (cdk)-activating kinase]. CAK itself is composed of three catalytic subunits, MAT1, cyclin H, and cdk7 (115). Immunoprecipitation of endogenous AR and CAK subunits in the prostate cancer cell line LNCaP demonstrated that AR interacts with cdk7. The ability of the AR interaction with CAK to enhance AR transcription in response to androgen was demonstrated in cotransfection experiments in prostate cancer cells in which transfection of all three CAK catalytic subunits resulted in a 2- to 3-fold increase in AR transactivation (114). In the case of CAK interaction with RARα, cdk7 functions to phosphorylate RARα at Ser-77, a residue known to be critical for RARα AF-1 activity (110). Phosphorylation of AR is known to modulate AR transcriptional activity, but it is not yet known whether CAK enhances AR transcription by phosphorylation of the AR NH_2 terminus or through other mechanisms. It has been found recently that AR interacts with the general elongation factor PITALRE [(pro-ile-thr-ala-leu-arg-glu) kinase] (116), suggesting that the interaction of AR with TFIIF and TFIIH may assist in the recruitment of elongation factors to AR target promoters. It should be noted that the ability of AR to bind its response element and recruit GTFs is not necessarily sufficient to allow transcription to occur. The suppression of NFκB transcription by GR results from the prevention of pol II phosphorylation after NFκB has bound to its response element and recruited the GTFs of the preinitiation complex (29, 117).

The AR coactivator ARA160 was initially isolated as a factor capable of inhibiting TBP activation of the human immunodeficiency virus 1 long terminal repeat (118, 119). However, ARA160 enhances the ligand-dependent transactivation of AR, GR, and PR (119). While the mechanism of these divergent effects is unclear, it is possible that ARA160 is capable of regulating TFIID by altering the DNA binding of TBP (118). ARA160 is a target of the FER nuclear tyrosine

kinases (120), suggesting that ARA160 may modulate AR transcription by coordinating a kinase signal cascade with the basal transcriptional machinery.

IV. AR Coactivators

Steroid receptor coregulators were initially postulated to exist on the basis of transcriptional interference (or squelching) in transfection experiments (121, 122). The ligand-induced transcriptional activity of a receptor was found to be decreased in the presence of a different transfected, ligand-bound receptor. The magnitude of the transcriptional interference, as well as the receptor domains that mediated the interference, were found to vary between receptors and between cell types (121). These observations suggested the presence of limiting mediators of steroid receptor transcription and indicated that these mediators vary between cell types. In confirmation of this hypothesis, biochemically defined receptor interacting proteins were subsequently identified (123, 124). In the past few years, a large number of nuclear and steroid receptor coactivators have been cloned and have been shown to augment receptor-mediated transactivation (reviewed in Refs. 16 and 28).

As shown in Table 1, many coactivators have been identified as enhancing the ligand-induced transcriptional activity of AR. However, the relative importance of these coactivators for any particular cell type remains unclear. A demonstration of the transcriptional effect of a newly isolated coregulator is typically done in transient transfection experiments that examine the ability of the putative coregulator to alter the transcriptional activity of an endogenous or transfected nuclear receptor on an artificial reporter construct. The milieu of endogenous coregulators will obviously influence the ability of an exogenous coactivator to enhance transcription and may account for many of the differences observed between cell lines (for example, Refs. 119 and 125). Even within the same cell line, cell density and culture conditions are known to alter steroid responsiveness (126). Additionally, the relative promoter strength or design of the expression vectors used, and the receptor-coregulator ratio, may affect the ability of the of the coregulator being examined to counteract the influences of the endogenous coregulators. These combined factors may explain the divergent results that have been reported for several coactivators (40, 127–129). Genetic manipulation of the mouse will be helpful in determining the extent to which the function of any one coregulator can be complemented by others and will assist in defining the relative importance of a given coregulator in particular tissues and in the transcriptional regulation by different steroid receptors. To date, relatively few coregulators have been targeted for disruption. Disruption of SRC-1 results in partial hormone resistance, particularly to thyroid hormone (130, 131). SRC-3 was shown to have particular importance in mammary gland development in SRC-3 null mice (132). In contrast, mice null for the PBP/DRIP205/TRAP220 (the PPARγ binding protein) coregulator, the member of the DRIP/TRAP complex that serves as a coactivator for a number of nuclear receptors (37, 133, 134), die at midgestation (135). While this demonstrates the importance of the DRIP/TRAP complex, this complex has not yet been characterized as a coregulator of AR.

One of the major mechanisms through which coregulators were initially envisaged to function was by forming a bridge between the DNA-bound nuclear receptor and the basal transcriptional machinery, a characteristic now considered to be one of the classifications of a type I coregulator (29). By stabilizing or recruiting the RNA pol II holoenzyme complex to the nuclear receptor target gene, such a coactivator would be able to enhance transcription. Although a wide range of interacting proteins has been shown to coactivate nuclear receptors, relatively few coactivators have been demonstrated to function in precisely this manner. CBP and the p300/CBP-associated factor (p/CAF) have been copurified with the RNA pol II holoenzyme complex (32, 136), and in GST pull-down assays SRC-1 has been shown to interact with TBP and TFIIB (33). As indicated below, a number of AR coactivators have been characterized as interacting with CBP and/or p/CAF and may therefore link AR to the basal transcriptional machinery through these proteins. It has been postulated that coregulators exist in partially assembled holocomplexes in the nucleus, similar to the RNA pol II holocomplex (29, 137). These coregulator holocomplexes are suggested to be composed of specific combinations of coactivators that are differentially located in the nucleus (29). In this model, a dynamic association between coactivator holocomplexes mediating chromatin modification or recruitment of the basal transcription factors occurs with the DNA-bound receptor to allow transcription from the target gene (29, 137). Support for this model in terms of steroid receptors has come from chromatin immunoprecipitation, chromatin reconstitution, and fluorescent recovery assays examining the dynamics of coactivator association with ERα (138–140). In the presence of estradiol (E2), DNA-bound ERα rapidly associates with SRC-3, PBP, and p300 and subsequently recruits pol II (139). After the initiation of transcription, ERα, SRC-3, and PBP cease to be associated with the promoter, presumably to begin another cycle of reinitiation (139). Similar results have been found for PR in chromatin reconstitution experiments (141). As indicated above, the manner in which AR interacts with SRC proteins is different from ER, and PBP has not yet been characterized as an AR coregulator. It is therefore possible that the initial coactivator holocomplex that associates with the DNA-bound AR is different from the initial ERα holocomplex.

Coregulator mutations that prohibit the appropriate multiprotein complex assembly would be expected to inhibit steroid receptor transcriptional activation, possibly in a dominant manner. The type I coregulator ARA54, a coactivator of AR and PR (142), functions as a dimer (143). A COOH-terminal truncation of ARA54 and a COOH-terminal truncation carrying a glutamic acid to lysine mutation at amino acid 472 function as dominant negative mutants of AR transcription. In addition, these mutations inhibit androgen-induced prostate cell growth (143). Mutations of the coregulator ARA70 that prevent dimerization and interaction with other coregulatory proteins also exert a dominant-negative affect on AR transactivation (M. Rahman and C. Chang, unpublished observations).

Coactivators may also function to facilitate ligand binding,

TABLE 1. AR coactivators

Coactivator	Region	Comments	Selected references
ANPK (PKY)	DBD	Serine/threonine kinase that does not phosphorylate AR. Enhances AR protein stability.	(160)
ARA24 (Ran)	NH_2-term.	Interacts with the NH_2-terminal domain that contains the polyglutamine repeat. Expansion of the AR polyglutamine tract from 25 to 49 results in a 50% reduction in AR-ARA24 interaction.	(74)
ARA54	LBD	Enhances AR(T877S) transcription in response to DHT, E2, and HF. wtAR transcription is only enhanced by ARA54 in the presence of DHT. Contains a RING finger and B-box domain. Also coactivates PR.	(142)
ARA55 (Hic5)	LBD	Contains a LIM domain. The mouse homolog is inducible by TGFβ1. Enhances wtAR transcription in response to DHT and AR(T877A) in response to DHT, E2, and HF. Also coactivates GR and PR.	(263, 272)
ARA70 (RFG, ELE1)	DBD-LBD	Enhances the transactivation of both wtAR and AR(T877A) in response to DHT and E2; enhances wtAR and AR(T877S) in response to androstenediol, HF, and casodex. Also coactivates PPARγ, shows marginal enhancement of ER and GR. May function as a bridging factor to p/CAF and TFIIB. Functions synergistically with ARA160 to enhance AR transcription. A chromosomal translocation resulting in the production of an ARA70 NH_4-terminal-Ret thymidine chimeric protein is oncogenic in papillary thyroid carcinomas.	(40, 41, 107, 125, 127, 186, 202, 322)
ARA160 (TMF)	NH_2-term.	Shows a greater than additive interaction with ARA70. Also enhances transcription by GR and PR.	(119)
ARA267 (NSD1)	NH_2- and COOH-term.	Contains SET and PHD domains. Also interacts with RAR, RXR, ER, and TR.	(224, 225)
ARIP3 (PIASαx)	DBD	Facilitates the interaction between the AR NH_2- and COOH-terminals. Represses AR mediated transcription of the probasin promoter at a high ratio (1:200 AR:coactivator).	(79)
BAG-1L	[a]	Also functions to regulate hsp70	(184)
β-Catenin	[a]	Enhances the transcription of AR(T877A) in response to androgen. Enhances wtAR transcription in response to T, androstenedione, and E2. β-Catenin also reduces the antagonistic effect of bicalutamide on AR in the presence of androgen. Activated by the Wnt pathway to complex with TCF transcription factors.	(162, 170)
BRCA1	NH_2- and COOH-term.	Breast cancer susceptibility gene. Interacts with CBP. Enhances AR transcription synergistically with ARA70 and ARA55. Disease-associated mutations of BRCA1 reduce its ability to enhance AR transcription.	(314, 323–325)
Caveolin-1	NH_2-term. and LBD	Membrane protein associated with caveoli membrane structures.	(326)
CBP	NH_2-term. DBD	Facilitates AR NH_2/COOH-term. interaction. Possesses acetyltransferase activity. Interacts with members of the SRC family. Coactivates multiple transcription factors. Mutated in RTS.	(21, 80, 144, 241–243, 310, 327)
Cyclin E	NH_2-term.	Enhances AR transcriptional activity independently of cell cycle progression.	(328)
E6-AP	[a]	Contains separable coactivation and ubiquitin ligase domains. Also interacts with PR, GR, and ER.	(254)
FHL2 (DRAL)	Requires intact AR	Expressed predominantly in the heart; expression also seen in the epithelia and stroma of the prostate. LIM only protein without an LXXLL motif.	(275)
Gelsolin	LBD	Enhances AR transcription in prostate and muscle cells. Also functions as an actin filament severing and capping protein.	(K. Nishimura and C. Chang, manuscript in preparation)

TABLE 1. Continued

Coactivator	Region	Comments	Selected references
HMG-1/-2	[a]	HMG-1 and HMG-2 represent separate gene products with extensive sequence identity. Also enhances transactivation by PR and GR. Enhances DNA binding of AR, PR, ER, and GR. Is found as an abundant chromatin-associated protein that does not bind a specific DNA recognition sequence.	(212)
hsp40 (dnaJ, ydj1p)	LBD	Member of the chaperone heterocomplex. Mutation of hsp40 in yeast reduces AR transcriptional activation.	(329)
PGC-1 (LEM6)	[a]	General nuclear receptor coactivator. Originally identified in mouse as a cold-induced coregulator in brown fat. In human tissue, the predominant site of expression is in skeletal muscle.	(330–332)
PIAS1	DBD-LBD	Expression in the rat testes coincides with the onset of spermatogenesis. Also coactivates GR but functions as a corepressor of PR.	(159)
RAF (IDE)	NH_2-term.	Enhances AR and GR DNA binding.	(87, 333)
Rb	NH_2-term.-DBD	Tumor suppressor. Enhances transcription of wtAR and AR(T877S). Interacts with the TR coactivator Trip230 to repress TR transcription.	(313, 334)
RIP140	NH_2-term.-DBD (LBD[b])	Functions as a coactivator at low receptor-coactivator ratios, but as a repressor at a high ratio. Influences the transcriptional activity of ER, PPARγ, and PPARα.	(63, 80, 322)
SNURF (RNF4)	DBD	RING finger protein; may recruit the chromatin remodeling factor HMGI(Y). Also interacts with ER and PR.	(335, 336)
SRA	[a]	Also enhances transcription by PR, GR, and ER. Enhances transactivation through the AF-1 domain of GR and PR. Functions as a RNA transcript and associates with a SRC-1 containing coregulator complex.	(337)
SRC-1 (NCoA-1)	NH_2-term.-DBD (LBD[b])	Unlike other nuclear receptors which interact with SRC-1 through their LBD, AR interacts through its NH_2-terminal and DBDs. Enhances AR NH_2/COOH-term. interactions. Interacts with CBP/p300. General nuclear receptor coactivator. Possesses weak acetyltransferase activity.	(31, 62, 80, 128, 144, 262, 338)
SRC-3 (Rac3, ACTR, AIB1, p/CIP, TRAM1)	[a]	Also enhances transcription by TR, PR, and RAR. Interacts with CBP/p300. Possesses acetyltransferase activity.	(41, 67, 146, 153, 339)
Supervillin	NH_2- and COOH-term.	Actin-binding protein. Also interacts with GR.	(175)
TIF2 (GRIP1, NCoA-2, SRC-2)	NH_2-term.-DBD	General nuclear receptor coactivator. Mutations of AR that interrupt NH_2/COOH domain interactions also disrupt AR interactions with TIF2.	(62, 151, 340, 341)
Tip60	Hinge-LBD	Member of the MYST/SAS family of histone acetyltransferases. Also coactivates PR and ER.	(298)
Ubc9	DBD-hinge	Covalently links the ubiquitin-like molecule SUMO-1 to target proteins. This activity is separable from coactivation. Also interacts with GR.	(342)
Zac-1	LBD	Can function as a coactivator of AR in HeLa cells but as a corepressor in 1471.1 cells. Also interacts with ERα, TR, and GR. In HeLa cells, coactivation is synergistic with TIF2.	(343)

term., Terminal; HF, hydroxyflutamide; wtAR, wild-type AR.
[a] Although interaction with AR has been demonstrated, the precise domain of AR that interacts with the coregulator has not yet been determined.
[b] This domain has been found to interact with the coregulator but this interaction is not essential for coregulation.

promote receptor nuclear translocation, or mediate signal transduction. Figure 2 depicts the multiple mechanisms that type I and type II coregulators may use to ultimately influence AR transcriptional activity. A number of AR coactivators can be grouped into families on the basis of structural and functional homology. Such families include members of the SRC family and some filamentous actin (f-actin)-binding proteins. However, not all AR coactivators have been found

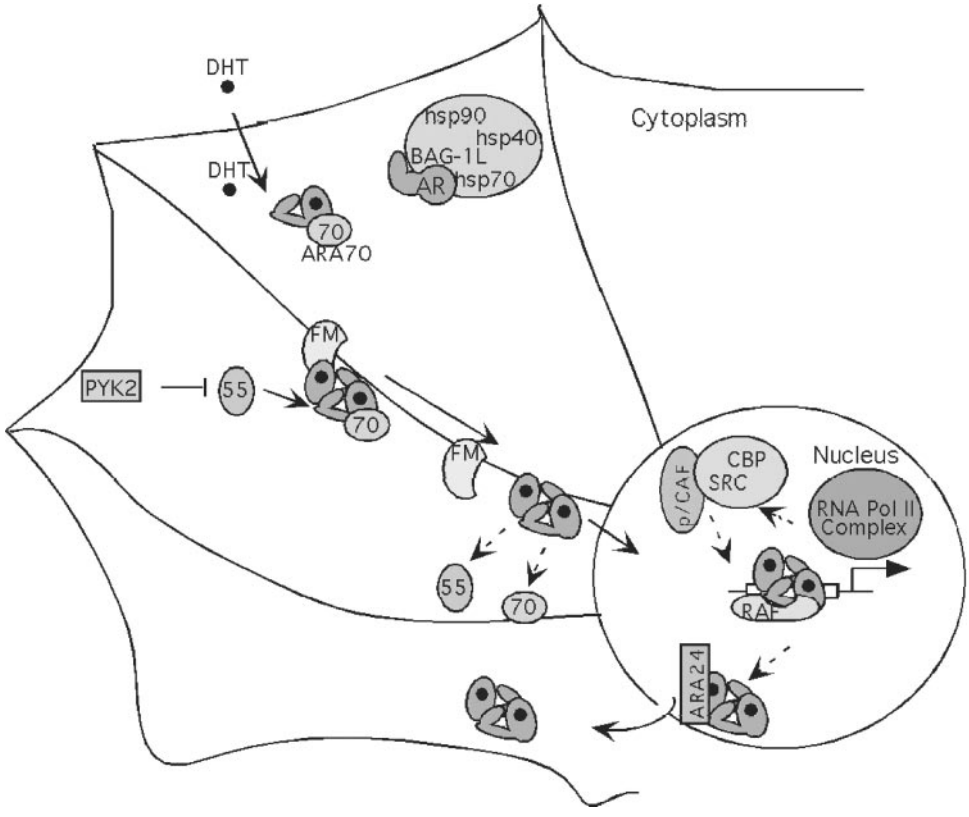

FIG. 2. Integration of different coactivator functions to enhance AR transcriptional activity. The chaperone heterocomplex, including the type 2 coactivators hsp40 and BAG-1L, assist in the appropriate folding of AR to a conformation permissive to ligand binding. DHT binding promotes receptor dimerization and NH_2/COOH-terminal interaction. Ligand binding is stabilized by type 2 coactivators such as ARA70. Interaction of some coactivators, such as ARA55, may be altered by kinases. As depicted, activation of the PYK2 kinase blocks ARA55 association with AR. Type 2 coregulators may also influence the ability of AR to be translocated to the nucleus, here shown by the f-actin binding protein filamin (FM). Binding to the promoter of target genes is assisted by certain type 1 coactivators (shown here is RAF). Separate type 1 coactivator complexes are recruited to facilitate transcription. After transcriptional initiation, AR may be recycled from the promoter. See text for a detailed discussion.

to be members of distinct protein families. These coactivators will be grouped, for the purpose of this review, by the mechanisms through which they have been found to enhance AR transcriptional activity. It is important to note that many coactivators may ultimately be found to use multiple mechanisms to influence AR transcription and that further characterization will reveal that some coactivators integrate a number of functions.

A. The steroid receptor coactivator (SRC) family

The members of the SRC family of nuclear receptor coregulators are among the most extensively characterized; because their characteristics have been reviewed recently (16), they will be discussed here only briefly. SRC-1 was initially isolated from a yeast two-hybrid screen as a protein that interacted with the PR LBD (128) and has subsequently been shown to enhance the ligand-dependent transcription of a number of nuclear receptors, including AR (62, 80, 144). The other SRC family members, TIF-2 and SRC-3, share a similar structural organization to SRC-1. All SRC coactivators are characterized by NH_2-terminal tandem basic helix-loop-helix and PAS (Per/Arnt/Sim homology) domains, contain three LXXLL motifs in the central portion of the protein, and carry a COOH-terminal glutamine-rich region. The SRC coactivators are able to recruit additional nuclear receptor coregulators including CBP and p/CAF (105, 145, 146). Additionally, SRC-1 has been found to interact with TFIIB and TBP (33). As described above, the interaction between SRC coregulators and AR differs from that of ER, GR, RAR, PPARα, and PPARγ (96, 103–105, 147, 148). AR interaction does not require that SRC-1 or TIF-2 carry intact LXXLL motifs, although other nuclear receptors require that at least a subset of SRC-1 or TIF-2 LXXLL motifs be present for interaction and coactivation (62, 63, 148, 149). While the AF-2 domain of AR is capable of interaction with SRC-1 and TIF-2, this interaction is weak compared with the LBD-AF-2 of GR and ER (62, 63). Mutagenesis studies additionally suggest that SRC coregulators are recruited to AR by the AR NH_2-terminal and DBD (62). SRC-1 and SRC-3 have both been characterized as HATs with the acetyltransferase domain located in the COOH terminus of the protein partially overlapping the glutamine-rich region (31, 146). While TIF-2 contains a COOH-terminal domain that is 38% identical with the HAT domain of SRC-1 at the amino acid level (146), it has not yet been established that TIF-2 is also an acetyltransferase. Increased histone acetylation is correlated with transcriptional activity (150), and the presence of HAT activity in coactivators suggests that they may play a role in establishing or maintaining a transcriptionally open chromatin struc-

ture at the promoter of nuclear receptor target genes. Because of the ability of at least some of the SRC family members to recruit the basal transcriptional machinery and function as HATs, the SRC proteins are considered to be type I coregulators (28, 29).

Although all members of the SRC family have been shown to enhance AR transcription in transfection assays (41, 62, 151), targeted disruption of SRC-1 in mice does not cause a significant androgen-insensitive phenotype (131), suggesting that other coactivators are able to substantially compensate for the loss of SRC-1. Male SRC-1 null mice show normal fertility (131), suggesting that the extremely androgen-sensitive process of spermatogenesis is not substantially altered. However, the testes of SRC-1 null mice were observed to be 19% smaller as a proportion of body weight compared with wild-type controls. Androgen responsiveness in SRC-1 null males was assessed by measuring prostate growth in castrated mice in response to androgen administration. After 7 d of testosterone (T) treatment, prostate plus urethral weight was 34% less than wild-type control-treated mice, again suggesting a mild androgen resistance (131). The SRC family of coactivators has recently been found to be functionally redundant to each other for enhancement of ERα transactivation (152). It is therefore possible that TIF-2 and/or SRC-3 can compensate for the loss of SRC-1 *in vivo*. The TIF-2 mRNA level is increased in the testes of the SRC-1 knockout mice, possibly compensating for the absence of SRC-1 and enabling spermatogenesis to continue (131). SRC-3 is known to localize to the AR-positive Sertoli cells of the testes (153, 154) and may also contribute to the maintenance of AR function in the absence of SRC-1. However, given the number and diversity of AR coregulators, it is possible that other, non-SRC coactivators may also contribute to the compensation for the lack of SRC-1.

B. The PIAS [protein inhibitor of activated signal transducer and activator of transcription (STAT)] family

The PIAS family comprises a number of related genes, the first member of which was cloned by its ability to interact with the STAT1 transcription factor (155). The STAT transcription factor family members are phosphorylated by the JAK nonreceptor tyrosine kinases in response to cytokine or growth factor stimuli, such as interferon, interleukins, and epidermal growth factor. STAT phosphorylation causes factor dimerization and translocation to the nucleus where STAT dimers regulate target gene transcription (156). PIAS1, however, functions to modulate this activation pathway by interacting with phosphorylated STAT1 to prevent DNA binding (155). Similarly, PIAS3 binds to phosphorylated STAT3 to inhibit its ability to bind DNA (157). The PIAS proteins, however, function not only to inhibit DNA binding of their interaction partners. PIASxβ (Miz1) has been shown to interact with the homeobox protein Msx2 to enhance the affinity of Msx2 for its DNA recognition sequence (158).

Two PIAS family members have been shown recently to interact with and coactivate AR. PIAS1 was isolated in a yeast two-hybrid screen as a factor capable of interacting with AR in an androgen-dependent manner. The interaction with AR occurs through the PIAS1 NH_2 terminus, which contains three LXXLL motifs (159). PIAS1 is predominantly expressed in the testes with expression observed in the Sertoli and Leydig cells as well as in spermatogenic cells (159). In addition, PIAS1 may be a target of another AR coactivator and kinase ANPK (androgen receptor-interacting nuclear kinase) (160). PIASxα (ARIP3) has also been found to be an AR coregulator and, like PIAS1, is primarily expressed in the testes (79). PIASxα also interacts with AR and appears to enhance AR transactivation through facilitating AR NH_2/COOH-terminal interaction rather than DNA binding affinity (79). While both PIAS1 and PIASxα contain multiple LXXLL motifs, it has not yet been determined whether the LXXLL motifs are important for interaction with AR. If so, this would be in contrast to members of the SRC family, which apparently do not require intact LXXLL domains to enhance AR transcription (62, 63). It is presently unclear whether the PIAS inhibition of STAT and coactivation of AR represent distinct regulatory pathways or whether PIAS proteins mediate cross-talk between cytokine (161) and androgen signaling in the testes. Because the PIAS proteins influence the DNA binding ability of the STAT transcription factors, they may be considered type I coregulators. However, it remains to be established whether the PIAS coregulators demonstrate other characteristics of type I coregulators in the context of AR, such as recruitment of chromatin-remodeling proteins or GTFs, or whether, when bound to AR, they function as type II coregulators.

C. Filamentous actin (f-actin)-binding proteins

Actin forms a major structural component in eukaryotic cells. The organization and reorganization of f-actin in the cytoskelton and membrane skeleton are involved in diverse cellular aspects and processes including cell morphology, migration, adhesion, and apoptosis (42, 162–164). f-Actin-binding proteins mediate the ability of actin to form bundles or arrays defining the morphology of cells and regulate actin polymerization and depolymerization through severing actin filaments or sequestering actin monomers (165). Several proteins initially characterized as actin-binding proteins or involved in actin-binding complexes have been found to coactivate transcriptional regulators, including AR (Fig. 3). Both type I and type II coregulators have been found to be actin-binding proteins.

β-Catenin plays an important role in cell-cell adhesion by linking the actin cytoskeleton to adherens junctions formed by cadherin and α-catenin (162, 166). In addition to this structural role, β-catenin is a downstream effector of the Wnt signaling pathway that regulates a number of cellular processes including cellular differentiation, proliferation, and migration (167). Activation of the Wnt pathway results in inactivation of the glycogen synthase kinase-3 (GSK3) kinase, causing an increase in available cytoplasmic β-catenin (Fig. 3), which forms a complex with members of the TCF/LEF (T cell factor and lymphoid enhancer factor) family of transcription factors in the nucleus and allows transcriptional activation by TCF/LEF (168), suggesting that, in this context, β-catenin functions as a type I coregulator. The activities of β-catenin in cell adhesion and gene expression can be separated in that β-catenin mutants that are unable to interact

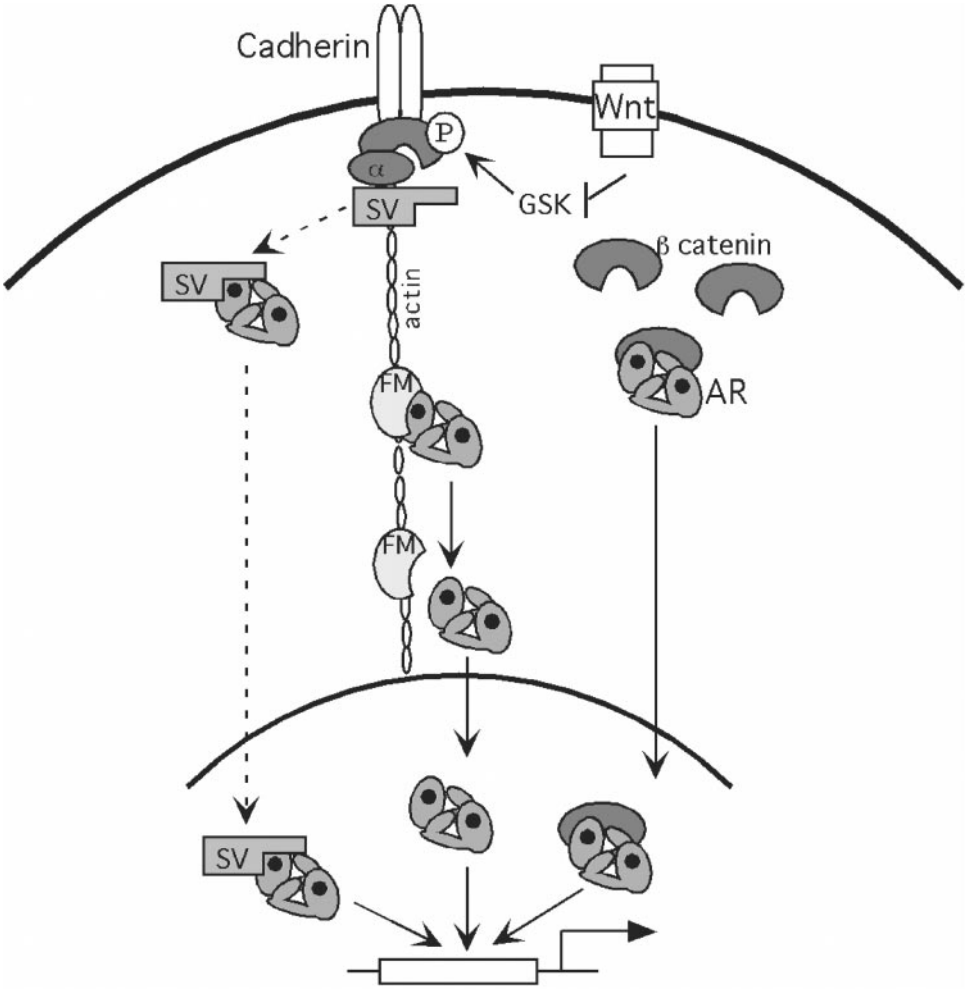

FIG. 3. Model of actin-associated proteins as AR coactivators. When phosphorylated by GSK, β-catenin is associated with α-catenin (α) and cadherins. Inactivation of GSK results in an increased cytoplasmic availability of β-catenin, allowing it to associate with AR and function as a type I coregulator (162, 166, 170). Supervillin (SV) associates with actin and cadherins (176). The precise mechanism through which supervillin enhances AR transcription has not yet been determined. However, because supervillin can localize to the nucleus under some cellular conditions (176), it is possible that it functions in a manner similar to β-catenin. Filamin (FM) functions as a type II coregulator to faciliate AR translocation to the nucleus upon binding to androgen.

with α-catenin are still able to transduce the Wnt signal (169). In addition to the TCF/LEF transcription factors, β-catenin has been shown recently to function as a transcriptional coactivator of AR in prostate cancer cells (170). However, it has not yet been determined whether the coactivator function of β-catenin with AR occurs in response to Wnt signaling or other extracellular stimuli.

The f-actin-binding protein gelsolin regulates actin polymerization and depolymerization through its ability to sequester actin monomers and sever and cap actin filaments (165). The activity of gelsolin is inhibited by the phosphoinositide phosphatidylinositol 4,5-bisphosphate (171) and activated by calcium (172). However, gelsolin can also be activated to sever actin filaments in a calcium-independent manner through cleavage by the apoptosis effector caspase-3 (164). Hippocampal neurons and neutrophils from gelsolin knockout mice are resistant to apoptotic stimuli (164, 173, 174), suggesting that the cleavage of gelsolin is a critical element in apoptosis. In a yeast two-hybrid screen, gelsolin has been identified as an AR interacting protein. Gelsolin interacts with AR in a T-dependent manner and enhances AR transactivation in the prostate cancer cell line DU145 (K. Nishimura and C. Chang, manuscript in preparation). The mechanism through which gelsolin enhances AR transcription and whether this function is separate from the gelsolin actin-severing activity remains to be determined.

Supervillin, an actin-binding protein with structural homology to gelsolin and villin, has also been identified as coactivator of AR and GR (175–177). The prostate cancer cell lines DU145 and PC-3 express a low level of endogenous supervillin. Cotransfection of supervillin with AR into these cells results in a 2- to 3-fold enhancement of AR transcription in response to 1 nM T (175). Supervillin shares 50% homology to the regions of gelsolin and villin that bind f-actin. However, supervillin lacks the amino acids found in gelsolin to be involved in actin severing, suggesting that supervillin lacks this activity (176). Cytologically, supervillin is localized to the plasma membrane at sites of intercellular contact (176). In MDBK epithelial cells grown at low density, supervillin is also localized in the cytoplasm and nucleus, showing a punctate distribution. At high density, supervillin is localized almost exclusively at the plasma membrane (176). These observations suggest that supervillin may transduce signals from sites of cellular adhesion to the nucleus during cellular proliferation or migration (Fig. 3). Alternatively, supervillin may have a dual function in cytoskeletal architecture and gene transcription, analogous to β-catenin.

Nuclear translocation of a subset of nuclear receptors ap-

pears to be mediated partly by a cytoskeleton-associated network. While disruption of microtubules or actin-containing microfilaments does not influence the ability of PR to translocate to the nucleus (178), disruption of the cytoskeleton blocks okadaic acid inhibition of GR nuclear localization in response to dexamethasone (179). Microtubule disrupting agents inhibit VDR nuclear localization and transactivation in response to 1,25-dihydroxyvitamin D_3, suggesting VDR utilizes a nuclear import mechanism that is associated with the cytoskelton (180, 181). Recently, the f-actin cross-linking protein filamin has been found to interact with the AR hinge domain (42) to function as a type II coregulator (Fig. 3). Mutant filamin inhibits AR transcriptional activity, and AR is unable to translocate to the nucleus and activate transcription in response to androgen in the filamin-negative M2 cell line. AR is able to translocate and activate a reporter gene upon androgen treatment in M2 cells stably transfected with filamin, demonstrating that this cell line is not lacking other factors necessary for AR transcription (42).

D. Coactivators that mediate ligand binding and receptor stability

The ability of nuclear receptors to bind ligand and the protein stability of nuclear receptors are apparently interrelated. The ligand binding ability of nuclear receptors requires appropriate folding of the receptor, a process that is facilitated through the chaperone heterocomplex (182). Upon ligand binding, AR dimerizes allowing the NH_2 and COOH termini of the receptor to interact. Pulse chase experiments indicate that the rate of AR protein turnover is decreased in the presence of ligand and that ligands with a higher affinity to AR confer a greater stabilizing effect (183). Additionally, AR mutations that reduce the NH_2/COOH terminus interaction increase the ligand dissociation rate and decrease AR protein stability (62, 183). Therefore, coregulators that influence AR protein folding, ligand binding, and NH_2/COOH-terminal interaction could affect AR protein stability and thus the observed transcriptional activation. Coregulators that function primarily in this manner can be classified as type II coregulators.

Several AR coactivators that modulate at least some of these processes have been identified. One of the components of the Hsp90 chaperone heterocomplex, BAG-1L, enhances AR transactivation in the presence of androgen, presumably by promoting the appropriate folding of AR (184). SRC-1 peptides that interact with the ER LBD have been reported to decrease the dissociation rate of ER agonists, suggesting that at least part of the mechanism through which SRC-1 enhances nuclear receptor transcription may be through stabilizing the interaction between the receptor and its ligand (185). However, because AR apparently interacts with SRC-1 in a different manner than ER, SRC-1 may enhance AR-mediated transcription primarily through other mechanisms, such as its HAT activity (discussed below) (31, 62). The AR coactivator ARA70 may play a unique role in AR ligand binding. ARA70 was initially identified as an AR coactivator that interacted with AR and induced AR-mediated transcription in response to both T and DHT (40). While ARA70 was originally characterized as an AR coactivator in prostate cancer cells, ARA70 has been shown by others to enhance AR transcription up to 8-fold in the fibroblastic COS-1 cell line (186). The interaction of ARA70 with DHT-bound AR enhances AR protein stability above DHT binding alone (S. Yeh and C. Chang, unpublished observations). In transfection experiments, ARA70 enhances AR transcription in response to the normally weak androgen Δ5-androstenediol (125). E2 normally binds AR with a 100-fold lower affinity than DHT (126) and does not normally activate AR transcription in transfection assays at concentrations up to 100 nM (41, 187). However, in the presence of exogenous ARA70, AR transcription is activated in DU145 prostate cancer cells in the presence of 1–10 nM E2 (41). Using PC3 cells, Greenberg and colleagues (188) also demonstrate that ARA70 can enhance E2-induced AR transactivation. Similarly, Weigel and colleagues (189) have shown that ARA70N (amino acids 1–401 of ARA70) promotes AR transcription in the presence of 10 nM E2 in HeLa cells. ARA70N slows the dissociation of E2 from AR, suggesting that the AR-ARA70 interaction stabilizes the binding of E2 to AR (T. H. Thin and C. Chang, unpublished observations). The physiological importance of the induction of AR transactivation by E2 in the presence of ARA70 has not yet been established. Because the level of E2 required to induce AR+ARA70 transcription is within the normal physiological range for premenopausal women (190), it is possible that AR transcription may be induced by E2 in tissues with high endogenous ARA70 expression or in pathological conditions associated with increased ARA70 expression. Although the E2 level in adult males is substantially lower than premenopausal females, local tissue levels of E2 can be relatively high. Bovine prostatic fluid has been found to contain 0.5 nM E2 (191). Several studies have found an elevated level of aromatase, the enzyme responsible for metabolizing T to estrogen, in the stroma of benign prostatic hypertrophy and some prostate cancer samples (192–195), although this is not a universal observation (196, 197). It is therefore possible that E2 in the prostate may be elevated above adult male serum levels, particularly in benign prostatic hypertrophy. Under these circumstances, AR activity may be induced by E2. The expression of ARA70 itself is induced by E2 and inhibited by antiestrogens (198), suggesting that an increase in local E2 levels may enhance AR transcriptional activity in response to any agonistic ligand by an increase in the abundance of ARA70. Pharmacological doses of estrogens have been used to suppress pituitary LH release and lower serum androgen levels in the treatment of prostate cancer (199). Although estrogen therapy is not generally considered to be the treatment of choice due to cardiovascular side effects, estrogen treatment of prostate cancer continues in developing countries due to its low cost (199, 200). It remains to be determined whether prolonged estrogen treatment in these patients influences ARA70 expression and contributes to prostate cancer progression.

In addition to enhancing AR transactivation in response to normally weak agonists, ARA70 has also been shown to enable the AR antagonists hydroxyflutamide and casodex to behave as AR agonists (201, 202). This is of particular relevance to prostate cancer where androgen antagonists are often used as part of androgen ablation therapy. In a pro-

portion of patients treated with antiandrogens, antiandrogens fail to suppress tumor growth and may in fact promote tumor growth (203). One mechanism through which this effect could be mediated is by an elevation of ARA70 within the tumor. Partial support for this model comes from analysis of the CWR22 xenograft system in mice. The CWR22 prostate xenograft model mimics human prostate cancer progression in that the human prostate cancer-derived CWR22 cells are initially androgen dependent in mice but androgen-independent tumors recur several months after castration. In this system, ARA70 mRNA levels decrease shortly after castration but are elevated in the recurrent, androgen-independent tumor (204).

E. Coactivators that influence nuclear-cytoplasmic trafficking of AR

Nuclear receptor transcriptional activity could potentially be increased by type II coactivators that facilitate the nuclear localization of ligand-bound receptors. This could be effected either by retaining the receptor in the nucleus or by enhancing the rate of transit to the nucleus. However, relatively little is known about receptor interacting proteins that mediate the subcellular distribution of nuclear receptors. Although two nuclear receptor coactivators, TRIP230 and ASC-1, are known to alter their subcellular distribution in response to physiological conditions or cell cycle progression (205, 206), there is no evidence that these coactivators facilitate the nuclear localization of steroid receptors. However, as discussed above, mutation of the f-actin-binding protein filamin prevents AR nuclear translocation in the presence of androgen, suggesting that filamin is important in the normal nuclear-cytoplasmic trafficking of AR (42). Recently we have found that the AR coactivator ARA70 increases the amount of nuclear localized AR upon ligand treatment in transfected COS-1 cells (S. Yeh and C. Chang, manuscript in preparation). However, because ARA70 enhances the protein stability of ligand-bound AR, it is unclear whether the increased localization is the result of an increased amount of ligand-bound AR protein. ARA70 is normally localized to the cytoplasm and remains cytoplasmic after androgen-bound AR translocates to the nucleus. ARA70 has been reported to interact with other coactivators such as p/CAF (127, 207), suggesting that it possibly forms a cytoplasmic molecular platform that is involved with the transition of the unliganded receptor associated with the Hsp90 chaperone heterocomplex to associating with at least a subset of coregulators upon ligand binding. The ARA70N translocates to the nucleus with androgen-bound AR, implying that the COOH-terminal domain of ARA70 contains a cytosolic retention signal. ARA70N is a stronger transcriptional coactivator than the full-length ARA70, possibly because it continues to provide a molecular platform for AR coactivators while in the nucleus.

The AR coactivator ARA24 (74) is identical to the general nuclear export factor RanGTPase (208) although the manner in which ARA24/Ran enhances AR-mediated transcription has not yet been determined. ARA24/Ran is responsible for the nuclear export of the importin proteins that mediate nuclear import (208, 209). It is possible that an increase in ARA24/Ran results in a more rapid return of importins to the cytoplasm, increasing the efficiency of translocation of proteins into the nucleus. ARA24/Ran also exports mRNA complexed with ribonuclear proteins (208), and therefore an elevation of ARA24/Ran could enhance the export of AR mRNA to the cytoplasm for translation. It is also possible that accelerated export of nuclear AR results in more efficient receptor recycling and thus a greater responsiveness to androgen. However, ARA24/Ran has also been found to be involved in nonexport functions such as nucleation of microtubules during mitosis (210). This raises the possibility that ARA24/Ran enhances AR transcription through mechanisms separate from its nuclear export function.

F. Chromatin remodeling and coactivators

The packaging of chromosomal DNA is broadly defined as chromatin of which the basic unit is the nucleosome. The nucleosome core particle is an octomer made up of two copies of each of the histone H2A, H2B, H3, and H4. Higher order DNA packaging is mediated by DNA architectural proteins, and the largest eukaryotic family of architectural proteins is the high-mobility group (HMG) proteins (211). The highly homologous HMG-1 and HMG-2 proteins can enhance transactivation by AR and other steroid receptors by stimulating receptor DNA binding, possibly by stabilizing the receptor response element in an energetically favorable conformation for receptor binding (212, 213).

The higher order folding of chromatin is disrupted in the promoters of transcriptionally active genes. The known chromatin remodeling or modifying complexes act upon the nucleosome by disrupting the histone-DNA interaction or through controlling the acetylation status of histones. This disrupted chromatin structure allows transcription factors to bind more readily to DNA and facilitates transcriptional activation. Transcription by nuclear receptors is thought to be a multistep process wherein the agonist-bound receptor binds to the target DNA recognition sequence and coactivators assist in establishing or maintaining an open chromatin structure either through direct modification of nucleosomes or by recruiting chromatin modifying complexes (214, 215). As indicated in the introduction, coregulators that participate in the modification of chromatin can be considered type I coregulators. Experiments in yeast have suggested that chromatin modification is itself a sequential process. Chromatin immunoprecipitation of the *HO* promoter has shown that an enhancer-bound transcription factor first recruits the SWI/SNF nucleosome remodeling complex, followed by recruitment of histone acetylation complexes (216, 217). It is possible that an analogous mechanism operates with nuclear receptor-directed transcription.

The SWI/SNF complex contains a DNA-dependent ATPase subunit necessary for chromatin modification and is one of the best characterized of the chromatin remodeling complexes (reviewed in Ref. 218). This complex functions to perturb the conformation of the nucleosome in an ATP-dependent manner, resulting in a greatly diminished interaction between the histones and DNA (219, 220). Components of the SWI/SNF complex have been shown to interact with ER and GR, and mutations in the SWI/SNF genes in yeast prevent transcriptional activation by GR (221, 222). It is possible that the recruitment of the SWI/SNF complex to

steroid receptors is facilitated by coactivators. β-Catenin interacts with Brg-1, a component of the SWI/SNF complex, and this interaction is necessary for β-catenin-mediated enhancement of transactivation by the TCF/LEF transcription factors (223). It is possible that it functions in a similar manner to coactivate AR. Another candidate for such an activity may be NSD1, a coregulator that contains conserved motifs found in proteins involved in chromatin modification (224). The human homolog NSD1, ARA267β, has recently been identified (225). NSD1 contains a SET domain, named after the *Drosophila* proteins in which it was first identified [Su-(var)3–9, Enhancer of Zeste, and Trithorax] (224, 226). The SET domains of trithorax and the human transcription factor ALL-1 have been found to interact with SWI/SNF components (227), raising the possibility that NSD1 also functions in this manner. NSD1 has been found to physically interact with RAR, RXR, TR, and ER, although the consequence of this interaction in mammalian cells has not yet been determined (224). Using mammalian two-hybrid and GST interaction assays, an isoform of NSD1/ARA267β, referred to as ARA267α, also interacts with AR and enhances DHT-induced transcription (225). The ARA267α isoform lacks the most NH_2-terminal 269 amino acids of NSD1/ARA267β, possibly due to the presence of a secondary transcription initiation sequence. In transfection assays, ARA267α enhances AR transcription of the endogenous prostate-specific antigen gene in LNCaP cells and from the mouse mammary tumor virus promoter in transfected PC3 cells (225). However, it remains to be determined whether the SET domain of NSD1/ARA267 functions to recruit the SWI/SNF complex to AR.

The acetylation of the lysine residues of the NH_2-terminal histone tails is correlated with active genes. Acetylation reduces the positive charge of the histone tails, which may result in the disruption of chromatin structure by reducing or preventing nucleosome-nucleosome contacts (228). The coactivators p/CAF, CBP/p300, SRC-1, and SRC-3 have all been demonstrated to have HAT activity (30, 31, 146, 229). The particular histone substrate specificity of each of these coactivators is different. While SRC-1 and CBP/p300 are able to acetylate all of the histones in nucleosomes, p/CAF and SRC-3 preferentially acetylate nucleosomal histone H3 (230). However, the functional consequences of this target specificity has yet to be determined. A biochemical approach has been used to demonstrate that p/CAF is part of a large multiprotein complex, and the HAT activity of the p/CAF complex is significantly higher toward nucleosomal histones than p/CAF alone (231). Although the p/CAF complex apparently does not contain CBP/p300 or SRC family members, p/CAF has been shown to interact with SRC-1, SRC-3, and CBP (146, 231–233). Progesterone-bound PR preferentially recruits an SRC-1 complex in T47D cells, suggesting the p/CAF complex may be recruited to steroid receptors via other coactivators (232).

G. AR coactivators as mediators of signal transduction

Transcriptional activity of AR has been found to be influenced by growth factors and cytokines through the stimulation of multiple signal transduction cascades (Fig. 4) (reviewed in Refs. 234 and 235). The stimulation of kinase cascades may affect AR transcription through phosphorylation of AR, AR interacting proteins, or coregulators. The growth factor receptor-mediated phosphorylation of two AR interacting proteins, Smad3 and STAT3, has been found to influence AR transcription. However, because STAT3 and Smad3 are transcription factors, they are not considered to be type I or type II coregulators. Phosphorylation of the transcription factor STAT3 in response to IL-6 allows STAT3 to interact with AR and enhance AR transcription (236, 237). TGFβ-induced phosphorylation of the transcription factor Smad3 also results in interaction between Smad3 and AR, but the overall effect of this interaction may be cell type specific, possibly as a result of differential availability of other AR and/or Smad3 interacting proteins such as Smad4 (H.-Y. Kang and C. Chang, manuscript in preparation). In the prostate cancer cells DU145 and PC3, Smad3 enhances AR transcriptional activity (19). However, in CV-1 cells, Smad3 has a suppressive effect on AR activity (18).

The direct phosphorylation of AR has been shown to influence its ability to interact with coregulators. AR is a direct target of the kinase Akt, one of the kinases of the PI3K signal transduction pathway. Phosphorylation of AR by Akt results in a decrease in AR transcriptional activity and is associated with a decrease in the ability of AR to interact with ARA70 (48). In contrast, stimulation of MAPK by overexpression of ErB2/Her2/Neu enhances AR transcription through phosphorylation of AR and facilitates AR-ARA70 interaction (52). MAPK phosphorylation of ERβ or the orphan receptor SF-1 also stimulates coactivator recruitment by these receptors (55, 238).

A number of nuclear receptor coregulators, including CBP and β-catenin, have been shown to mediate the effects of signal transduction pathways. In theory, the function of either type I or type II coregulators could be influenced by alterations in phosphorylation or acetylation in response to extracellular signals. A number of coregulators themselves perform enzymatic activities such as phosphorylation or acetylation, modifying either the chromatin surrounding the promoter of the target gene or other coregulators. The prototypic coactivators of this type are CBP and the closely related p300. CBP was initially identified as a coactivator of CREB that regulates cAMP-inducible promoters (239). Subsequent studies have shown that CBP can function as a coactivator of other transcription factors, such as NFκB (240), and of nuclear receptors including AR (241–243). CBP additionally interacts with the SRC coactivators (243–245) and has been purified with the RNA pol II holoenzyme complex (32, 136). Biochemical studies have suggested that in the case of PR, SRC-1 and/or TIF-2 may bind the receptor and recruit a CBP-containing complex (that may contain RNA pol II) to the target promoter (232). It has not yet been determined, however, whether other AR coactivators function in a similar manner. The observation that the DRIP/TRAP complex does not contain CBP (38, 246), and may recruit RNA pol II by a separate mechanism (38), suggests that multiple coactivator complexes may exist in the cell, although it is unclear to what extent these complexes interact.

Acetyltransferase activity has been demonstrated for CBP/p300 (30, 247) and the CBP-associated factor and nu-

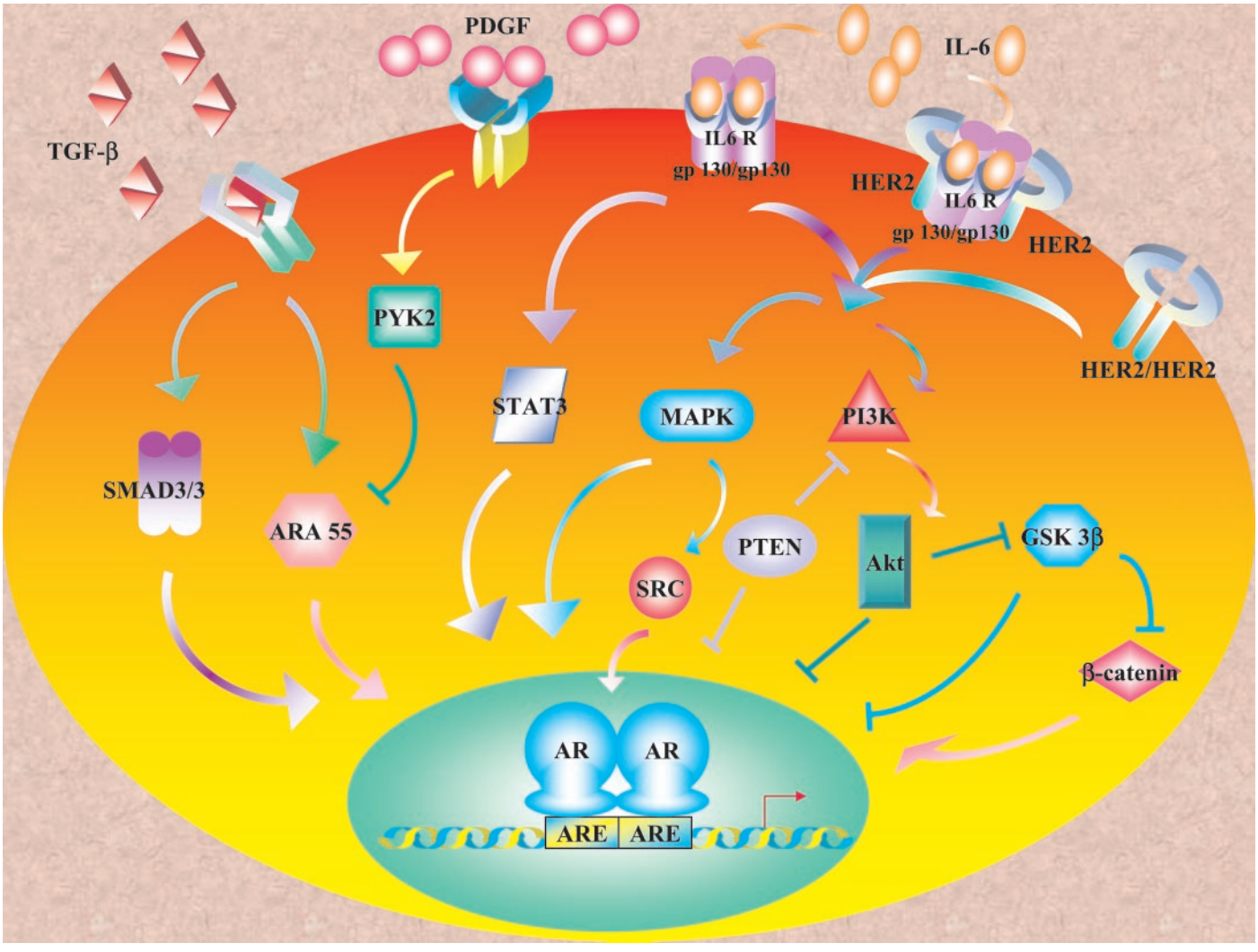

FIG. 4. Multiple signal transduction pathways are involved in the regualtion of AR and AR coregulator function. The activation of the MAPK and PI3K signal cascades occurs in response to multiple growth factor stimuli. For simplicity, IL-6 and Her2 induction of these pathways is depicted here. MAPK can directly phosphorylate AR to enhance AR interaction with coactivators and can phosphorylate coactivators, such as SRC family members, to facilitate transcription. Akt phosphorylation of AR represses AR transcription, at least in part, through reduction of AR-coactivator interaction. In addition to SRC, the coregulators β-catenin and ARA55 are targets of phosphorylative regulation as discussed in the text.

clear receptor coactivator p/CAF (229), SRC-1 (31), and SRC-3 (146). The acetylation targets originally identified for these coactivators were histones, suggesting that these coactivators may function in part through chromatin modification. However, CBP/p300 has been found to acetylate nonhistone proteins. Acetylation of SRC-3 by CBP disrupts the interaction between SRC-3 and ER, resulting in a reduction in hormone-mediated transcription (248). The general transcription factor TFIIE, involved in the recruitment of helicase to the promoter, and TFIIF, the factor involved in targeting RNA pol II to the promoter, have both been shown to be acetylated by p300 and p/CAF, although the functional consequences of these modifications have not yet been determined (249). Although CBP/p300 acetylation enhances the DNA binding ability of p53 (250), it has not yet been determined whether nuclear receptors themselves are targets for acetylation by their coregulators. CBP acetyltransferase activity is enhanced by phosphorylation by cyclin E-cdk2 (251). Because CBP acetyltransferase activity is not required for its ability to coactivate all of the transcription factors with which CBP interacts (233), CBP phosphorylation may provide a mechanism for differential transcriptional enhancement. Recently, CBP/p300 has been found to be methylated by CARM1 (252, 253). CARM1 increases the ability of CBP/p300 to enhance RAR/RXR transcription; however, CARM1 inhibits the ability of CBP to enhance CREB transactivation (252). It remains to be determined whether CBP methylation influences AR transcriptional activity.

Ubiquitin ligase activity has been identified for two AR coactivators, ARA54 and E6-AP (254, 255). The ubiquitination of cellular proteins is important for multiple cellular processes, including cell cycle regulation and response to extracellular signals (256, 257). The major role of ubiquitination is to target substrate proteins for proteosomal degradation (258), and it is therefore somewhat unexpected that ubiquitin ligase activity has been identified in proteins that

enhance steroid receptor transcription. ARA54 was initially identified as a coregulator of AR and PR and contains a RING finger domain (142). Recently, ARA54 has been found to be able to ubiquitinate itself *in vitro*, contributing to its proteosomal degradation (255). The RING finger of ARA54 mediates the interaction between ARA54 and ubiquitin-conjugating enzymes and is necessary for its autoubiquitination (255). However, it has not yet been determined whether ARA54 targets other proteins for ubiquitination and degradation. E6-AP was originally characterized as a ubiquitin ligase (259, 260), and mutation or loss of E6-AP is associated with the inherited disorder, Angelman's syndrome (257). E6-AP functions as a coactivator of AR, PR, GR, and ER, as well as the transcription factor Sp1 (254). However, mutants of E6-AP lacking ubiquitin ligase activity are still able to coactivate PR to the same degree as wild-type E6-AP in transfection assays, suggesting that the coactivation and ubiquitin ligase functions of E6-AP are distinct (254). It is possible that coactivators with ubiquitin ligase activity contribute to nuclear receptor transcription through targeting the degradation of corepressors, as has been reported for nuclear receptor corepressor (NcoR) (261). Alternatively, after the initiation of transcription by steroid receptors, targeted degradation of the preinitiation complex by ubitiquitin ligase coactivators may facilitate reinitiation of transcription.

Although phosphorylation is known to modify the transcriptional activity of the AR (50–52), the potential involvement of kinase and phosphatase modulation of SRCs has only recently been addressed. SRC-1 can be phosphorylated at seven sites, two of which have been demonstrated to be phosphorylated by ERK-2, a member of the MAPK family (262). Stimulation of the MAPK signal transduction pathway enhanced the ability of SRC-1 to coactivate PR (262). As indicated above, the AR coactivator β-catenin mediates signaling through the Wnt pathway to coactivate TCF transcription factors (162, 170). However, it is not known whether β-catenin also mediates AR transcription in response to growth factor stimulation. The coactivator ANPK is itself a Ser/Thr kinase that does not phosphorylate AR (160). It is possible that ANPK enhances AR transcription as part of a yet undetermined signal pathway.

ARA55, a coactivator of AR, GR, and PR (263, 264), potentially mediates the transduction of signals from cellular focal adhesions with the extracellular matrix (ECM) to the nucleus. In prostate-derived DU145 cells, ARA55 is a stronger coactivator for AR than for GR or PR (263). Although ARA55 is able to enhance AR transcription in response to the antiandrogen hydroxyflutamide, it does so to a lesser extent than ARA70 (207). ARA55 has been localized immunocytochemically to the nuclear matrix and focal adhesions (264, 265). Focal adhesions are the points at which the cell membrane contacts the ECM via the transmembrane integrin receptors. The cytoplasmic domain of the integrins interacts with microfilaments or intermediate filaments through a variety of cytoskeletal proteins (266, 267). The focal adhesion plaque is formed by a clustering of ligand-bound integrins and cytoskeletal proteins with focal adhesion kinase (FAK) through a process mediated by the RhoGTPase. Integrin-mediated phosphorylation and activation of FAK stimulates the MAPK pathway (267). Growth factor receptors have been shown to be recruited to the focal adhesions, resulting in an enhanced cellular response to exogenous growth factors (268). ARA55, in addition to its function as an SRC, has been shown to interact with FAK (265) and with the FAK-related PYK2 kinase (269). Activation of PYK2 results in an increase of cellular phosphorylated ARA55 and in phosphorylated ARA55 coimmunoprecipitating with PYK2 (269), suggesting that ARA55 functions in a signaling pathway downstream of PYK2 or FAK. ARA55 also interacts with the cytoplasmic tyrosine kinase Csk, although the functional consequence of this interaction has not been established (265). It is currently unknown whether phosphorylated ARA55 serves a solely cytoplasmic function or whether it translocates to the nucleus to modulate gene transcription. The integrins play a role in diverse cellular processes including anchorage-dependent growth, differentiation, and apoptosis (266), and ECM or integrin alterations have been implicated in a wide variety of cancers, including those of the breast and prostate (270, 271). The potential importance of ARA55 in these processes is demonstrated by the observation that human tumor-derived cell lines have a low or absent level of ARA55 (272, 273). Overexpression of ARA55 in immortalized human fibroblasts results in growth retardation and a senescent morphology and pattern of gene expression (274). The convergence of growth factor receptors with integrins in focal adhesions associated with ARA55 phosphorylation by FAK or PYK2 suggests that cycles of ARA55 phosphorylation or dephosphorylation could be involved in regulating cellular growth and migratory responses. In this model, the loss of ARA55 expression in tumors would remove a growth-regulatory process and favor an amplified response to exogenous growth factor stimulation. One mechanism through which ARA55 may function in this manner is through the alteration of the ability of ARA55 to function as a coactivator, possibly by alteration of the phosphorylation status of ARA55. In normal cells in the absence of growth factor or integrin stimulation, ARA55 may by hypophosphorylated and able to function as an AR coactivator to maintain normal androgen-mediated transcription in androgen target tissues, such as the prostate. Upon phosphorylation of ARA55 in response to exogenous stimulation, ARA55 may no longer function as an AR coactivator contributing to a modulation of the growth response. Tumor cells lacking ARA55 would be expected under this model to be more susceptible to proliferative or migratory responses with growth factor stimulation or abnormal integrin signaling. Partial support for this model comes from the observation that overexpression of PYK2 inhibits androgen-induced AR transcription (L. Wang and C. Chang, unpublished observations).

ARA55, in common with FHL2, is a LIM domain protein (264, 274, 275). LIM domains are cysteine- and histidine-rich regions that mediate protein-protein interactions (276). The four LIM domains of ARA55 are similar in sequence and organization to the LIM cytoskeleton binding proteins paxillin and zyxin (277), consistent with the localization of ARA55 to the nuclear matrix and focal adhesions (264, 265). LIM domain-containing proteins have been found to function as bridging molecules between transcription factors (278). A number of additional coregulators have been identified that interact with LIM

proteins to modulate their effect on transcription (279–281). It remains to be determined whether ARA55 recruits LIM coregulators or other transcription factors as part of the mechanism through which it regulates AR transactivation and conveys extracellular signals.

V. AR Corepressors

Most of the coregulators identified to date have been shown to enhance transcription of a subset of both classical steroid receptors and the type 2, RXR heterodimerizing receptors. However, transcriptional repression by these two receptor types appears to operate through distinct mechanisms. When not bound to an agonist, the type 1, classical steroid receptors are complexed with heat shock proteins preventing DNA binding *in vivo* and are therefore transcriptionally silent. In contrast, the type 2 receptors are capable of binding to DNA in the absence of ligand, resulting in transcriptional repression (44). Corepressors were originally identified as proteins associated with unliganded type 2 nuclear receptors that mediate transcriptional repression, possibly through the formation of a nonproductive interaction with general transcription factors (282) or through recruitment of histone deacetylase complexes (283–285). The two best characterized corepressors, NCoR and silencing mediator of retinoid and thyroid hormone receptor (SMRT), do not interact with ER, GR, or PR in the absence of ligand (286, 287). However, both NCoR and SMRT interact with ER when bound to the mixed agonist tamoxifen, an ER ligand that acts as an agonist or antagonist in a tissue-specific manner, and overexpression of either corepressor abolishes tamoxifen agonist activity (288). Similarly, NCoR and SMRT preferentially interact with PR in the presence of the antagonists RU486 and ZR98299 and can repress the partial agonist action of RTI-020 (287). The interaction between AR and NCoR or SMRT has not yet been examined, although it might be expected that these corepressors could only interact with an antagonist-bound AR by analogy to other steroid receptors. NCoR and SMRT interact with nuclear receptors through motifs similar to the LXXLL motifs found in some coactivators (289, 290). The corepressor interaction motifs are able to interact with a subset of the same receptor LBD residues that interact with coactivators (289–292). The binding of an agonistic ligand alters the conformation of the LBD, repositioning the coregulator interacting residues to stabilize the binding of coactivators and sterically inhibit NCoR or SMRT binding (289, 292). AR interacts differently than ER or PR to some LXXLL motif-containing coactivators (62, 63), at least partly due to the nature of the AR NH_2-terminal interaction with the AR LBD (78). AR NH_2/COOH terminus interaction is induced by some antiandrogens, including cyproterone acetate (293), but it is not known whether the AR NH_2-terminal would block the ability of NCoR or SMRT to interact with the LBD.

Three corepressors of androgen-bound AR have been identified to date, cyclin D1, calreticulin, and HBO1 (Table 2). However, relatively little is known about the mechanism of their repressive effect. Cyclin D1 reduces AR transcription in the presence of the synthetic androgen R1881 (294). The D-type cyclins bind to and activate the cyclin-dependent kinases CDK4 and CDK6 to promote cell cycle progression through the G_1 phase. The CDK4-cyclin D1 complex functions to phosphorylate and inactivate Rb (retinoblastoma gene product). Mutations in cyclin D1 that abolish its ability to interact with CDK4 do not influence the ability of cyclin D1 to reduce AR transcription. Similarly, cyclin D1 is able to repress AR transcription in Rb-negative cells (294). These observations suggest that cyclin D1 inhibits AR transactivation through a mechanism independent of its function in cell cycle regulation. The calcium-binding protein calreticulin has also been characterized as a corepressor of AR. Calreticulin inhibits AR transcription in response to R1881 and prevents AR binding to its response element (86). Cytologically, calreticulin is localized to the endoplasmic reticulum and nucleus (295), although the physiological role of calreticulin-mediated repression of AR remains to be determined.

The AR corepressor HBO1 is a member of the MYST protein family that is characterized by a homologous zinc finger and carries an acetyltransferase domain (296). The MYST family includes both transcriptional silencers, such as the yeast SAS2 and SAS3 genes, and transcriptional activators, including the AR coactivator Tip60 (Table 1) (297, 298). Acetyltransferase domains are more typically thought to be associated with coactivators, and HBO1 only weakly acetylates histones (299). However, it is possible that HBO1 functions to acetylate other nonhistone proteins involved in AR transcriptional regulation and that acetylation by HBO1 reduces the ability of these proteins to facilitate androgen-induced AR transactivation.

TABLE 2. Corepressors of the AR

Corepressor	Region	Comments	Selected references
Calreticulin	DBD	Inhibits DNA binding and transcription. Also functions as a corepressor of RAR:RXR and GR. Nuclear localization is enhanced in some cell types by interaction with holo-GR.	(86, 295, 344)
Cyclin D1	[a]	Reduces AR ligand-dependent transcription in a cell cycle-independent manner. Functions as a coactivator of ER.	(294, 345)
HBO1	DBD-LBD	Member of the MYST/SAS family of proteins. Reduces AR transcription in the presence of DHT but does not influence ER or TRβ transactivation. Carries a functional HAT domain.	(296)

[a] Although interaction with AR has been demonstrated, the precise domain of AR that interacts with the coregulator has not yet been determined.

VI. AR Coregulators and Cancer

Androgens, functioning through the AR, are essential for the normal development and maintenance of the prostate (300, 301). However, the progression of prostate cancer is also sensitive to androgens. The removal of testicular androgens by castration has long been recognized to result in tumor regression (302), and surgical and/or pharmacological androgen ablation remain the predominant form of treatment for advanced prostate cancer (199, 203). Androgen ablation therapy is often combined with treatment with nonsteroidal antiandrogens, such as hydroxyflutamide, to block residual adrenal androgen action. While 70–80% of patients initially respond to androgen ablation therapy, tumors ultimately become resistant and may, in fact, proliferate in response to antiandrogens (203). Because AR is generally expressed in prostate tumors and their metastases (303), aberrant regulation of AR activity by coregulators may contribute to prostate cancer progression or the acquired agonist effect of antiandrogens. Alterations in β-catenin expression have been found in multiple tumor types (304–306), and mutations of β-catenin have been identified in primary prostate cancers (307). One of these mutant β-catenin alleles (β-catenin S33F) enhances AR sensitivity to the normally weak adrenal androgens androstenedione and DHEA, allowing AR transcriptional activation in response to these ligands comparable to that induced by DHT or T (170). β-Catenin S33F also enhances AR transcription in response to E2 (170). These observations suggest that mutation of β-catenin in the progression of prostate cancer could enable the cancer cells to survive in the presence of low serum levels of testicular androgens (170, 307). The AR coactivator ARA70 has been extensively characterized as having the capacity to enhance AR transcriptional activity in response not only to normally weak adrenal androgens (40, 125), but also to the antiandrogens hydroxyflutamide and casodex (202). In the CWR22 prostate xenograft system, in which the CWR22 tumors progress from androgen dependent to androgen independent after castration, ARA70 mRNA is elevated in the recurrent androgen-independent tumors (204). It is possible that ARA70 expression is elevated in a subset of human prostate tumors and may contribute to tumor progression after androgen ablation therapy by allowing AR to become transcriptionally active in response to adrenal androgens or antiandrogens. The possibility that ARA70 is amplified or overexpressed in prostate tumors is currently under investigation. The amplification of coregulator genes is not without precedent in tumors. SRC-3 and PBP/DRIP205/TRAP230 are frequently amplified and overexpressed in breast tumors, suggesting that these coregulators may contribute to breast carcinogenesis through their function as ERα coactivators (67, 308). The agonistic effect of antagonists in prostate cancer could also be due conceivably to the reduction of corepressor expression. In a mouse model of mammary tumors, the acquisition of tumor proliferation in response to the antiestrogen tamoxifen was accompanied by a decrease in the expression of the corepressor NCoR (309). An analogous mechanism may function in prostate cancer.

Mutation of the coactivator CBP causes the human autosomal dominant disorder Rubinstein-Taybi syndrome (RTS) (310). RTS is characterized by facial abnormalities, broad toes and thumbs, and mental retardation, as well as an elevated incidence of malignant and benign tumors of the brain and neural crest derivatives (311). However, male RTS patients do not show symptoms of androgen insensitivity, and RTS-associated tumors occur at similar frequencies in both genders (311), suggesting that this syndrome results primarily from the disruption of CBP coactivation of transcription factors other than AR.

AR has also been shown to be coactivated by the known tumor suppressor genes Rb and BRCA1 (312–314). Epidemiological evidence suggests that aberrations in the interaction between AR and the breast cancer susceptibility gene BRCA1 may contribute to breast cancer progression in some patients. Women who inherit germline BRCA1 mutations and who carry a less transcriptionally active AR allele show an earlier age of breast cancer development (315). Androgens acting through AR have been shown to inhibit breast cancer proliferation clinically and in animal models. We have shown that BRCA1 physically associates with AR to regulate endogenous genes in breast cancer cells (314), and the antiproliferative effects of androgens in breast cancer may be mediated in part through BRCA1 coactivation of AR. Rb functions in the control of cellular differentiation and proliferation (316). Inactivating mutations of Rb are frequently (60%) observed in both early-stage and low-grade prostate tumors and advanced disease (317). The prevalence of Rb mutations early in prostatic tumorigenesis may indicate that Rb normally functions with AR during the controlled development of the prostate or in the maintenance of the prostate. The phosphatase PTEN (phosphatase and tensin homologue deleted from chromosome 10) functions as a tumor suppressor, and loss of PTEN function is observed in a number of human cancers, including prostate cancer (318–320). PTEN has been found to suppress AR transcriptional activation (49) by reducing the rate of AR nuclear translocation and/or altering AR protein stability (H.-K. Lin and C. Chang, manuscript in preparation). Because type II coregulators such as ARA70 and filamin influence the ability of AR to translocate to the nucleus in response to androgen (42), it is possible that PTEN exerts its effect on AR through the modulation of AR-coregulator interaction. Finally, the loss or reduction of ARA55 expression in tumor-derived cell lines of multiple origins and in some primary prostate tumors (263, 272), as well as the observation that overexpression of ARA55 induces growth inhibition and senescence in immortalized cells (274), raises the possibility that ARA55 is itself a tumor suppressor.

VII. Conclusion and Future Directions

The continuing study of AR coregulators has suggested multiple mechanisms through which the transcriptional activity of AR may be regulated. However, for many coregulators, the mechanism of action and relative *in vivo* importance have yet to be established. Coregulators are typically identified on the basis of interaction studies and their influence is gauged in transient transfection studies. The mechanism of action is often inferred from the presence of con-

served protein motifs or other characterized functions of the protein. This leaves many unanswered questions about a coregulator's role in development, in response to normal physiological stimuli, and in pathological conditions. Continuing investigation of AR coregulators will hopefully further define their roles in these processes.

However, the available information on AR coregulators suggests a tantalizing array of mechanisms through which they may function to regulate AR transcriptional activity. The ability of AR to interact directly with components of the general transcriptional machinery and with coregulator complexes that modify the chromatin of the target gene or form a bridge between the receptor and the GTFs provides insight into the process of transcriptional initiation and the perpetuation of transcription from target promoters. Initial observations suggest a stepwise association of coregulators and coregulator complexes with the DNA-bound AR. However, it remains to be determined to what degree these complexes exist preassembled in the cell and their relative importance for different AR target genes. Recent investigation of coregulators suggests that they may play an increasingly important role as physiological integrators of signal transduction. A number of coregulators are known to mediate growth factor signaling, such as the PIAS family. Still others have been shown to be phosphorylation targets of kinase cascades or are kinases themselves. The action of coregulators such as ARA70 can broaden the spectrum of ligands capable of evoking AR-mediated transcription with implications for the biological effects of steroids in both normal and pathological conditions. There is also the perhaps surprising involvement of actin-binding proteins in AR transcription, possibly facilitating communication from the ECM to the plasma membrane and ultimately to the nucleus. It is possible that such communication is important in androgen-mediated developmental processes or in the metastasis of prostate cancer. The differential tissue distribution of AR coregulators has provided an additional factor in examining tissue differences in androgen action other than the level of AR protein. Animal models, including targeted disruption of coregulators, will be important for determining the relative importance of AR coregulators in a particular tissue or pathological condition. Further clinical studies examining the relative expression level, phosphorylation status, or presence of mutations in AR coregulators in histological samples such as prostate cancer specimens, will also help contribute to an understanding of the involvement of AR in human disease. A comparison of the role of a coactivator in normal and disease states is important to establish a more complete picture of the relative importance of a coregulator *in vivo*, as exemplified by SRC-1. The lack of SRC-1 is substantially compensated by other coactivators in knockout mice, but overexpression of SRC-1 is associated with a population of recurrent prostate cancers (131, 321). The interaction between AR and its coregulators is a clearly developing field, and the observations already made indicate that the biology of the androgen receptor is more complex and interesting than was suspected when it was initially cloned (5, 7–9).

Acknowledgments

The authors wish to thank members of the Chang laboratory for helpful and valuable discussions on the manuscript. We also thank Pu-Yeh Kan for assistance with Fig. 4.

Address all correspondence and requests for reprints to: Chawnshang Chang, Ph.D., George Whipple Laboratory for Cancer Research, Department of Pathology, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, New York 14642. E-mail: chang@urmc.rochester.edu

This work was supported by the George Whipple Professorship Endowment and NIH Grants CA-55639 and CA-715670.

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