0163-769X/04/$20.00/0
Printed in U.S.A.

Endocrine Reviews 25(1):45–71

doi: 10.1210/er.2003-0023

Coregulator Function: A Key to Understanding Tissue Specificity of Selective Receptor Modulators

CAROLYN L. SMITH AND BERT W. O'MALLEY

Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030

Ligands for the nuclear receptor superfamily control many aspects of biology, including development, reproduction, and homeostasis, through regulation of the transcriptional activity of their cognate receptors. Selective receptor modulators (SRMs) are receptor ligands that exhibit agonistic or antagonistic biocharacter in a cell- and tissue context-dependent manner. The prototypical SRM is tamoxifen, which as a selective estrogen receptor modulator, can activate or inhibit estrogen receptor action. SRM-induced alterations in the conformation of the ligand-binding domains of nuclear receptors influence their abilities to interact with other proteins, such as coactivators and corepressors. It has been postulated, therefore, that the relative balance of coactivator and corepressor expression within a given target cell determines the relative agonist *vs.* antagonist activity of SRMs. However, recent evidence reveals that the cellular environment also plays a critical role in determining SRM biocharacter. Cellular signaling influences the activity and subcellular localization of coactivators and corepressors as well as nuclear receptors, and this contributes to gene-, cell-, and tissue-specific responses to SRM ligands. Increased understanding of the effect of cellular environment on nuclear receptors and their coregulators has the potential to open the field of SRM discovery and research to many members of the nuclear receptor superfamily. (*Endocrine Reviews* 25: 45–71, 2004)

Abbreviations: AF-1 and -2, Activation function 1 and 2; AIB1, amplified in breast cancer 1; AP-1, activator protein 1; AR, androgen receptor; CARM1, coactivator-associated arginine (R) methyltransferase-1; CBP, cAMP response element binding protein (CREB)-binding protein; ChIP, chromatin immunoprecipitation; CNS, central nervous system; DRIP, vitamin D receptor-interacting protein; E2, 17β-estradiol; EGF, epidermal growth factor; ER, estrogen receptor; ERE, estrogen response element; GR, glucocorticoid receptor; GRIP, GR-interacting protein; 4HT, 4-hydroxytamoxifen; IKK, IκB kinase; LBD, ligand-binding domain; MEF, mouse embryo fibroblast; MEK, MAPK kinase; NCoR, nuclear receptor corepressor; PCOS, polycystic ovarian syndrome; PGC-1, PPARγ coactivator-1α; PPAR, peroxisome proliferator-activated receptor; PR, progesterone receptor; RAC3, receptor associated coactivator; RAR, retinoic acid receptor; RIP, receptor-interacting protein; SARM, selective AR modulator; SERM, selective ER modulator; SGRM, selective GR modulator; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor; SPRM, selective PR modulator; SRC, steroid receptor coactivator; SRM, selective receptor modulator; TIF, transcriptional intermediary factor; TR, thyroid hormone receptor; TRAP, TR-associated protein.

Endocrine Reviews **is published bimonthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

I. Introduction

NUCLEAR RECEPTORS COMPRISE a large family of eukaryotic transcription factors, and those for whom ligands have been identified are broadly exploited to manipulate various aspects of human biology (1, 2). There is a well-developed pharmacology for many of the nuclear receptors, and the identification of natural and high-affinity synthetic agonistic ligands for these receptors has enabled many studies of the biological effects of these nuclear receptors *in vitro* and *in vivo*. The availability of antagonists also has been important. In experimental studies they proved to be useful tools for validating that an effect under consideration is indeed mediated by a nuclear receptor of interest. Clinically, they have been used to block or inhibit undesirable physiological actions of receptors. For instance, tamoxifen, due to its ability to inhibit estrogen receptor (ER) action, is used widely in the treatment and prevention of breast cancer.

Careful examination of the selective biological effects of tamoxifen (*e.g.*, estrogen-like activity in the uterus but antiestrogen-like effects in the breast) led to the emergence of the concept of selective ER modulators or SERMs (see below). The molecular mechanisms through which selective effects are obtained has been the topic of intense investigation with the result that not only do we have at least a basic under-

standing of how the selective nature of SERM activities is achieved, we have now progressed to a point at which the stage is set to pursue the identification and development of selective receptor modulators (SRMs) for a host of other nuclear receptors. The driving force in this research is the desire to obtain agents that can better separate desired nuclear receptor effects from those that are undesirable. Indeed, the lessons learned from SERMs provide a framework in which to pursue the development of other SRM ligands with high binding affinity to the receptor of interest. The experience with SERMs suggests that compounds with selective activities are likely to be of intense clinical and economic interest, thereby stimulating significant research in this area in both the basic science and pharmacological arenas.

II. Biology of Selective Receptor Modulators (SRMs)

A. Selective ER modulators (SERMs)

Estrogens have long been recognized to play critical roles during development and reproduction, as well as in the growth and maintenance of the skeleton. In addition, evidence of the contribution of estrogens to the normal function of the cardiovascular system and central nervous system (CNS), including cognition and potential delayed onset of Alzheimer's disease, and a variety of other tissues and organs (*e.g.*, colon) indicates that this class of steroids, and by extension its receptors, ERα and ERβ, plays a significant role in normal biology and pathophysiology. Estrogens are widely used clinically to control reproduction (*i.e.*, oral contraceptives) and for hormone therapy and the management of menopausal symptoms in women. Although beneficial in these contexts, estrogen use also has been implicated as a risk factor in breast and uterine cancer, particularly since the first published report from the Women's Health Initiative (3), suggesting that a greater measure of flexibility to control unwanted side effects would be desirable. Consequently, the recognition of SERMs as agents able to elicit estrogenic effects in a tissue-specific manner has expanded the potential population that could benefit from ER ligand therapies.

SERMs have been important for their clinical potential as SRMs as well as serving as the focus of a vast body of research defining the molecular mechanisms through which cell and tissue selectivity is achieved. The *trans* isomer of tamoxifen is the prototypic SERM (4–8). Although one of its first proposed uses was to regulate fertility, it has been employed primarily as an agent used to treat and, more recently, prevent breast cancer (9–12). The ability of tamoxifen to inhibit ER action has long been considered integral to its utility in the breast cancer arena, and this is consistent with numerous studies and clinical trials demonstrating an effect of tamoxifen in ER-positive cells or breast tumors and an absence of any significant activity in those lacking ER expression (8, 13). However, the demonstration of tamoxifen's estrogen-like effects in the rodent uterus and skeleton (7, 14) suggested that this drug may have distinct biological properties depending on the tissue environment. The subsequent observation of the estrogen-like effects of tamoxifen in the human skeleton (15) was important to the conceptualization of SERMs as potential drugs for indications other than breast cancer.

Tamoxifen's activity profile has long been thought to be a reflection of its partial agonist/antagonist activity. For instance, in the absence of endogenous estrogens, tamoxifen frequently exhibits weak estrogenic activity, such as modest stimulation of uterine wet weight and bone density in ovariectomized rats, whereas in the presence of estradiol, it can serve as an antiestrogen, inhibiting responses to a level corresponding to the comparatively modest agonist activity of tamoxifen itself (7, 16). Much effort has been devoted toward understanding the molecular mechanisms through which selective ER actions are achieved, and this is the focus of much of this review. It is appropriate, however, to recognize that the success of tamoxifen as a SERM has been a driving force in the search for new SERMs as well as selective modulators for other nuclear/steroid receptors. Raloxifene-like tamoxifen exhibits antiestrogen activity in the breast and estrogen activity in the skeleton. However, raloxifene lacks the significant uterotropic activity associated with tamoxifen and therefore represents an improved agonist/antagonist profile (6, 17–19). However, because neither tamoxifen nor raloxifene possesses significant estrogen-like activity in the CNS, there is clearly a market for other SERMs to fill this niche. Indeed, a number of other compounds, including lasofoxifene, arzoxifene, and bazedoxifene, are under development, which may one day be of clinical use for chemoprevention of breast cancer or treatment and prevention of osteoporosis (20–22). It is also noteworthy that SERMs likely exist in nature. For instance, the estrone metabolite, $\Delta^{8,9}$-dehydroestrone sulfate suppresses hot flushes in postmenopausal women, an estrogenic action. However, it is unable to significantly affect certain other parameters associated with estrogenic responses such as total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol (23).

B. Selective tissue estrogenic activity regulators (STEARs)

Although distinct from SRMs, a new classification of compounds with tissue-selective biological activity, called selective tissue estrogenic activity regulators (STEARs), has arisen. This class of compounds does not interact directly with receptors such as the ERs, progesterone receptor (PR), or androgen receptor (AR), thus distinguishing them from SRMs. Instead, a precursor (prohormone) compound relies on tissue-selective metabolism to generate hormonal metabolites that have a tissue-specific functional profile. An example of such a drug is tibolone, which currently is the leader in worldwide sales for hormone therapy outside of the United States (24). The oral form of this steroid prohormone is inactive; once in the body, however, it is metabolized to 3α- and 3β-hydroxy-derivative forms, which are estrogenic, and a Δ^4-isomer, which has weaker androgenic and progestational activities (25). Moreover, tibolone is a sulfatase inhibitor (*e.g.*, blocks conversion of estrone sulfate into estrone) and can also stimulate local sulfotransferase activity (26). The resultant activity profile in humans shows estrogenic activity in bone, as well as CNS vasomotor suppression of hot flushes, but no significant uterotropic or mammotropic activities (27). With the exception of the notable CNS effects, tibolone has a SERM profile, but achieves this without di-

rectly modulating the estrogen, androgen, or PRs as it is the various metabolites of tibolone that exert their effects directly on these receptors (24, 26).

C. Selective PR modulators (SPRMs)

PRs control a number of processes critical for reproduction (28). In addition to the natural ligand progesterone, there presently are good synthetic PR agonists and antagonists available for use in humans, and these are used to control reproduction and the function of various reproductive organs such as the uterus. Although the need for a SPRM may be less obvious than for SERMs and selective AR modulators (SARMs) (see below), it has been suggested that a SPRM may be useful for treatment of endometriosis. Current therapies for endometriosis include the use of GnRH antagonists, PR agonists, or androgens. However, these options are not without side effects. GnRH antagonists, by virtue of their ability to induce a hypoestrogenic state, induce hot flushes and urogenital symptoms and may increase the risk of osteoporosis. Progestins may produce breakthrough bleeding, bloating, breast tenderness, mood changes, and breast epithelial proliferation, and androgen use is associated with seborrhea, acne, hirsutism, negative changes in lipid profiles, and, potentially, virilization. As an alternative, an agent that can more specifically target the endometrium may be an effective treatment for endometriosis, while minimizing side effects. A SPRM in combination with an ER ligand also may be useful in menopausal hormone therapy, particularly with respect to hormonal effects on the breast.

Several potential SPRMs have been identified [*e.g.*, dexamethasone-oxetanone and J867 (29, 30)]. The biological properties of some of these agents have been shown to exert partial PR agonist activity in that they can stimulate the proliferation and differentiation of epithelial endometrial cells in juvenile, estrogen-primed rabbit uterus, although not as effectively as progesterone (30). In contrast, in the presence of progesterone these agents partially inhibit the activity of the PR and thus also possess antagonist properties. Various other measures of progestin/antiprogestin activity, such as induction of cervical ripening and parturition in pregnant guinea pigs, and the ability to inhibit mammary gland proliferation, indicate that the activity of these agents falls between that of antiprogestins such as onapristone (ZK 98,229) and mifepristone (RU486) and progestins such as progesterone and R5020. Differences in the action of SPRMs in comparison with antiprogestins also have been observed at the gene level; whereas medroxyprogesterone acetate (a progestin) and ZK137,316 (an antiprogestin) inhibit vascular endothelial growth factor expression in endometrial fibroblasts, the SPRM J867 is unable to do so (31). Moreover, at least one agent, J1042, is able to induce a marked reduction in endometrial thickness in cynomolgus macaques, suggesting it or a related compound may have potential as an endometriosis therapy (32). Recently, it has been shown that the SPRM asopril can inhibit estrogen-dependent uterine growth but is devoid of the breast stimulatory effects of progesterone. Such a compound is being used to treat endometriosis and uterine fibroids, while sparing the breast of significant stimulation (33).

D. Selective AR modulators (SARMs)

AR expression is widespread throughout the body, and androgens play a desirable role in promoting and maintaining bone strength, increasing muscle mass, decreasing fat tissue, and enhancing libido (34). Although androgen therapies are currently available, they are primarily based on delivery of testosterone or its derivatives by injections or skin patches (35). Neither approach is optimal because injections result in undesirable fluctuations in serum testosterone levels, and skin patches are associated with irritation and rashes. Oral preparations of currently available androgens are not recommended because of their relatively low efficacy and potential hepatic toxicity. There is, therefore, a desire to develop a form of androgen therapy that is easily administered orally and that will avoid the considerable fluctuations of serum androgens observed for injectables. More importantly, the goal exists to obtain androgenic therapies that do not exert undesirable side effects such as alterations in lipid profiles (*e.g.*, high- and low-density lipoproteins), fluid retention, liver toxicity, prostatic hypertrophy, and gynecomastia. The more severe side effects associated with supraphysiological doses of androgens taken by body builders and athletes, such as increased aggression, decreased testicular size, and azoospermia, are unacceptable under all conditions. Simply stated, the goal of preservation of positive androgen effects in some tissues, while minimizing negative side effects in other tissues, has stimulated a search for SARMs.

Although the use of estrogens and SERMs is widespread, there has not been an equivalent trend observed for androgen therapies. This is not due to a lack of indication for androgen treatment, as this type of therapy would be of benefit for treatment of men with primary or secondary hypogonadism, osteopenia and osteoporosis, HIV wasting and cancer-related cachexia, anemias, various muscle dystrophies, and, potentially, male contraception. Although there is a paucity of well-designed studies supporting an indication for androgen therapy in women, androgen therapy has been advocated for improvement of bone strength, libido and other sexual parameters, as well as a sense of well-being in postmenopausal women (36).

Indeed, the current lack of SARM-based therapies results from a lack of a suitable agent. However, the greater understanding of the molecular events through which SERM actions are achieved established a rational basis for identifying and characterizing SARMs. Ideally, such an agent would be orally active, and, as a treatment for hypogonadism, should be capable of stimulating muscle mass and strength, bone strength, libido, and virilization but with minimal hypertrophic effects on the prostate. For osteopenia or osteoporosis indications, a SARM with anabolic activity in bone and possibly muscle, but with relatively little activity on sex-accessory tissues, would be desirable. SARMs for females might target libido and other sexuality parameters while avoiding virilization. Progress has been made in this area, and several compounds that possess a mixture of agonist- and antagonist-like activities in transient transfect assays measuring AR *trans*-activation of a target gene in cells have been identified (37, 38). Assessments of the *in vivo* SARM activity of these

compounds is underway in animals as well as in humans, and they show a promising tissue-selective activity profile. Animal experiments with one such SARM, LGD2226, revealed that it prevented loss of bone mineral density associated with orchidectomy in rats and exerted anabolic activity in the levator ani muscle; in contrast, LGD2226 did not stimulate prostate weights above those observed for intact rats (39).

E. Selective peroxisome proliferator-activated receptor modulators (SPARMs)

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that bind to DNA as heterodimers with the retinoid X receptors. The PPARs have been shown to play an important role in regulating genes involved in lipid metabolism and may well be exploited in the management of various other aspects of health and disease including inflammation and cancer (see Refs. 40–43 for review). The synthetic ligands for the PPARγ receptor, thiazolidinediones, have been exploited for their antidiabetic activity, but due to their side effects (*e.g.*, weight gain and increase in LDL-cholesterol), the search for additional PPARγ agonists has continued (40). Several potential compounds have been identified. One of these, N-9-fluorenylmethyloxycarbonyl (FMOC)-L-leucine improves insulin sensitivity without greatly activating PPARγ adipogenic pathways (44). A second compound, the SR-202 phosphonophosphate, inhibits thiazolidinedione-induced PPARγ activity as measured by *trans*-activation assays and 3T3-L1 cell differentiation to adipocytes, yet is still able to improve insulin sensitivity *in vivo* (45). Most recently, a novel non-thiazolidinedione-selective PPARγ modulator, nTZDpa, which activates a unique profile of PPARγ target genes in comparison with full agonists in white adipose tissue, was shown to ameliorate hyperglycemia and hyperinsulinemia in mice fed a high-fat diet while reducing body weight gain and adipose depot size (46). Moreover, nTZDpa did not cause cardiac hypertrophy, an effect associated with several other PPARγ agonists (47). Although it is not yet clear the extent to which the effects of either of these two compounds are mediated by PPARγ *in vivo*, these agents represent an advancement in the separation of insulin sensitization and adipocyte differentiation properties associated with thiazolidinediones, and they or agents modeled on their structures have potential as a future source of antidiabetic drugs.

F. Other SRMs

With our increased understanding of the mechanisms through which SRM activities are achieved (see following sections), it is now possible to extend the pharmacological concept of executable modulation to other members of the nuclear receptor superfamily. For example, the identification of a selective glucocorticoid receptor (GR) modulator (SGRM) with good antiinflammatory activity and reduced side effects (*e.g.*, diabetogenic, osteoporotic) would be highly beneficial. The recent identification of the nonsteroidal GR ligand, AL-438, indicates that selective modulation of GR activity is obtainable (48). This compound retains potent antiinflammatory activity, as measured by a carrageenan-induced paw edema assay, yet has reduced the severity of side effects associated with glucocorticoid use. In rats, AL-438 did not induce hyperglycemia, nor did it exert the same negative effects on bone associated with use of the potent glucocorticoid, prednisolone. These differences are accompanied by differential regulation of GR target genes *(i.e.*, osteocalcin and aromatase) by prednisolone in comparison with AL-438.

The appearance of tissue selectivity may also be achieved through the use of ligands that distinguish between receptor isoforms and, consequently, regulate biological processes in an apparently tissue-specific manner. For example, therapeutic use of thyroid hormone (T_3) will reduce serum cholesterol, but this is accompanied by various cardiac side effects, such as tachycardia and increased cardiac output. Studies from thyroid hormone receptor-α (TRα) and TRβ knockout mice reveal that the effects of T_3 on the heart are primarily TRα regulated, whereas the effects on cholesterol are mediated via TRβ (49, 50). The development of thyromimetics that selectively target TRβ offers the opportunity to achieve cholesterol-lowering effects with a reduced risk of negative effects on the heart (51). One such agent, KB-141, caused a significant reduction in cholesterol, lipoprotein(a), and body weight with no effect on heart rate in a short-term treatment of primates and suggests that selective TRβ activation may have utility in the treatment of obesity and hypercholesterolemia (52).

III. Mechanisms of SRM Action on Steroid Receptors

A. General steroid hormone action

The basic molecular mechanisms of actions of the ligands for nuclear receptors such as ERα, ERβ, AR, PR, PPARγ, and others have been revealed through research conducted over the last 15^+ years. In general, the effects of these ligands are mediated via their cognate nuclear receptors, which are members of a superfamily of transcription factors. For clarity and because SERMs are the founding members of this class of compound, the basic model of steroid receptor action will be presented relative to the ERs. There are two ERs, ERα and ERβ (53, 54). It has long been established that 17β-estradiol (E2) binding to ERα induces a conformational change in the receptor's hormone-binding domain and enhances receptor dimerization and the ability of receptors to bind to estrogen response elements (EREs) generally located in the promoter region of target genes (1, 55). Studies of the effect of ligands on the structure of the ERβ hormone-binding domain reveal ligand-induced conformational alterations (56–58), and it is clear that ERβ also binds with high affinity to EREs (59, 60). Two distinct regions within ERα, apart from the centrally located DNA-binding domain, specifically contribute to transcriptional activity: the constitutively active, activation function-1 (AF-1), which is located in the amino terminus, and the ligand-regulatable AF-2 found within the hormone-binding domain. The ligand-binding domain (LBD) of ERβ exhibits a moderately high degree of homology to the corresponding region of ERα, and like all other steroid receptors, it possesses an AF-2 domain the activity of which is

sensitive to ligand. However, its AF-1 domain is poorly conserved with that of ERα and is less active (61); certain data suggest that the ERβ A/B domain possesses a repressive function (61–63). Once at the promoter, the "activated," ligand-bound receptor, either ERα or ERβ, interacts with coactivator proteins to form a multiprotein complex that activates the general transcriptional machinery and increases the expression of target genes through processes involving chromatin remodeling, formation of stable preinitiation complexes, and enhanced rates of RNA polymerase II reinitiation (2, 64–69).

The activities of the ERs, when assessed on EREs, can be inhibited by binding to antagonistic ligands, of which there are two types. Class I antiestrogens, such as 4-hydroxytamoxifen (4HT) and raloxifene, are referred to as partial or mixed agonists/antagonists, whereas type II antiestrogens, such as ICI 182,780, are called "pure" antiestrogens. These compounds bind to the ERs with high affinity (70–72); raloxifene and 4HT block the ligand-activated AF-2 domain and particularly in the case of 4HT, leave AF-1 able to initiate gene expression (73, 74). Depending on the cell type and promoter examined, AF-1 and AF-2 can mediate E2-induced transcription independently or synergistically (75, 76); their relative abilities to stimulate gene expression vary in a promoter- and cell type-specific manner (73, 77). As a result, 4HT and raloxifene stimulate gene expression in some, but not all, contexts, and these antiestrogens are therefore classified as SERMs. Importantly, the ability of 4HT or raloxifene to either activate or inhibit gene expression in a context-specific manner indicates that intrinsic cellular differences in processes or factors, such as cell-signaling pathways, accessory transcription factors, and/or transcription factor modulatory proteins, may account for the distinct interpretations of SERM biocharacter (*i.e.*, agonist *vs.* antagonist activity). For ERβ, 4HT and raloxifene also inhibit its AF-2 domain, and due to the relatively poor AF-1 activity of the receptor, these ligands generally block ERβ transcriptional activity measured on EREs (61, 78, 79). In contrast to 4HT and raloxifene, the pure antiestrogens, ICI 164,384 and ICI 182,780, inhibit ERα and ERβ transcriptional activity in a context-indiscriminate manner. It should be noted that 4HT and raloxifene also exert effects on the transcriptional activity of both ERα and ERβ tethered to DNA indirectly through interaction with other transcription factors such as activator protein 1 (AP-1) and Sp1 (80, 81). In this context, the agonist activities of these ligands also is apparent in a cell-specific manner.

B. Effect of ligand on receptor structure

The first results indicating that ligands affect the structure of steroid receptors were obtained from antibody epitope mapping and partial proteolysis experiments (64, 77, 82, 83). For the latter, receptors were incubated with various ligands, subjected to limited digestion with enzymes such as chymotrypsin or trypsin, and, depending on whether ERs were bound to estradiol or antiestrogens, protected polypeptide fragments of different sizes were obtained. This was taken as an indication that these different classes of ligands induced distinct conformational changes in the C-terminal LBD of the receptor, later termed helix 12 (58, 64, 77); similar approaches have revealed ligand-induced conformational changes for AR (84), PR (83), GR (85), and PPARγ (86).

Subsequently, the crystal structures of the LBDs of a number of nuclear receptors, including ERα and ERβ complexed with the agonistic ligands 17β-estradiol or diethylstilbestrol (87, 88), were solved, and, like other members of the steroid receptor superfamily, these regions were found to be composed of 12 α-helices. Surrounding a tightly packed central core composed of helices 5, 6, 9, and 10 are helices 2, 3, 4, 7, 8, and 11; helix 1 is not part of the conical nuclear receptor LBD "sandwich" motif (helices 2–11), and the position of helix 12 is variable. The region on the surface of the LBD to which coactivators bind via their NR boxes (see *Section III.C* for more details) is referred to as the coactivator-binding groove and is composed of residues from helices 3, 4, 5, and 12. The bottom and sides of this groove are nonpolar, but the ends are charged (87). When agonists occupy the LBD, helix 12 packs against helices 3, 5/6, and 11, thus forming part of the coactivator-binding groove. However, relative to the agonist-bound structures of ERα and ERβ, the position of the 12th helix in relation to the remainder of the LBD differs when mixed ER agonists/antagonists, such as 4HT or raloxifene, occupy the ligand-binding pocket (56, 87, 88). In these structures helix 12 is reoriented to partially occlude the coactivator-binding groove, therefore enabling it to block certain AF-2-dependent interactions with coactivators (56, 87). Thus, crystallography substantiates that ER ligands are determinants of the conformation of the LBD of the receptor. It is important to note that structures for the A/B domain or full-length ERs, including information on how ligands affect amino- and carboxy-terminal interactions, are not available. These will undoubtedly be important for fully understanding SERM action.

The conformation of ERβ LBD bound to the pure antiestrogen ICI 164,384 is distinct as revealed by crystallography; helix 12 is disordered and therefore does not appear in the structure (89). However, the bulky side chain of ICI extends out of the ligand-binding pocket and makes contact with a portion of the coactivator-binding groove, therefore likely precluding productive LBD interaction with coactivators. As noted above, ICI antiestrogens, unlike SERMS such as tamoxifen and raloxifene, do not possess partial agonist activity. This has been suggested to result from the ability of ICI 164,384 to inhibit ERα dimerization (90). However, recent data suggest that this is not the case; the ERβ-ICI 164,384 crystal structure reveals a dimer (89), and fluorescence resonance energy transfer experiments demonstrate that both ICI 164,384 and ICI 182,780 induce ERα LBD dimerization (91). The ICI compounds also have been shown to promote a modest nuclear to cytoplasmic shuttling of ERα and induce the degradation of this receptor (92–94). The ICI 182,780 antagonist also has been shown to significantly retard the intranuclear mobility of ERα and to render the receptor resistant to extraction, both suggesting a tight association with a subnuclear compartment (95, 96). The potent antiestrogen activity of the ICI compounds therefore resides in their ability to efficiently block coactivator interactions as well as other aspects of receptor function and expression required for transcriptional activity.

A second approach used recently to characterize the effect

of ligands on the conformation of steroid receptors utilizes affinity selection of phage-displayed peptides (reviewed in Ref. 97). Full-length recombinant ERα or ERβ immobilized on tissue culture plates incubated with phage in the absence or presence of various ER ligands revealed that ligand-induced ER-phage interactions varied depending on the ligand and type of receptor (57, 98, 99). These patterns of interactions are consistent with the ability of each ligand to induce a receptor conformation that exposes a unique peptide-binding surface. These results are notable not only because they are consistent with the above mentioned crystallography studies, but also because they reveal structural differences in the context of full-length ERs instead of only the LBDs, as is the case for the crystallographic analyses. In addition, they clearly reveal differences in the structures of ERs bound to either 4HT or raloxifene, which are difficult to discern in the crystallographic analyses. This is important for understanding the molecular basis for differences in the biological activities of these two SERMs. Peptide-based approaches also have been used to evaluate the conformation of AR (100).

C. Coactivators

1. Coactivator interactions with ERs. The hypothesized ability of coactivators to bind to steroid receptors in an agonist-dependent manner was exploited in the initial predictions of coactivators and corepressors (101–103). Upon cloning of the first authentic steroid receptor coactivator (SRC)-1, its interaction with PR or ER was demonstrated to be promoted by agonist and inhibited by antagonist (104). SRC-1 (NCoA-1) was the progenitor molecule for the SRC-1/p160 family of coactivators, which includes SRC-2 [transcriptional intermediary factor 2 (TIF2)/GR-interacting protein 1 (GRIP-1)] and SRC-3 [(ACTR/pCIP/receptor associated coactivator (RAC3)/TRAM-1/amplified in breast cancer 1 (AIB1) (104–112)]. This slightly confusing nomenclature will vary in this review, depending on the laboratory source for the data. Subsequent analyses have defined the NR box motifs (LxxLL, where L = leucine and x is any amino acid) found within many coactivators including the p160s as critical for their ability to bind to steroid receptors via their coactivator-binding groove within the LBD (113, 114). The structure of the co-crystal complex of the ER agonist, diethylstilbestrol, with the ERα LBD and a NR box-containing portion of the GRIP1 coactivator reveals that residues within helix 12 as well as within helices 3 and 5 are important for mediating interactions between ERs and coactivators (87, 88, 115). As discussed above, helix 12, by virtue of the ability of ligands to alter its position relative to the remainder of the LBD, plays a critical role in regulating coactivator interactions with this region of the receptor (87, 116, 117). Thus, agonists promote coactivator binding to ERs by inducing a LBD structure favorable for this interaction. However, it is important to note that steroid receptors and coactivators can utilize other regions within their structures to bind to one another. For example, SRC family coactivators as well as cAMP-response element binding protein (CREB)-binding protein (CBP) and p300 also bind to the A/B domain of ERα and ERβ in a hormone-independent fashion (118–120); in the case of at least GRIP1 this interaction with the A/B domain is not dependent on the coactivator's LxxLL motifs.

2. Coactivators functional roles. Experiments performed in yeast provided some of the first indications that there may be competition among transcription factors (squelching) for binding to a limiting pool of accessory factors necessary for gene expression (121). Work in transient transfection systems utilizing cotransfection of PRs and ERs extended this concept to the nuclear receptor superfamily and strongly suggested that, in order to activate gene expression, receptors had to interact with some unknown factors in the cell (122). After initial biochemical identification of several ER-interacting proteins (103, 123), molecular biological approaches, chief among them yeast two-hybrid assays, resulted in the cloning of more than 50 coactivators in a relatively short period of time (66, 68, 69). In general, coactivator proteins (note: there is one RNA coactivator; see Ref. 124) do not bind to DNA, but interact indirectly through association with other DNA-binding proteins (*e.g.*, nuclear receptors). Once recruited to the promoter, coactivators enhance transcriptional activity through a combination of mechanisms, including efficient recruitment of basal transcription factors such as template-activating factors and TATA-binding protein. In addition, nuclear receptor-interacting coactivators possess themselves, or recruit other nuclear proteins that possess, enzymatic activities crucial for efficient gene expression including the ATP-coupled chromatin-remodeling SWI-SNF complex, a number of acetyltransferase proteins (*e.g.*, CBP/p300, pCAF, and p160s), methyltransferases [*e.g.*, coactivator-associated arginine (R) methyltransferase-1 (CARM1) and PRMT-1/2] and ubiquitin ligases (*e.g.*, E6-AP and Rsp5) (125–128). A general model of coregulator interactions with ERα is presented in Fig. 1 (see accompanying legend for more details).

A detailed review of the biochemistry and molecular biology of coregulating molecules (both coactivators and corepressors; see below) is beyond the scope of this review, and the reader is referred to several recent reviews (2, 66, 69). In general terms, ligand-activated nuclear receptors bind to DNA and through interaction with the SWI/SNF chromatin remodeling machine and acetyltransferases there is a disruption of the local nucleosomal structure. The TR-associated protein (TRAP)/vitamin D receptor-interacting protein (DRIP) complex is recruited then to target gene promoters and makes direct contact with components of the basal transcription machinery to bring about transcriptional initiation. Additional coactivator molecules carry out subsequent downstream reactions in the transcription process, such as RNA processing (129, 130) and turnover of the receptor-coactivator complex (131).

Several recent reports primarily employing chromatin immunoprecipitation (ChIP) assays suggest that receptor and coregulator association in gene promoters is temporally regulated. For instance, ERα appears to cycle off and on the pS2 promoter in certain cells (*e.g.*, MCF-7) in response to continuous E2 stimulation (132). *In vitro* transcriptional assays indicate that SRC-1 must be recruited to the promoter before p300 for efficient gene expression (133). In addition, a recent report has shown that SRC-1

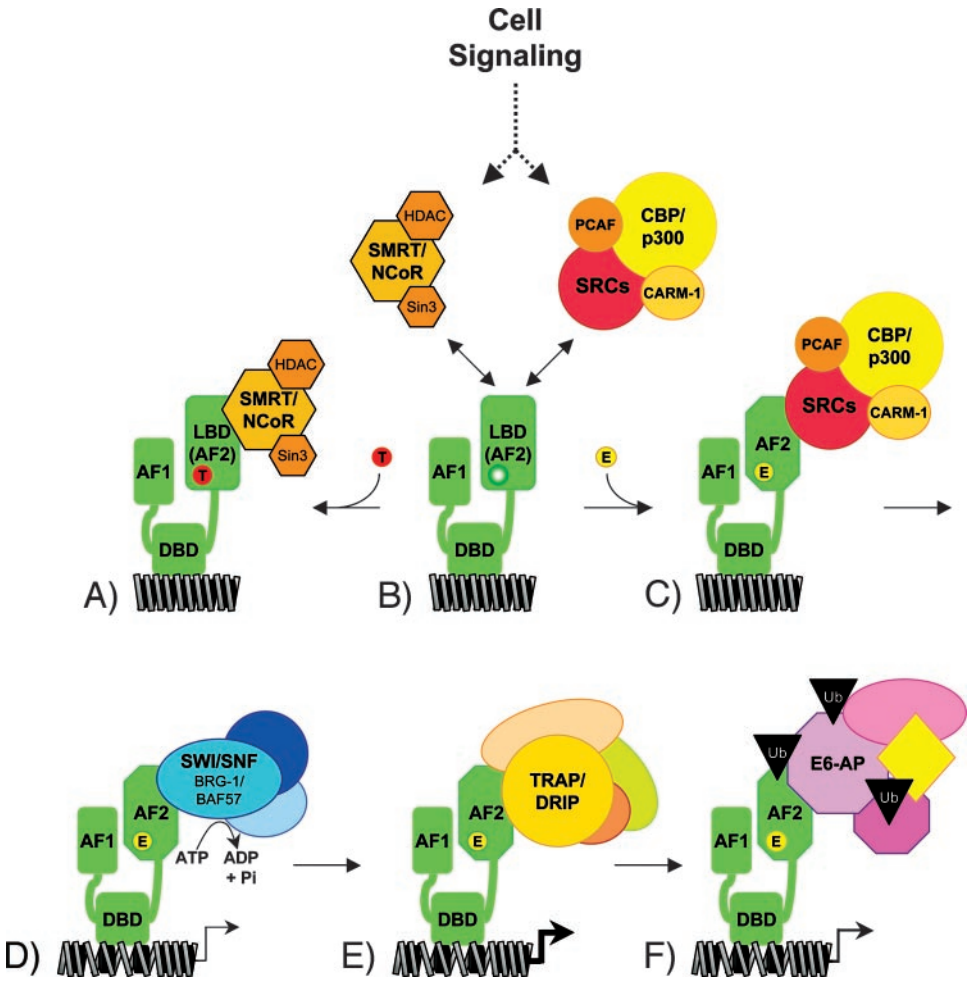

FIG. 1. Model of nuclear receptor-dependent gene expression. This represents a hypothetical schematic of the exchange of coregulators involved in activation of a gene by a steroid hormone receptor, such as ERα. Coactivators and corepressors exist in complexes in the cell and do not appear to bind to receptor as monomers. A, In the presence of antiestrogens, such as tamoxifen (T), the receptor interacts with a complex of corepressor proteins, including SMRT and/or NCoR, that maintains the gene in an inactive state. B, In the unliganded state, ERα may bind to either corepressor or coactivator complexes. Intracellular signaling can influence the extent of interaction with these complexes and therefore the relative magnitude of basal receptor activity: less activity when bound to corepressor complexes and more activity when the equilibrium is shifted to coactivator complex interaction. C–E, When estrogen (E) activates the receptor, a series of coactivator complexes bind and exchange in a programmed sequence to deliver functions needed to activate the gene (see series of reactions, panels C–E). This arguably involves the sequence of histone acetylation (or other modifications) carried out by histone acetylases (CBP/p300 and SRCs), followed by a complex containing BRG-1/BAF57, which unwinds DNA and remodels the chromatin, followed by a complex involved in initiation of transcription. These early complexes all may include SRC-1 or one of the other members of the SRC-1 family. After initiation, reinitiation/maintenance of transcription is carried out by TRAP220 and the TRAP/DRIP complex of proteins, which, in turn, interact with RNA polymerase II itself. F, Finally, coactivator complexes and the receptor itself are turned over at the promoter by proteasome-dependent processes. The presence of protein complexes containing ubiquitin ligases, such as E6-AP and MDM2, which polyubiquitinate proteins and target them for degradation by the 26S proteasome, have been noted. The turnover leads to down-regulation of receptor/coactivator levels, but this turnover also is required for efficient continued transcription of the gene. DBD, DNA-binding domain; HDAC, histone deacetylase; Ub, ubiquitin.

and the DRIP205/TRAP220 coactivator cycle off and on the pS2 promoter after E2 treatment; notably when SRC-1 is bound, TRAP220 is absent and vice versa (134). Although the rapid kinetics of receptor and coactivators are evident in cellular imaging experiments (95, 135), it is unknown what controls the dynamic association of nuclear receptors and coregulators with target genes, or whether these processes can be regulated in a cell-specific fashion. Nonetheless, the ability of steroid receptors to activate transcription is a product of the ability of the receptor to interact with coactivators and other proteins required for gene expression, and the effect of various enzymatic activities on the formation, function, and disassembly of the receptor-coactivator complex.

D. Corepressors

Although there are far fewer nuclear receptor corepressors, these molecules serve important roles in negatively regulating receptor-dependent gene expression. Unliganded retinoic acid receptor (RAR) and thyroid-hormone receptor (TR) repress basal transcription in the absence of their cognate ligands, and this function is mediated, at

least in part, by two, large (~270 kDa) nuclear proteins, silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) and nuclear receptor corepressor (NCoR). These corepressors bind to the unliganded receptors via CoRNR boxes, which consist of LxxxI/HIxxxI/L motifs (136–139). Upon hormone binding, these corepressors dissociate from receptor and enable TR and RAR to associate with coactivator(s) and stimulate gene expression (138). Accordingly, the occupancy of the LBD, and therefore its conformation (140), dictates whether these receptors interact with coactivators or corepressors and activate or repress transcription (141). Although these corepressors do not appear to possess intrinsic repressive activity, they, like coactivators, also function as part of larger protein complexes that include histone deacetylases, which enhance tight nucleosome-DNA interactions and inhibit transcription factor recruitment and gene expression.

Although corepressors bind very well to some nuclear receptors in the absence of their cognate ligands, this is less the case for steroid receptors such as ER, PR, GR, and AR. Rather, corepressors bind to these receptors in the presence of their respective antagonists, 4HT, RU486 (for PR and GR), and cyproterone acetate (119, 142–145). Both 4HT and raloxifene have been shown to recruit NCoR and SMRT to certain ER target gene promoters (132, 146). The molecular basis of the interactions between steroid receptors and corepressors is not well defined, but a CoRNR box-containing peptide can bind to ERs in the presence of tamoxifen, and mutations within helices 3 and 5 inhibit this interaction (147). Transient transfection assays first demonstrated that both NCoR and SMRT can selectively repress the agonist activity of 4HT and RU486 on ERα and PR, respectively (142, 143). Subsequently, it was demonstrated that injecting inhibitory antibodies to NCoR or SMRT promoted the agonist activity of 4HT (119) and that 4HT was a relatively potent ERα agonist in fibroblasts derived from NCoR null mice (148). Taken together, the data indicate that SMRT and NCoR are weak ERα corepressors, but that these proteins inhibit the agonist potential of antiestrogen-liganded ERα activity. Evidence for coregulator inhibition of agonist-bound steroid receptor by the DEAD box RNA helicase DP97 and a novel corepressor, LCoR, also has been obtained (149, 150). Whether corepressor binding represses the ligand-independent activity of these receptors is less clear, but is possible (151). The existence of a cellular equilibrium of coactivators and corepressors that can be shifted toward corepressor preference by antagonist is most likely. Interaction of NCoR and/or SMRT with AR, GR, and PR also has been demonstrated (142, 144, 145, 152, 153).

In addition to NCoR and SMRT, several other molecules have been associated with negative regulation of steroid receptor activity. SAP30 has been shown to be important for NCoR-mediated repression of antagonist bound ERα whereas Sharp may play a role in repressing estrogen-induced ERα activity indirectly via its effects on the SRA coactivator (154–157). Corepressor activity also has been shown for MTA (158) receptor-interacting protein (RIP140)/Nrip140 (159, 160), REA (161, 162), RTA (163), and DAX (164).

E. SRM hypothesis

With the identification of coactivators and corepressors, and the biochemical demonstrations that ligands regulate the interactions of receptors with coregulator proteins, it has become possible to more fully consider the role of coregulators in regulating receptor function. Early work demonstrated that overexpression of SRC-1 significantly enhanced 4HT-stimulated ER activity in a cell environment (*e.g.*, HepG2 cells) where 4HT exhibits agonist activity (143). However, exogenous SRC-1 does not robustly increase 4HT agonist activity in all cells, suggesting some component of cell specificity (143). Nonetheless, the ability of SRC-1 to modulate ER-4HT activity in HepG2 cells suggests that productive ER-SRC-1 interactions do occur in some cells in the presence of 4HT. It is possible that *in vitro* interactions between 4HT-occupied receptor and SRC-1 are generally not observed because studies often fail to consider the contribution of the AF-1 or DNA-binding domains (103). When the corepressors NCoR or SMRT were ectopically expressed in cells, the agonist activity of 4HT was reduced (142, 143, 148), and this is consistent with antiestrogens promoting interactions between ER and corepressors. Collectively, these data suggest that perturbing the expression of coactivators and corepressors within a cell affects the relative agonist and antagonist activity of the SERM, 4HT (see model in Fig. 2). Many studies have followed this line of reasoning, and the activity of a potential corepressor is typically assessed by determining the ability of the candidate molecule to reduce the agonist activity of the SERM, 4HT.

Similarly, NCoR and SMRT interact with PR in the presence of partial antiprogestins such as RU486, and overexpression of these corepressors reduced the partial agonist activity of these compounds (142, 153). Further evidence that the relative expression of coactivators and corepressors regulates the activity of partial antiprogestins was obtained in *in vitro* chromatin transcription assays using extracts from T47D and HeLa cells (165). The ratio of SRC-1 to NCoR and SMRT was 2.4 and 0.8 for these cells, respectively. Increasing the SMRT levels in T47D cell extracts reduced the agonist activity of RU486, whereas increasing SRC-1 levels in HeLa cell extracts enhanced the agonist activity of this ligand. Moreover, RU486-liganded PR bound to both SRC-1 and SMRT (165). For GR and PR, corepressors have been shown to shift the dose-response curve for antagonists and agonists to the right, whereas coactivators shift the curves to the left (29, 166). The coactivator responses are similar to the ability of increased PR or GR expression to left shift the dose-response curves, suggesting that variation in receptor or coregulator expression is a general mechanism for regulating the "sensitivity" of target cells to steroid hormones. Experiments with mutant forms of TIF2 reveal that coactivators can left shift GR activity in the presence of an antisteroid independent of the ability of TIF2 to bind to CBP, p300, or pCAF, suggesting that this effect is mechanistically distinct from events typically associated with chromatin remodeling and initiation of transcription (167). Taken together, these studies substantiate the currently accepted theory that the relative expression of coactivators and corepressors within a cell influences the ability of SRMs to regulate gene expression

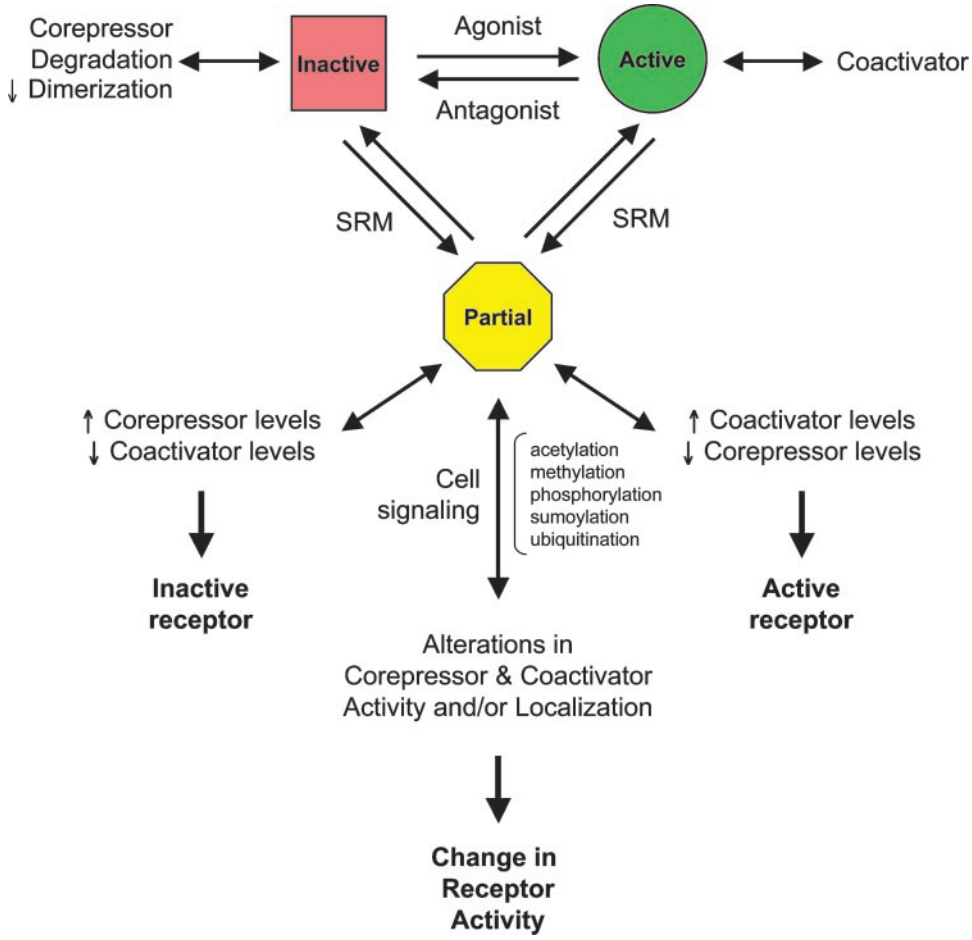

FIG. 2. Model of the contribution of coactivators and corepressors to relative SRM agonist/antagonist activity. In the presence of agonist, nuclear receptors in the active conformation interact well with coactivators and are transcriptionally active. In the presence of antagonist, receptors adopt an inactive conformation and preferentially interact with corepressors, resulting in loss of transcriptional activity. In the presence of SRMs, nuclear receptors adopt a conformation intermediate between the active and inactive states and therefore have the potential to interact with either coactivators or corepressors and exert partial activity. The activity of SRM-occupied receptors depends on the relative expression of coactivators and corepressors in a given cell environment and the effect of cell signaling on coregulator subcellular localization and/or activity.

(143). However, it is not yet clear whether the expression of a threshold level of a limited number of specific coactivators or corepressors dictates the relative agonist/antagonist activity of SRMs or whether the relative ratio of all coactivators to all corepressors is the critical determinant of SRM activity.

IV. Molecular Basis of Cellular Selectivity

A. Receptor-selective recruitment of coactivators

With the possible exception of SRM-induced action emanating from the plasma membrane and/or cytoplasm (*e.g.*, see Refs. 168 and 169), the ability of SRMs to regulate gene expression is dependent on their binding to an appropriate receptor. In the case of SERMs, SPRMs, and SGRMs, there are, however, two distinct receptors to which ligands can bind. In the case of estrogens, the identification of a second receptor gene brought about the realization that estrogen effects are mediated through either ERα or ERβ. Work from many investigators has characterized functional similarities and differences between these two receptors (170). Although both receptors bind estradiol and SERMS with similar affinity and interact with the same DNA response element, the transcriptional activity of these receptors is distinct. For instance, estradiol generally stimulates greater transcriptional activity via ERα than through ERβ (61, 171, 172). More pronounced differences are observed in the case of SERM-bound receptors. For ERE-dependent gene expression, tamoxifen is a partial agonist of ERα but is generally unable to stimulate ERβ transcriptional activity (61, 171, 173). Conversely, when assessing ER activity on AP-1 containing reporter genes, tamoxifen will stimulate ERα and ERβ transcriptional activity (174).

It has been postulated that differences in the activities between the respective forms of each of these receptors is due to differences in the abilities of the receptors to interact with coregulatory proteins. In support of this, a number of differences in the ability of ERα and ERβ to interact with coactivators have been noted. For estrogen-bound receptors, ERβ, but not ERα, binds well to the receptor-interacting component of the mammalian mediator complex, TRAP220 (175). In contrast, the PPARγ coactivator-1α (PGC-1)-related coactivator, PERC, coactivates ERα, but not ERβ, transcriptional activity (176). There are also several AF-1-specific coactivators, such as p68/p72 and MMS19; in the case of the former, they enhance ERα, but not ERβ, transcriptional activity (177–179).

There are differences between the relative affinities of ERα and ERβ for members of the p160 coactivator family. The ERα-specific agonists, propyl pyrazole triol and R,R-tetrahydrochrysenes bind to both ERα and ERβ, but induce interaction of p160 coactivators only with ERα, not ERβ (180). Other differences in binding are more subtle. Using an ap-

proach in which the NR box regions of the SRC family are assessed for their ability to bind to ERs in the presence of estradiol, ERα, compared with ERβ, was shown to bind with greater affinity to each of these coactivators (181). Real-time interactions between ERs and NR box domains of each of the p160s assessed by BIAcore technology substantiate that result (182). However, this approach also revealed that differences between ERs and full-length SRC family members were more similar with a relative strength of RAC3/SRC-3 greater than SRC-1 greater than TIF2/SRC-2. Differences in binding also can be observed in analyses of the ability of ERα and ERβ to interact with peptides encompassing each of the individual NR boxes of the p160 coactivator family (183, 184). For instance, NR box IV of SRC-1 binds much better to ERβ than to ERα, regardless of ligand (*e.g.*, estradiol, diethylstilbestrol, or genistein). In addition, the nature of the ligand influences the relative affinity of the receptor for particular NR boxes; interaction of the estradiol-bound ERβ is approximately two times greater than genistein-bound ERβ to SRC-2 NR box I, whereas genistein-bound ERβ binds approximately five times better to SRC-2 NR box III than the same receptor bound to estradiol. This is also consistent with genistein-bound ERβ binding better to p160 coactivators than genistein-bound ERα (185).

In the case of progestins and glucocorticoids, there are two hormone-binding receptors for each class of ligand. However, these do not arise from separate genes, but rather from variations in transcriptional start sites and translation initiation (186–189). The A form of PR and the B form of GR (PR_A and GR_B) represent N-terminal deletions of the larger proteins, PR_B and GR_A, respectively. Regardless of the route in which the different receptors are derived, they have distinct biological activity. In both cases, the activity of the B forms of each receptor is greater than the A receptor isoform (186, 190). Moreover, PR_A can also repress the transcription of other transcription factors, and this extends to other steroid receptors such as ERα (190–192). In the case of PRs, an inhibitor domain has been mapped within the amino terminus of PR_A (193) that is required for the ability of PR_A to bind with greater affinity than PR_B to the corepressor SMRT (194). In contrast, PR_B binds better to NR box peptides of SRC-1 and GRIP1, and this undoubtedly contributes to this receptor's greater transcriptional activity.

Another example of the specificity of nuclear receptor interactions with coactivators comes from a study examining the requirement of specific p160 coactivators for activation of an integrated chromosomal reporter gene, MMTV-CAT, by GR and PRs and their respective ligands (195). ChIP assays revealed that both receptors recruited SRC-3 to the target gene. In addition, PR recruited SRC-1 and CBP, and this was associated with acetylation of histone H4 on Lys^5. In contrast, ligand activation of GR led to recruitment of SRC-2 and pCAF followed by Ser^{10} phosphorylation and Lys^{14} acetylation of histone H3. Thus, even for identical cell and promoter contexts, closely related receptors can utilize different complements of coactivators in the process of activating gene expression. Differences in the interactions of PPARγ with p160s also have been observed (44). In the presence of rosiglitazone, TIF2 interaction with this receptor is greater than for SRC-1, whereas in the presence of the selective PPAR modulator, FMOC-L-leucine, which activates insulin-sensitizing but not adipogenesis pathways, PPARγ preferentially interacts with SRC-1. Taken together, these results suggest that p160s may substitute for one another to the extent that they promote overall target gene expression, but that differences in downstream events (*e.g.*, histone modification or biological responses) are associated with different coactivator usage.

B. Influence of DNA on coregulator interaction

Although consensus DNA response element sequences have been defined for members of the steroid receptor superfamily, it is clear that not all target genes contain the ideal sequence required to mediate receptor-DNA interactions. For instance, for the 38 estrogen-responsive genes reviewed by Klinge (196), most of the functional EREs located within the promoters or 3′-untranslated regions are not the traditional consensus sequence. Thus, many target genes contain response elements that bear little similarity to consensus EREs. It has been demonstrated that the sequence of the response element affects the affinity that a given receptor has for binding DNA. As might be expected, ERα binds with the greatest affinity to the consensus ERE sequence found within the vitellogenin A2 gene and less well to the imperfect EREs found within the vitellogenin B1, pS2, and oxytocin genes (197). This explains, at least in part, how the sequence of the response element can be one important determinant of the extent to which ERs can activate gene expression (197–200).

However, the conformation of transcription factors can be altered through binding to DNA (reviewed in Ref. 201). The specific sequence of the receptor response elements for ERs and TR can exert distinct allosteric effects on the conformation of ERα, ERβ, and TR. This has been shown by experiments in which DNA-bound ERs or TR are subjected to limited proteolysis (197, 202) as well as experiments in which LXXLL-containing peptides sensitive to the conformation of the ERα or ERβ vary in their relative ability to bind to receptor depending on the nature of the ERE (203). Just as ligand-induced changes in receptor conformation influence receptor interactions with coactivators, consensus and imperfect EREs also influence the relative ability of ERs to bind to coactivators. Using an approach based on the relative ability of ERs bound to various response elements to bind to coactivators in HeLa cell nuclear extracts, it has been shown that TIF2 interacts better with ERα bound to EREs from the vitellogenin A2, pS2, or oxytocin genes than from the vitellogenin B1 gene (198). In contrast, the response element sequence did not affect ERα interaction with AIB1 (198). A similar theme of ERE sequence affecting ERβ interaction with coactivators in U2OS nuclear extracts also has been documented (199). However, in this case, interaction between ERβ bound to EREs from either the pS2 or vitellogenin B1 genes bound to TIF2 and AIB1 less well than ERβ bound to the vitellogenin A2 gene (199). This also correlated with the inability of overexpressed AIB1 to enhance ERβ-mediated transcription of synthetic target genes containing either a pS2 or vitellogenin B1 ERE.

In addition to the nature of the steroid response element itself, the context in which the response element resides also

determines the ability of steroid receptors to interact with coactivators. The estrogen responsiveness of the pS2 gene is dependent upon both an ERE and an AP-1 site located adjacent to one another; mutation of either one of these sites significantly compromises, but does not block, induction of target gene expression by estrogens (204, 205). It is known that SRC-1 and TIF2 both can coactivate pS2 gene expression (204, 206). However, SRC-1 coactivation of estrogen-stimulated pS2 expression is more dependent on a functional AP-1 site than is coactivation by TIF2 (204). The difference in the relative ability of SRC-1 and TIF2 to coactivate pS2 expression is dependent on the sequence of the ERE; substitution of the pS2 ERE with a consensus ERE enables SRC-1 to coactivate gene expression regardless of whether or not the AP-1 site is mutated.

It should be noted that many estrogen-responsive genes do not appear to contain functional ERE sequences, and the ability of ERs to regulate gene expression is achieved via indirect tethering of the receptor to DNA via other transcription factors, such as AP-1 and Sp1 (reviewed in Refs. 80 and 81). ChIP experiments have demonstrated distinct patterns of tamoxifen-induced association of the promotors of the c-Myc and cathepsin D genes with coactivators and corepressors (146). In Ishikawa cells, tamoxifen stimulates the expression of c-Myc but not cathepsin D mRNA; this result correlates with the recruitment of coactivators to the c-Myc promoter and corepressors to the cathepsin D promoter. Intriguingly, ER regulation of c-Myc expression is dependent upon a discrete non-ERE-containing site (207), whereas ER regulation of cathepsin D is directly mediated via an ERE (208). Although not formally proven, these data raise the interesting hypothesis that tamoxifen cell specificity may be influenced by the mechanism by which ER is tethered to the promoters of target genes, and therefore the ability of the receptor to recruit coactivators or corepressors. It is interesting to note in this study that raloxifene recruited corepressors to both target gene promoters, thus clearly distinguishing itself from tamoxifen.

C. Effect of cell signaling on receptor-coregulator interactions

A number of cellular signaling pathways influence the ability of coregulators to exert their effects on nuclear receptor-dependent gene expression. A summary of enzymatic, protein-protein, and regulatory effects for a selected group of coactivators is located in Table 1. More details are presented in the following sections.

1. Nuclear receptor phosphorylation. Activation of ERs, PRs, ARs, and other nuclear receptors is accompanied by an increase in receptor phosphorylation and associated with an increase in the transcriptional activity of the receptors (209–211). Although in most cases, the molecular mechanisms through which changes in receptor phosphorylation alter gene expression are unclear, several reports have shed light on the potential range of mechanisms that likely contribute to this mode of regulating gene expression. For example, it long has been known that ERα is phosphorylated in response to estrogen treatment in cells as well as stimulation with various growth factors, and that this phosphorylation is associated with an increase in the receptor's transcriptional activity (209, 212). Several studies relate this to alterations in coregulator interactions. For instance, in breast cancer cells, phosphorylation of Ser^{118} in human ERα leads to reduced interaction with the SMRT corepressor (119), whereas another laboratory has demonstrated that this posttranslational modification is required for the interaction of this receptor with the amino-terminal-specific coactivator, p68 (177). Although an early report indicated that phosphorylation of the A/B domain of ERα did not affect interactions between GAL-A/B and the p160 and CBP/p300 coactivators (118), recent work has shown that phosphorylation of serines 104, 106, and 118 does positively modulate this interaction, particularly in the context of the full-length receptor (213). It is not clear to what extent, if any, that the phosphorylation-induced loss of interaction between ERα and corepressors contributes to the phosphorylation-induced interaction between this receptor and coactivators. Phosphorylation of serines in the amino terminus of ERβ also has been shown to enhance interactions between this receptor and SRC-1 and CBP (214, 215). Therefore, to the extent that nuclear receptors can be differentially phosphorylated in various cellular environments, this can directly affect the ability of coregulators to interact with these receptors and affect their transcriptional activity.

2. Coregulator phosphorylation. Although there is a long history of steroid receptor phosphorylation and the effect of this posttranslation modification on receptor function (209, 210), it has been recognized only recently that both coactivators and corepressors are also substrates for kinases (Fig. 3). For instance, SRC-1 is a phosphoprotein in which seven phosphorylation sites have been identified (216), two of which (Thr^{1179} and Ser^{1185}) can be phosphorylated *in vitro* by the MAPK, Erk2 (216). Although treatment of cells with a steroid receptor ligand, progesterone, does not increase SRC-1 phosphorylation, cAMP treatment of cells does; however, this probably occurs as a result of cAMP stimulating the activity of the Erk1/2 kinases (217). SRC-1 phosphorylation contributes to its ability to coactivate cAMP- or progesterone-induced PR transcriptional activity, as evidenced by reduced coactivation by a SRC-1 phosphorylation mutant and the ability of the MAPK kinase (MEK)1/2 inhibitor U0126 to reduce SRC-1 coactivation (217). This is not due to any effect on the intrinsic transcriptional activity of SRC-1, or the ability of this coactivator to bind to PR or CBP, but rather a reduction in SRC-1 interaction with the pCAF coactivator, and a loss of SRC-1 functional cooperation with CBP has been observed (217). Phosphorylation of SRC-1 is also important for its coactivation of AR ligand independently activated via IL-6 signaling (218). In contrast, mutation of all seven known SRC-1 phosphorylation sites does not specifically inhibit coactivation of ERα function stimulated by either E2- or cAMP-signaling pathway; rather this results in a general reduction in coactivation function (219).

Likewise, Erks phosphorylate GRIP1 (SRC-2) at Ser^{736} (220), and treatment of cells with epidermal growth factor (EGF) or TGFα increases the intrinsic transcriptional activity of GAL-GRIP1 (220). Moreover, mutation of Ser^{736} to an

TABLE 1. Interacting factors that possess enzymatic activity

Enzymatic activity	Interacting factor	Target	Examples of effects on gene expression
Acetylase	CBP/p300, SRC-1/3, pCAF	ACTR, nuclear receptors, histones	Acetylation of ACTR by CBP/p300 promotes dissociation of the p160 from ER and attenuates transcription (274); acetylation of ER may decrease receptor transcriptional activity whereas AR acetylation may enhance its activity (274, 329); histone acetylation alters chromatin structure and facilitates transcription (330).
Helicase/splicing factor	p68, p72, CoAA, PGC-1	Steroid-responsive genes and their transcripts	Overexpression of p72 and CoAA influences extent of exon inclusion *vs.* exclusion (129, 225).
Kinase	cdk7/TFIIH	Nuclear receptors, coactivators, corepressors	Interaction of TFIIH with ER or AR results in AF-1 phosphorylation via cdk7 (331, 332); other phosphorylation events also affect nuclear receptor and coregulator function [*e.g.*, SRC-1 phosphorylation facilitates coactivation of PR via enhanced interaction with pCAF and CBP (217); phosphorylation of SMRT induces its translocation to the cytoplasm (235)].
Methylase	CARM1, PRMT-1/2	CBP, histones	Methylation of CBP shifts the binding preference of CBP from CREB to nuclear receptors (271); CBP methylation enhances its coactivation function (273); CARM1 methyltransferase activity increases ER transcriptional activity (272).
NEDD8-activating enzyme	Uba3	Nuclear receptors	Stimulation of a neddylation pathway increases ERα ubiquitination and turnover (333, 334).
Phosphatase	Cdc25B		Overexpression of cdc25 enhances AR transcriptional activity; this does not require its phosphatase activity (335).
SUMO-1 conjugating or ligase enzyme	PIAS proteins, Ubc9	Nuclear receptors, coactivators	Sumoylation of SRC-1 or GRIP1 enhances their interaction with nuclear receptors (255, 256, 260); sumoylation of the N terminus of AR inhibits its transcriptional activity (336).
Ubiquitin-conjugating or ligase enzyme	E6-AP, RPF1, p300	Nuclear receptors, coactivators, corepressors, histones	Ubiquitination of nuclear receptors targets them for destruction by the 26S proteasome (131, 242, 249); coactivators and corepressors are also targets of the proteasome (253, 265); p300 possesses ubiquitin ligase activity (337).

Selected factors that can interact with nuclear receptors and/or coregulators and also possess enzymatic activity. In some, but not all, cases the activity is required to modify steroid receptor-dependent gene expression. Space constraints preclude a complete listing of the effects on gene expression.

alanine residue reduces the ability of EGF to stimulate the intrinsic transcriptional activity of this coactivator, as well as the ability of GRIP1 to coactivate PR stimulated by either R5020 or EGF. Similar results are obtained with ERα in which mutation of the ERK2 phosphorylation site on GRIP1 inhibits the ability of this coactivator to stimulate ERα transcriptional activity stimulated by EGF and E2. The mechanism by which this occurs is not well defined, but there is evidence to suggest that functional interactions between GRIP1 and CBP are compromised by mutation of the Ser^{736} site. Note that the $serine^{736}$ to $alanine^{736}$ mutant form of GRIP1 is nuclear in the absence or presence of EGF (220). In addition, GRIP1 also can be phosphorylated by c-Jun N-terminal kinase 1 *in vitro* (220), and this appears to occur through sites other than Ser^{736} or Ser^{554} (220). AIB1 (SRC-3) can also be phosphorylated *in vitro* with Erk2, and transfection of MCF-7 cells with a constitutively active form of MEK1 results in increased intrinsic transcriptional activity of AIB1 as well as enhanced interaction with p300 (221). Moreover, SRC-3 is found as a complex with IκB kinase (IKK), the kinase responsible for phosphorylation and subsequent degradation of IκB and consequently the activation of nuclear factor-κB (NF-κB); IKK complexes can phosphorylate SRC-3 *in vitro* (222). Intriguingly, elevated SRC-3 phosphorylation is observed in cells treated with TNFα, an agent that activates the IKK/NF-κB pathway, and this correlates with a shift of compartmental equilibrium for SRC-3 from the cytoplasm to the nucleus, suggesting that phosphorylation influences the subcellular localization of SRC-3 (222). A second report confirms that p/CIP (SRC-3) subcellular localization is regulatable, as evidenced by the treatment of serum-starved cells with growth factors or phorbol esters promoting the translocation of p/CIP from the cytoplasm to nuclear compartments (223).

Phosphorylation of coactivators also may affect their ability to interact with steroid receptors. For instance, TRBP/ASC-2/RAP250/AIB3/PRIP is a coactivator that in addition to binding to nuclear receptors via a single LXXLL motif (224) associates with CBP/p300, the DRIP130 component of DRIP/TRAP complexes, the RNA recognition motif-containing coactivator CoAA, DNA-dependent protein kinase, and poly(ADP-ribose) polymerase complexes (224–227). Although wild-type TRBP binds well to both ERα and ERβ, mutational analyses of the region surrounding the LXXLL motif within TRBP revealed that the amino acid located at the -3 position relative to the NR box could influence the relative binding of TRBP mutants to ERα *vs.* ERβ (228). More-

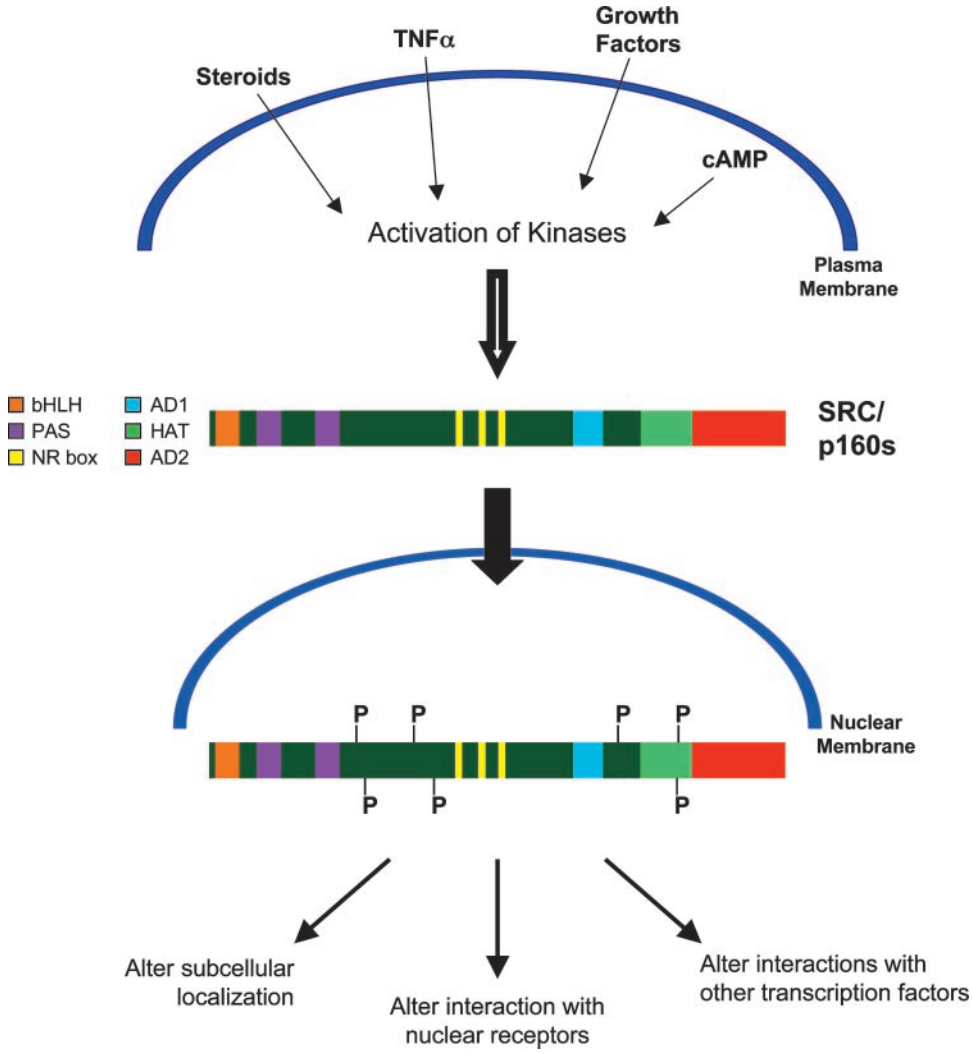

FIG. 3. Effect of phosphorylation events on coregulators. Signaling pathways activated by steroids through nongenomic signaling pathways (*e.g.*, src kinase or novel G protein-coupled receptors). TNFα or growth factors via their receptors, or elevation of intracellular cAMP (via stimulation of cells with neurotransmitters or pharmacological agents) can communicate with coactivators and corepressors, resulting in the phosphorylation of coregulators in the cytoplasm or nucleus. In the case of the p160 coactivator, SRC-3 (schematic of structural and functional domains is given, see *color key*), phosphorylation (P) takes place in the cytoplasm and is associated with the translocation of SRC-3 from the cytoplasm to the nucleus. bHLH, Basic helix-loop-helix; HAT, histone acetyltransferase; PAS, Per/Arnt/Sim domain.

over, *in vitro* experiments demonstrate that MAPK phosphorylation of the wild-type −3 position amino acid, Ser^{884}, inhibits the interaction between a TRBP peptide and both ERα and ERβ, thereby suggesting that phosphorylation of TRBP may be a negative regulator of its coactivator function (228). Likewise, phosphorylation of the AR coactivator, ARA55, by the proline-rich tyrosine kinase 2 (Pyk2) reduces ARA55 coactivation of AR-dependent gene expression (229). This appears to be related, at least in part, to a reduction in interaction between AR and ARA55.

Finally, phosphorylation of CBP/p300 may also affect the activity of these cointegrators of nuclear receptor function. For example, phosphorylation of p300 on Ser^{89} by PKCδ reduces its coactivation ability as well as its histone acetyltransferase activity measured on histones *in vitro* (230). Moreover, phosphorylation of this same serine residue by the AMP-activated protein kinase reduces interaction between p300 and the nuclear receptors PPARγ, TR, RAR, and retinoid X receptor (231). Thus, phosphorylation of Ser^{89} by either of these two kinases is a negative regulatory event that may become important during fluctuations in cellular metabolism reflected by elevated AMP (for AMP kinase) or during differentiation or apoptosis (for PKCδ). Conversely, phosphorylation of CBP or p300 by members of the MAPK signaling pathway, p42/p44 and MAPK kinase kinase 1, positively regulates the activity of these coactivators (232–234). In addition, cAMP can also increase the intrinsic transcriptional activity of CBP (233).

Just as phosphorylation can regulate the ability of coactivators to affect nuclear receptor activity, by either affecting coactivator function or the ability to interact with receptor, so too can phosphorylation influence the interaction of corepressors with nuclear receptors. The SMRT corepressor may be phosphorylated by the MEK1 kinase, MAPK kinase kinase-1 (as well as MEK-1) resulting in loss of repression due to reduction in the interaction between SMRT and TR, which is accompanied by nuclear export of SMRT to the perinuclear or cytoplasmic compartments (235). Phosphorylation by the p38 MAPK also increases the activity of the PGC-1 coactivator, and this appears to be the result of a phosphorylation event that impairs the functional interaction between PGC-1 and a putative repressor of this coactivator's activity; estrogen-related receptor-α has been shown to possess this activity (236–238). A nuclear to cytoplasmic shift in the localization of the corepressor RIP140 by the 14–3-3 protein and by cytoplasmic sequestration of SMRT and NCoR by p65 and

IκBα also may affect gene expression positively by reducing levels of corepressors in their functional compartments of interest (239, 240).

3. Regulation of coregulator function by ubiquitinylation or sumolyation. Steroid receptors, such as ER, PR, PPARα, and GR can be down-regulated by their cognate ligands in cells. The ability of ligands to induce polyubiquitination of these steroid receptors and the ability of inhibitors of the 26S proteasome such as MG132 and lactacystin to block ligand-dependent degradation of these steroid receptors argues that ligand-dependent degradation of these receptors occurs via the 26S proteasome (131, 241–244). The 26S proteasome is a multiprotein entity, which possesses protease activity that ultimately leads to the cleavage and degradation of polyubiquitinated target proteins (see Refs. 245 and 246 for review). In seeming contradiction to the ability of proteasome inhibitors to increase receptor levels, they also block the transcriptional activity of ERα and a number of other members of the nuclear receptor superfamily including progesterone, T_3, ARs, and RARs (131, 247, 248). There is not a simple correlation between the ability of ligands to down-regulate nuclear receptor expression and the requirement of proteasome activity for receptor-dependent gene expression. Notably, the transcriptional activity of the human GR, which is a ligand-dependent target of the 26S proteasome, is not compromised by proteasome inhibitors such as MG132 or lactacystin (243, 249–251). In contrast, the transcriptional activity of the AR is blocked by proteasome inhibitors, even though ligand stabilizes this molecule instead of inducing its down-regulation (248). Thus, the mechanisms by which proteasome inhibitors block transcription are not defined. This inhibition may signify that receptor turnover is required for its efficient transcriptional activity and/or that proteasome activity is required for other aspects of the transcription process. In this regard, MG132 blocks association of the phosphorylated RNA polymerase II with the pS2 gene promoter in MCF-7 cells, and also reduces the frequency with which ERα and other factors cycle off and on this promoter in response to estradiol treatment (252).

In this regard, the p160 family of coactivators, as well as CBP, have been shown to be targets of the ubiquitin-dependent 26S proteasome pathway by virtue of their increased expression in cells treated with MG132 and ubiquitination *in vivo* (131, 253). However, proteasome activity is not required for the intrinsic transcriptional activity of these molecules. This would suggest that reductions in steroid receptor activity induced by treatment with inhibitors of the proteasome are not linked to loss of coactivator activity *per se*, but instead result from a block in some other reaction required for receptor-dependent gene expression. In support of this, it has been demonstrated that proteasome inhibitor treatment of prostate cancer cells blocks cytoplasmic-to-nuclear translocation of AR (but not GR) after ligand treatment. Moreover, mammalian two-hybrid assays suggest that the MG132 may reduce ligand-dependent interactions between the AR LBD and the ARA70 and TIF2 coactivators (248). Proteasome function also appears to be required for the dynamic occupancy of the prostate-specific antigen promoter by AR in prostate cells; ChIP assays reveal the presence of the S1 subunit of the 19S proteasome subcomplex at the prostate-specific antigen promoter and that MG132 blocks release of AR from the promoter (254). Similar results, as described above, have been obtained for ERα (252).

It should be noted that the nuclear interaction domain of GRIP1 and SRC-1 can be modified by sumoylation (255, 256). SUMO (small ubiquitin-like modifier) modification results from the covalent linkage of SUMO to specific lysines of target proteins (257–259). It does not, however, promote degradation of target proteins, but instead appears to regulate protein-protein interactions and protein targeting. Mutation of GRIP1 sumoylation sites reduces the colocalization of AR with GRIP1 in the nucleus after testosterone treatment (260). Moreover, GRIP1 sumoylation mutants are relatively poor coactivators of AR-dependent gene expression. There are two major sumoylation sites in SRC-1. Sumoylation was reported to increase PR-SRC-1 interaction and prolong SRC-1 retention in the nucleus (256).

A number of molecules associated with the proteasome-dependent degradation pathway have been identified as either coactivators [*e.g.*, E6-AP (127), the SUG1 ATPase which is a member of the base portion of the 19S proteasome regulatory complex (261), and yRsp5/hRPF1 (128)] or corepressors [*e.g.*, the ubiquitin ligase BRCA1 (262, 263)] of nuclear receptor function. The extent to which the activities of these proteins relate to proteasome function and contribute to their effect on nuclear receptor transcriptional activities in most cases has not been determined. One notable exception to this is for BRCA1, which strongly inhibits ERα transcriptional activity in T-47D (breast) and DU145 (prostate) but not in three cervical cell lines, C33A, CaSki, or SiHa (262). This appears to be related to the ability of wild-type BRCA1 to down-regulate expression levels of p300, but only in the cells in which BRCA1 inhibits ERα transcriptional activity [*e.g.*, breast and prostate but not cervical cells (264)]. Interestingly, this effect of BRCA1 does not appear to influence expression levels of CBP, suggesting a high degree of specificity considering the similarity of CBP and p300 structure and function (264).

Proteasomal regulation of the corepressor NCoR has been documented (265). Treatment of cells with MG132 increases NCoR expression levels, indicating that this protein is degraded by the 26S proteasome. This degradation results from the interaction of mSiah2, the mammalian homolog of *Drosophila Seven in absentia* (*sina*) with the amino terminus of NCoR. Although experiments examining the ability of mSiah2 to affect SMRT degradation revealed no such regulation, they were performed with the originally identified version of SMRT, lacking the amino terminus later shown to be part of the full-length protein (265, 266). Intriguingly, expression of mSiah2 is variable, being most abundant in the nervous system (267). Therefore, in cells in which mSiah2 is relatively high (*e.g.*, N18 neuroblastoma cells) NCoR expression is relatively low, whereas in 293T kidney cells in which mSiah2 expression is relatively low, NCoR expression is relatively abundant (265). Transfection of 293T cells with a mSiah2 expression vector significantly decreases the half-life of NCoR protein.

4. Coactivator methylation. The AD2 region of p160 coactivators binds to methyltransferases (126, 268), and this interaction was suggested to be important for methylation of histones, a process involved in activation of gene expression (269, 270). An arginine residue within the CBP KIX domain also has been shown to be methylated by CARM1, and this inhibits the interaction between CBP and the kinase inducible domain of CREB (271). This could promote CBP coactivation of nuclear receptor activity by increasing the pool of CBP available for functional interaction. Under some conditions, the methyltransferase activity of CARM1 is important for the ability of this factor to coactivate ERα transcriptional activity (272). Another report indicates that CARM1 can methylate CBP on three arginine residues, Arg^{714}, Arg^{742}, and Arg^{768}, conserved between CBP and p300 (273). These residues are not located in the nuclear receptor or p160-interacting domains of CBP, but this methylation is important for strong CBP coactivation of ERα and GRIP1 transcriptional activity (273). The mechanism for this is not clear, but enhanced CBP/p300 coactivation potential within a cell could potentially contribute to the agonist activity of SRMs.

5. Acetylation. Although no published evidence to date implicates acetylation in the regulation of SRM activity, this posttranslational modification through its ability to regulate protein-protein interactions could affect the interaction of coregulators with nuclear receptor receptors and, in so doing, perturb the agonist/antagonist potential of this class of compounds. The SRC-1, RAC3, CBP, and p300 coactivators possess acetyltransferase activity that was originally thought to be directed solely toward histones. However, one report implicates histone acetyltransferase activity in the disassembly of receptor-coactivator complexes leading to the attenuation of gene transcription (274). This is accomplished through acetylation of ACTR (SRC-3) by CBP/p300 on lysine residues adjacent to an NR box, with the result of a loss of interaction between this p160 and ERα. In this context, acetylation could be a mechanism that inhibits the agonist potential of SRMs through reducing ERα interaction with a coactivator. p300 Has been shown *in vitro* to acetylate ERα on lysines 302 and 303, residues located within the hinge region (275). Mutation of these sites to arginines, threonines, glutamines, or alanines increases E2-induced ERα transcriptional activity, again suggesting that acetylation results in a loss of transcriptional activity (275). Paradoxically, template-activating factor 1β inhibits ERα acetylation and transactivation, suggesting that regulation of receptor-dependent gene expression by acetylation is complex and not yet well understood (276). Whether or even how these acetylation events may be regulated in a cell-specific manner is presently unknown.

6. Coactivator effects on splicing. An ever-expanding role of coregulators in transcription has become evident. The original SRCs (such as SRC-1 and SRC-3) have been thought to work primarily via remodeling of promoter chromatin and initiation of transcription, a role also ascribed to CBP/p300/pCAF. The TRAP/DRIP complex of coactivators then plays a role in reinitiation of transcription and links the coactivational complex to general transcription factors and RNA polymerase II for efficient transcription of pre-mRNA. One might question, however, of what avail would be a high level of transcription if splicing were to become limited or inaccurate? Because the structural genes are designed to produce protein products and not unspliced pre-mRNAs, a growing suspicion has developed that transcription and alternative splicing might be somehow linked via information in the promoter regions of genes (277). Given the estimates that more than 60% of transcripts from eukaryotic genes are alternatively spliced (some of which can be regulated by steroid hormones), and given that nuclear receptors are the "great family of gene regulators," it would not be unreasonable that nuclear receptors coordinate their transcriptional regulatory effects with simultaneous effects on alternate splicing of the target gene mRNAs. In fact, a recent report in cultured cells substantiates this hypothesis, demonstrating that steroid hormones can affect the processing of pre-mRNA synthesized from steroid-sensitive promoters, but not from steroid-unresponsive promoters (129). This effect on alternative splicing is ligand and receptor dependent and receptor selective. The mechanism of the regulatory effect of receptors on RNA processing appears to be due to recruitment of subsets of certain coactivators, because addition of the coactivator CoAA (225) stimulated ER-mediated exon exclusion whereas the coactivator p72 (178) stimulated exon inclusion in the same target gene (129). SRC-1 had no significant effect on splicing. Similar results were demonstrated first for PGC-1 (130) and subsequently for the p68 and p72 coactivators/RNA helicases, which have been shown to act as splicing factors (278, 279). With the availability of these data, we now can conclude that steroid hormone receptors can simultaneously control gene transcription activity and exon content of the product RNA by recruiting coactivators involved in both processes (129, 278). It would not be unexpected if future experiments were to show that steroid receptors can have effects on other steps in mRNA processing and its export from the nucleus.

7. Triggering coactivators. In addition to receptors acting as proteins that serve to specifically recruit coactivators to target gene promoters, they also can influence the transcriptional activity of the coactivators. The intrinsic transcriptional activity of the PPARγ coactivator PGC-1 is relatively low when measured as a GAL4-PGC-1 fusion protein. However, expression of PPARγ can significantly increase the apparent transcriptional activity of PCG-1 (280). This increase in activity, which does not require the PPARγ AF2 activity, is reflected in a PPARγ-induced change in the conformation of PGC-1 and an increase in the association between PGC-1 and the SRC-1 and p300 coactivators (280). These data therefore suggest that receptor binding to coactivator can induce allosteric changes in coactivators that trigger their activity via increased recruitment of other coactivators, thereby increasing receptor-dependent transcription.

The AR also has been shown to stimulate the transcriptional activity of a DNA-bound coactivator (281). The intrinsic transcriptional activity of GAL4-GRIP1, GAL4-CBP, and GAL4-p300 all can be stimulated by AR expression. Although this AR activity is not dependent on the AF-2 domain of the receptor, it does require both the amino- and carboxy-

terminal regions of AR. Moreover, AR is unable to trigger the activity of an AD1-deletion mutant of GRIP1, although this form of GRIP1 still retains the capacity to coactivate AR-dependent gene expression, suggesting that unique domains of GRIP1 are required for coactivation of AR-dependent gene expression and GRIP1 activity triggered by AR (281). A similar model has been proposed for ERα stimulation of AP-1-dependent gene expression in which ERα interacts with AP-1 indirectly through binding to CBP/p300 and p160 coactivators (80). However, in neither of these cases has the mechanism by which AR or ER increases coactivator activity and reporter gene expression been determined.

D. Relative coregulator expression

1. Coactivator expression in normal tissues. Based on the original hypothesis in which the relative levels of coactivators and corepressors were envisioned to control the relative agonist and antagonist activity of SRMs, it was predicted that significant differences in coactivator and corepressor expression found in various cell and tissue types would be important determinants of SRM activity (142, 143). With the continually expanding number of nuclear receptor coregulatory molecules, this has become an increasingly difficult hypothesis to test. Nonetheless, several examples of coactivators with distinct expression cell patterns have been described. For example, the DNA-binding domain-interacting coactivator, GT198, is expressed in a tissue-selective fashion; mRNA levels are very high in testis, modest in spleen and thymus, but absent in brain, heart, kidney, liver, lung, and thyroid (282). The human PGC-1 coactivator is also expressed in a tissue-restricted manner. Although detected in heart, skeletal muscle, kidney, and liver by Northern blot, it is absent in brain, colon, thymus, spleen, small intestine, placenta, lung, and peripheral blood lymphocytes (283). The expression pattern of the AR coactivator, FHL2, is restricted to the myocardium of the heart and prostate epithelial cells (284).

However, the expression pattern of most coactivators and corepressors examined appears to be quite broad. For example, the NCoR and SMRT corepressors are widely expressed, and the p160 family coactivators have been detected in most cell and tissue types (104, 139, 285, 286). There are, however, exceptions and variations to this theme. As an example, SRC-3 expression is undetectable in the ventromedial hypothalamus of mice and rats, and in 4-wk-old mouse uterus, although it is expressed in many other tissues examined (287–289). It should be noted, however, that low levels of SRC-3 have been demonstrated for human proliferating endometrium with increased expression in the late secretory phase (290), and other investigators have demonstrated the mRNA in immature and mature rat uteri (291). In addition to absolute changes in coactivator expression, relative differences that may be important for tissue/cell-specific responses to ligands, particularly SRMs, have been noted. For instance, similar expression levels of CBP, p300, AIB1, GRIP1, p300, NCoR, and SMRT have been measured for Ishikawa uterine and MCF-7 breast cells (146). However, SRC-1 expression was much greater in the Ishikawa cells, and this correlated with the agonist activity of tamoxifen in this cell line (146). Moreover, increasing SRC-1, but not GRIP1 or AIB1, expression in MCF-7 cells conferred tamoxifen agonist activity, whereas decreasing SRC-1 expression via short interfering RNA inhibited the agonist activity of tamoxifen in Ishikawa cells. Thus, relative as well as absolute changes in coactivator expression can affect SRM activity.

The demonstration that changes in coactivator expression could lead to alterations in SERM responses, as mentioned above, raises the issue of whether changes in coregulator expression within a given cell or tissue type could lead to altered responsiveness of that tissue to ligand. This has been addressed with biopsies obtained from human endometrium, which demonstrated that although SRC-1 and TIF2 levels did not change over the menstrual cycle, AIB1 expression increased as the cycle progressed (290). A subsequent report outlining differences in the relative expression of some coactivators and corepressors suggests that more studies are required to get a clear picture of the patterns of coregulator expression in this tissue (292). Specific influences may alter coactivator expression and, intriguingly, some of these responses appear to be cell and/or tissue specific. For example, estradiol decreases and T_3 increases SRC-1 mRNA expression in GH3 rat pituitary cells, an effect matched in the pituitaries of T_3-treated male rats (285). However, neither estradiol nor tamoxifen affects the expression of p160, RIP140, or p300 mRNAs in rat uterus (291), whereas another report indicates that estradiol decreases and antiestrogens increase AIB1 expression in MCF-7 cells (293). Estrogen also increases the expression of RIP140 mRNA in MCF-7, but not Ishikawa cells (294). The basis for the differences in response to estradiol in the different tissues and cells is unknown. Retinoic acid also has been shown to increase AIB1 expression in breast (MCF-7) and leukemia (HL-60 and NB4) cells; however, several lines of evidence support the conclusion that the retinoid effects in MCF-7 cells are not direct but, rather, are mediated through alterations in TGFβ production (288, 293). Indeed, TGFβ increases AIB1 expression in A549 and MCF-7 cells (293, 295). Decreases in coactivator expression also have been observed. Stimulation of protein kinase A is associated with a reduction in TIF2 protein, but not mRNA expression, whereas the deacetylase inhibitor sodium butyrate reduced p300 expression (296, 297). These are not transcriptional effects but, rather, reflect increased protein degradation which, at least for p300, is proteasome dependent (297). Thus, other signaling events within the cell may affect nuclear receptor transcriptional responses via alteration in the expression of coregulators. When the above data are viewed together, the relative quantitative changes in the cellular fingerprint of coactivator proteins in normal differentiated cells are rather minimal, usually varying by a factor of 1.

2. Alterations in coregulator expression associated with pathology. The identification of AIB1 as a coactivator with increased expression in breast and ovarian cancer was the first indication that alterations in coactivator and corepressor expression may be associated with disease (111). As its name implies, the gene for AIB1 (amplified in breast cancer-1) was first reported to be amplified in approximately 10% of human breast cancers, and in a survey of 105 human tumors, 64% were found to express elevated levels of AIB1 mRNA in

comparison with normal mammary epithelium. Another study has found the AIB1 gene to be amplified in 4.8% of breast (n = 1157) and 7.4% of ovarian (n = 122) cancers (298). Genetic amplification in cancer tissues suggests that AIB1/SRC-3 may be an oncogene. Since that time, a number of studies have examined the expression of coregulators in breast and prostate cancer and, in general, suggest that a change in the expression of selected coactivators is associated with tumorigenesis and/or progression. For example, increases in the SRA and AIB1 coactivators in breast tumors in comparison with adjacent normal tissue have been reported (299), as have changes in SRA and AIB1 expression relative to ER and PR status (300, 301). This suggests that elevated coactivator levels may increase the sensitivity of tumors to estrogens and growth factors. Another recent study indicates that, in patients receiving tamoxifen adjuvant therapy, high AIB1 expression correlates with poor disease-free survival, which is indicative of tamoxifen resistance (302). Importantly, patients with high levels of AIB1 and the growth factor receptor HER2 had worse outcomes than all other patients combined. In contrast, high levels of AIB1 in patients not receiving tamoxifen therapy were associated with better prognosis and longer disease-free survival. Taken together, a poor response to tamoxifen therapy appears to relate to high levels of both HER2 and AIB1 expression, and this suggests that AIB1 may be an important therapeutic target. Another study has linked low NCoR mRNA expression to a relative reduction in relapse-free survival (303).

Elevated TIF2 and AIB1 expression also have been found in the endometrium of women with polycystic ovarian syndrome (PCOS) in comparison with control fertile women, potentially contributing to the sensitivity of the PCOS endometrium to estrogen and the association of PCOS and endometrial adenocarcinoma (290, 304). The expression of the MTA1 corepressor and a MTA1 variant has been found at greater levels in breast tumors, particularly in those that are ER negative (158, 305). Although this might seem to confer an advantage, increased MTA1 variant expression mislocalizes ERα to the cytoplasm, increases nongenomic estrogen signaling, and correlates with a more malignant phenotype (305).

Results indicating that expression of TIF2 and CBP are higher in intraductal carcinomas than normal mammary gland whereas NCoR levels are lower in invasive *vs.* intraductal carcinoma also have been obtained (306) and suggest that changes in coregulator expression may occur during cancer progression. Based on current models of the molecular basis of SRM tissue-selective action, it has been predicted that alterations in coregulator expression are associated with the acquisition of hormone independence and/or antihormone resistance. Although decreases in NCoR expression have been found for tamoxifen-resistant MCF-7 breast cancer cells (119), relative changes in the expression of AIB1 and SRA were not observed for *de novo* tamoxifen-resistant breast cancer (307). Although it may be tempting to speculate, based on these studies, that alterations in corepressors are responsible for acquisition of abnormal hormone responses, prostate cancer recurrence after androgen deprivation therapy has been associated with increased expression of SRC-1 and TIF2 coactivators (308). Collectively, the results of studies characterizing coregulator expression in hormone-responsive tissues are intriguing, but the data are still incomplete. Studies of a larger number of tumors and corresponding normal samples would be helpful. Moreover, more complete surveys of coactivator and corepressor protein content for each specimen also may help elucidate the identity of the coactivators and/or corepressors critical for tumor development and progression.

V. Lessons Learned from Coregulator Knockout Mice

The generation of knockout mice for the coactivators SRC-1 (309), TIF2 (310), SRC-3 (287, 311), RAP250 (312), and E6-AP (313), and the corepressors NCoR (148) and Nrip1/RIP140 (314), provides animal and cell models in which to examine the absolute and relative roles of these coregulatory proteins *in vivo*. Although the first published account of a coactivator knockout suggested that increased levels of TIF2 in brain and testes could partially offset loss of SRC-1 expression in SRC-1 null mice (309), and a recent study demonstrates that SRC-2 can compensate in reproductive behavior for genetic loss of SRC-1 [*e.g.*, in SRC-1 null mice (289)], detailed studies of these knockout models reveal that this need not be the case. The studies completed to date have revealed that coactivators are not functionally redundant, even within the same coactivator family. For instance, within the p160 coactivator family, only SRC-1 knockout mice have a phenotype of generalized resistance to steroid hormone action (309); TIF2 and SRC-3 null mice do not. In contrast, only TIF2 null mice are affected by a significant gonadal and fertility impairment, whereas the general phenotype of SRC-3-deficient mice is one of impaired growth (287, 310, 311). Thus, even at the organismal level, these coactivators are not functionally equivalent, although limited cross-compensation can occur when a given coactivator is eliminated.

Although some differences in the biology of coactivators are related to their tissue expression profiles (*e.g.*, SRC-1 null, but not SRC-3 null, mice have a uterine phenotype; this undoubtedly relates to the relatively low SRC-3 expression in wild-type mice), differences in the biological roles of coactivators expressed within a given tissue or organ can be observed. For example, the mammary glands of virgin SRC-1 null mice exhibit decreased ductal growth and branching (309). However, TIF2, SRC-3, and E6-AP expression within the mammary gland is not required for virgin mammary gland development, even though these coactivators are expressed in this tissue; this suggests that coactivators play spatial and temporal specific roles *in vivo* (287, 310, 311, 315). It is interesting to note that E6-AP is expressed in prostate as well as mammary gland, and lack of E6-AP expression does compromise prostate growth responses to androgen stimulation. Thus, this coactivator contributes to steroid-induced growth responses in a tissue-specific manner (315). Interesting differences between the SRC-1 and TIF2 knockouts have been recently documented for adipose tissues (316). TIF2$^{-/-}$ mice are protected against obesity and exhibit enhanced adaptive thermogenesis, whereas SRC-1$^{-/-}$ mice, as a consequence of their reduced energy expenditure, are prone to obesity. These responses can be influenced in response to a

high-fat, Western-style diet which increases the ratio of TIF2:SRC-1 expression in adipose tissue, with the resulting increase in TIF2 expression leading to higher fat accumulation and decreased energy expenditure (316). These metabolic patterns appear due to the relative preferences of TIF2 and SRC-1 for PPARα and PGC-1.

Although the SRC-1 and TIF2 null mice have revealed aspects of their unique and overlapping functions, analyses of SRC-1 knockout mice relative to T_3 action has revealed a paradoxical aspect of the function of this coactivator; specifically, it can play both positive and negative regulatory roles in gene expression. SRC-1 null mice are resistant to T_3 as evidenced by elevated levels of serum TSH despite increased T_3 levels (317). Moreover, the reduction in TSH levels associated with administration of T_3 to wild-type animals is blunted in SRC-1 knockouts. This is reflected in a reduced ability of T_3 to suppress TSHβ mRNA in these animals (318). Although loss of SRC-1 expression also affects the expression of another gene positively regulated by T_3, spot 14 (S14), several other T_3 target genes (*e.g.*, GH, malic enzyme, type I iodothyronine 5′-deiodinase and others) are unaffected by loss of this coactivator, providing another example of gene-selective regulation by coactivators (318).

Crosses between SRC-1 null mice and mice deficient in either TRα or TRβ reveal specific requirements of this coactivator relative to the ability of these receptors to activate or repress TR target genes in the liver (319). For instance, 5′-deiodinase expression is unaffected by loss of SRC-1 alone. However, loss of SRC-1 expression in SRC-1/TRβ double null mice further reduces expression of this gene to levels below those observed for TRβ null mice. In contrast, TRα negatively regulates this gene, and loss of TRα expression increases 5′-deiodinase expression by 2400%. Nonetheless, loss of TRα and SRC-1 results in only a 3-fold increase in gene expression over wild-type controls. This suggests that the loss of TRα-dependent repression is balanced by the loss of SRC-1 on TRβ-positive regulation of this gene. Thus, animals deficient for either TRα or TRβ along with SRC-1 reveal a role for this coactivator that would be unsuspected from the phenotype of the SRC-1 null animal alone (319). Crosses between the SRC-1 and TIF2 null mice also reveal information on the biological function of these two coactivators (320). Unlike SRC-1 null mice, TIF2 null mice are not resistant to thyroid hormone as measured by T_3, T_4, and TSH levels, and this initially suggested that TIF2 did not play a role in thyroid hormone action. However, double-heterozygous mice (SRC-$1^{+/-}$/TIF$2^{+/-}$) are as resistant to T_3 as SRC-1 null mice, although SRC-1 heterozygotes (SRC-$1^{+/-}$) are not. The appearance of a TIF2 phenotype on a reduced SRC-1 expression background indicates that TIF2 does contribute to T_3 action and suggests aspects of limited functional redundancy between these two coactivators (320).

It should be noted that knockouts for the coactivators CBP (321, 322), p300 (323), and TRAP220/DRIP205 (324) have been generated. In contrast to the knockout models described above, lack of expression of these coactivators results in embryonic lethality, resulting from early defects in development. Consistent with the ability of these factors, particularly CBP/p300, to stimulate the activity of a broad range of transcription factors (325, 326), the knockout data indicate that these cofactors are critical to mammalian development, and this is supported by the phenotypes of CBP, p300, and TRAP220 heterozygous mutant mice, which exhibit gross defects in morphogenetic and cell differentiation processes (323, 327).

Evidence obtained from NCoR null mice reveals biological functions that require corepressor expression for normal activity and lays the groundwork to begin to examine the role of these molecules in the regulation of tissue-specific nuclear receptor ligand function. The NCoR null mice are embryonic lethal, with the majority dying at postnatal d 15.5 due to developmental defects in the erythrocytic, thymic, and neural systems (148). However, mouse embryo fibroblasts (MEFs) derived from NCoR null embryos provide a model system to examine the importance of this corepressor for the relative agonist/antagonist activity of tamoxifen. In transient transfection assays, 4HT stimulated ERα-dependent expression of a reporter gene in NCoR null, but not wild-type, MEFs (148); estrogen stimulation of the target gene was comparable between genotypes. Moreover, expression of exogenous NCoR in the null MEFs significantly attenuated the agonist activity of 4HT, consistent with the hypothesis that corepressors play an important role in defining the biocharacter of this antiestrogen. Although these results support the hypothesis that corepressors are important for the antagonistic activity of SRMs, the inability of the SMRT corepressor expressed within these cells to maintain the antagonist activity of 4HT suggests some selectivity between NCoR and SMRT function. More detailed studies of the relative expression of these and other corepressors, and analysis of endogenous target gene expression should help to clarify this issue.

VI. Concluding Remarks

The discovery and cloning of coregulator molecules have been key to our understanding of hormonal regulation of gene expression in spatial and temporal contexts. They were the missing link for transducing the transcriptional potential of nuclear receptors to that of the general transcription machinery. The diversity of coactivator and corepressor functions now has been extended well beyond initiation of transcription to RNA processing, transcription complex turnover, and environmental signaling via cell surface receptors (69). Their overexpression in many types of malignancies provides additional knowledge of the mechanisms employed by the cancer cell to achieve a selective growth advantage over normal cells. Importantly, discovery of the coregulators has provided also a key to understanding the pharmacology of tissue-selective actions of hormones and SRMs.

It is hoped that the future will hold an entirely new complement of SRMs for every occasion. SERMs, which inhibit breast and prostate response to estrogens while providing estrogen-like stimulation of bone, brain, and potentially the cardiovascular system, are already available. To date, however, we have not been able to discover SERMs that suppress hot flushes in postmenopausal women without stimulating uterine growth; nevertheless, an interesting combination of a new SERM plus conjugated equine estrogens (Premarin)

may provide this desired profile (328). In the near future, we may be using oral SARMs for treatment of male osteoporosis or muscle-wasting diseases without the concomitant stimulation of prostatic growth. SPRMs that do not stimulate mammary alveolar proliferation will be employed for the treatment of uterine endometriosis. New SGRMs may give us greater abilities to suppress inflammation without concomitant fat deposition, collagen destruction, osteoporosis, and diabetogenic effects. These same concepts of ligand-driven conformational diversity and selective tissue actions will also be exploited in the future for drugs that selectively regulate the many additional orphan receptors in the nuclear receptor family. By this process of translation of fundamental research to development of pharmaceutical therapies, the field of nuclear receptors will provide a significant return for the investment by the National Institutes of Health in basic research over recent decades.

Acknowledgments

The secretarial assistance of Barbara Hatton is gratefully acknowledged.

Address all correspondence and requests for reprints to: Carolyn L. Smith, Ph.D., Department of Molecular and Cellular Biology, One Baylor Plaza, Houston, Texas 77030. E-mail: carolyns@bcm.tmc.edu

This work was supported in part by an award from the National Institutes of Health (NIH; DK53002) (to C.L.S.), and NIH, National Institute of Diabetes & Digestive & Kidney Diseases/Atlas, and Texas Advanced Technology Program grants (to B.W.O.).

References

1. **Tsai M-J, O'Malley BW** 1994 Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486
2. **Glass CK, Rosenfeld MG** 2000 The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 14:121–141
3. **Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J: Writing Group for the Women's Health Initiative Investigators** 2002 Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 288:321–333
4. **Jordan VC, Robinson SP** 1987 Species-specific pharmacology of antiestrogens: role of metabolism. Fed Proc 46:1870–1874
5. **Gottardis MM, Robinson SP, Satyaswaroop PG, Jordan VC** 1988 Contrasting actions of tamoxifen on endometrial and breast tumor growth in the athymic mouse. Cancer Res 48:812–815
6. **Gottardis MM, Jordan VC** 1987 Antitumor actions of keoxifene and tamoxifen in the N-nitrosomethylurea-induced rat mammary carcinoma model. Cancer Res 47:4020–4024
7. **Jordan VC, Phelps E, Lindergren JU** 1987 Effects of anti-estrogens on bone in castrated and intact female rats. Breast Cancer Res Treat 10:31–35
8. **Harper MJ, Walpole AL** 1966 Contrasting endocrine activities of cis and trans isomers in a series of substituted triphenylethylenes. Nature 212:87
9. **Jordan VC** 1992 The strategic use of antiestrogens to control the development and growth of breast cancer. Cancer 70:977–982
10. **Williamson JG, Ellis JD** 1973 The induction of ovulation by tamoxifen. J Obstet Gynaecol Br Commonw 80:844–847
11. **Harper MJ, Walpole AL** 1967 A new derivative of triphenylethylene: effect on implantation and mode of action in rats. J Reprod Fertil 13:101–119
12. **Cole MP, Jones CT, Todd ID** 1971 A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI46474. Br J Cancer 25:270–275
13. **Early Breast Cancer Trialist Collaborative Group** 1998 Tamoxifen for early breast cancer: an overview of the randomized trials. Lancet 351:1451–1467
14. **Turner RT, Wakley GK, Hannon KS, Bell NH** 1987 Tamoxifen prevents the skeletal effects of ovarian hormone deficiency in rats. J Bone Miner Res 2:449–456
15. **Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC, Carbone PP, DeMets DL** 1992 Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. N Engl J Med 326:852–856
16. **Moon LY, Wakley GK, Turner RT** 1991 Dose-dependent effects of tamoxifen on long bones in growing rats: influence of ovarian status. Endocrinology 129:1568–1574
17. **Delmas PD, Bjarnason NH, Mitlak BH, Ravoux AC, Shah AS, Huster WJ, Draper M, Christiansen C** 1997 Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. N Engl J Med 337: 1641–1647
18. **Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J, Gluer CC, Krueger K, Cohen FJ, Eckert S, Ensrud KE, Avioli LV, Lips P, Cummings SR** 1999 Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple outcomes of raloxifene evaluation (MORE) investigators. JAMA 282:637–645
19. **Cauley JA, Norton L, Lippman ME, Eckert S, Krueger KA, Purdie DW, Farrerons J, Karasik A, Mellstrom D, Ng KW, Stepan JJ, Powles TJ, Morrow M, Costa A, Silfen SL, Walls EL, Schmitt H, Muchmore DB, Jordan VC, Ste-Marie LG** 2001 Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-year results from the MORE trial. Multiple outcomes of raloxifene evaluation. Breast Cancer Res Treat 65:125–134
20. **Ke HZ, Qi H, Crawford DT, Chidsey-Frink KL, Simmons HA, Thompson DD** 2000 Lasofoxifene (CP-336, 156), a selective estrogen receptor modulator, prevents bone loss induced by aging and orchidectomy in the adult rat. Endocrinology 141:1338–1344
21. **Suh N, Glasebrook AL, Palkowitz AD, Bryant HU, Burris LL, Starling JJ, Pearce HL, Williams C, Peer C, Wang Y, Sporn MB** 2001 Arzoxifene, a new selective estrogen receptor modulator for chemoprevention of experimental breast cancer. Cancer Res 61: 8412–8415
22. **Miller CP, Harris HA, Komm BS** 2002 Bazedoxifene acetate: selective estrogen receptor modulator, treatment and prevention of osteoporosis. Drugs Future 27:117–121
23. **Baracat E, Haidar M, Lopez FJ, Pickar J, Dey M, Negro-Vilar A** 1999 Estrogen activity and novel tissue selectivity of $\Delta^{8,9}$-dehydroestrone sulfate in postmenopausal women. J Clin Endocrinol Metab 84:2020–2027
24. **Kloosterboer HJ** 2001 Tibolone: a steroid with a tissue-specific mode of action. J Steroid Biochem Mol Biol 76:231–238
25. **Chetrite G, Kloosterboer HJ, Pasqualini JR** 1997 Effect of tibolone (Org OD14) and its metabolites on estrone sulphatase activity in MCF-7 and T-47D mammary cancer cells. Anticancer Res 17:135–140
26. **Chetrite GS, Pasqualini JR** 2001 The selective estrogen enzyme modulator (SEEM) in breast cancer. J Steroid Biochem Mol Biol 76:95–104
27. **Kloosterboer HJ, Ederveen AGH** 2002 Pros and cons of existing treatment modalities in osteoporosis: a comparison between tibolone, SERMs and estrogen (±progestogen) treatments. J Steroid Biochem Mol Biol 83:157–165
28. **Conneely OM, Mulac-Jericevic B, DeMayo F, Lydon JP, O'Malley BW** 2002 Reproductive functions of progesterone receptors. Recent Prog Horm Res 57:339–355
29. **Giannoukos G, Szapary D, Smith CL, Meeker JEW, Simons Jr SS** 2001 New antiprogestins with partial agonist activity: potential selective progesterone receptor modulators (SPRMs) and probes for receptor- and coregulator-induced changes in progesterone receptor induction properties. Mol Endocrinol 15:255–270
30. **Chwalisz K, Garg R, Brenner RM, Schubert G, Elger W** 2002

Selective progesterone receptor modulators (SPRMs): a novel therapeutic concept in endometriosis. Ann NY Acad Sci 955:373–388
31. **Classen-Linke I, Alfer J, Krusche CA, Chwalisz K, Rath W, Beier HM** 2000 Progestins, progesterone receptor modulators, and progesterone antagonists change VEGF release of endometrial cells in culture. Steroids 65:763–771
32. **Chwalisz K, Brenner RM, Fuhrmann UU, Hess-Stumpp H, Elger W** 2000 Antiproliferative effects of progesterone antagonists and progesterone receptor modulators on the endometrium. Steroids 65:741–751
33. **Chwalisz K, Lamar PR, Williamson S, Larsen L, McCrary K, Elger W,** Treatment of uterine leiomyomas with the novel selective progesterone receptor modulator (SPRM) J867. J Soc Gynecol Invest, in press
34. **Mooradian AD, Morley JE, Korenman SG** 1987 Biological actions of androgens. Endocr Rev 8:1–28
35. **Negro-Vilar A** 1999 Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. J Clin Endocrinol Metab 84:3459–3462
36. **Davis S** 1999 Androgen replacement in women: a commentary. J Clin Endocrinol Metab 84:1886–1891
37. **Zhi L, Tegley CM, Marschke KB, Jones TK** 1999 Switching androgen receptor antagonists to agonists by modifying C-ring substituents on piperidino[3,2-*g*]quinolinone. Bioorg Med Chem Lett 9:1009–1012
38. **Edwards JP, West SJ, Pooley CLF, Marschke KB, Farmer LJ, Jones TK** 1998 New nonsteroidal androgen receptor modulators based on 4-(trifluoromethyl)-2(1*H*)-pyrrolidino[3,2-*g*]quinolinone. Bioorg Med Chem Lett 8:745–750
39. **Risek B, Miner JN, Evans G, Turner RT, Bain S, Shen V, Chang W, Barnett RW, Schrader WT, Rosen J, Negro-Vilar A,** A nonsteroidal, selective androgen receptor modulator (SARM) with efficacy on bone in the orchiectomized adult rat. Program of the 83rd Annual Meeting of The Endocrine Society, Denver, CO, 2001, p 255 (Abstract P1-497)
40. **Schoonjans K, Auwerx J** 2000 Thiazolidinediones: an update. Lancet 355:1008–1010
41. **Kersten S, Desvergne B, Wahli W** 2000 Roles of PPARs in health and disease. Nature 405:421–424
42. **Desvergne B, Wahli W** 1999 Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 20:649–688
43. **Sporn MB, Suh N, Mangelsdorf DJ** 2001 Prospects for prevention and treatment of cancer with selective PPARγ modulators (SPARMs). Trends Mol Med 7:395–400
44. **Rocchi S, Picard F, Vamecq J, Gelman L, Potier N, Zeyer D, Dubuquoy L, Bac P, Champy M-F, Plunket KD, Leesnitzer LM, Blanchard SG, Desreumaux P, Moras D, Renaud J-P, Auwerx J** 2001 A unique PPARγ ligand with potent insulin-sensitizing yet weak adipogenic activity. Mol Cell 8:737–747
45. **Rieusset J, Touri F, Michalik L, Escher P, Desvergne B, Niesor E, Wahli W** 2002 A new selective peroxisome proliferator-activated receptor γ antagonist with antiobesity and antidiabetic activity. Mol Endocrinol 16:2628–2644
46. **Berger JP, Petro AE, Macnaul KL, Kelly LJ, Zhang BB, Richards K, Elbrecht A, Johnson BA, Zhou G, Doebber TW, Biswas C, Parikh M, Sharma N, Tanen MR, Thompson GM, Ventre J, Adams AD, Mosley R, Surwit RS, Moller DE** 2003 Distinct properties and advantages of a novel peroxisome proliferator-activated protein γ selective modulator. Mol Endocrinol 17:662–676
47. **Sorbera LA, Rabasseda X** 1998 Rosiglitazone maleate. Drugs Future 23:977–985
48. **Coghlan MJ, Jacobson PB, Lane B, Nakane M, Lin C-W, Elmore SW, Kym PR, Luly JR, Carter GW, Turner R, Tyree CM, Hu J, Elgort M, Rosen J, Miner JN** 2003 A novel antiinflammatory maintains glucocorticoid efficacy with reduced side effects. Mol Endocrinol 17:860–869
49. **Yen PM** 2001 Physiological and molecular basis of thyroid hormone action. Physiol Rev 81:1097–1142
50. **Gullberg H, Rudling M, Saltó C, Forrest D, Angelin B, Vennström B** 2002 Requirement for thyroid hormone receptor β in T_3 regulation of cholesterol metabolism in mice. Mol Endocrinol 16:1676–1777
51. **Yoshihara HAI, Apriletti JW, Baxter JD, Scanlan TS** 2003 Structural determinants of selective thyromimetics. J Med Chem 46: 3152–3161
52. **Grover GJ, Mellström K, Ye L, Malm J, Li Y-L, Bladh L-G, Sleph PG, Smith MA, George R, Vennström B, Mookhtiar K, Horvath R, Speelman J, Egan D, Baxter JD** 2003 Selective thyroid hormone receptor-β activation: a strategy for reduction of weight, cholesterol, and lipoprotein (a) with reduced cardiovascular liability. Proc Natl Acad Sci USA 100:10067–10072
53. **Jensen EV, Jacobson HI** 1962 Basic guides to the mechanism of estrogen action. Recent Prog Horm Res 18:387–414
54. **Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J** 1996 Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925–5930
55. **Parker MG** 1993 Steroid and related receptors. Cell Biol 5:499–504
56. **Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, Ljunggren J, Gustafsson J-A, Carlquist M** 1999 Structure of the ligand-binding domain of oestrogen receptor β in the presence of a partial agonist and a full antagonist. EMBO J 18: 4608–4618
57. **Paige LA, Christensen DJ, Gron H, Norris JD, Gottlin EB, Padilla KM, Chang C-Y, Ballas LM, Hamilton PT, McDonnell DP, Fowlkes DM** 1999 Estrogen receptor (ER) modulators each induce distinct conformational changes in ERα and ERβ. Proc Natl Acad Sci USA 96:3999–4004
58. **Van den Bemd G-JCM, Kuiper GGJM, Pols HAP, Van Leeuwen JPTM** 1999 Distinct effects on the conformation of estrogen receptor α and β by both the antiestrogens ICI 164, 384 and ICI 182, 780 leading to opposite effects on receptor stability. Biochem Biophys Res Commun 261:1–5
59. **Pace P, Taylor J, Suntharalingam S, Coombes RC, Ali S** 1997 Human estrogen receptor β binds DNA in a manner similar to and dimerizes with estrogen receptor α. J Biol Chem 272:25832–25838
60. **Cowley SM, Hoare S, Mosselman S, Parker MG** 1997 Estrogen receptors α and β form heterodimers on DNA. J Biol Chem 272: 19858–19862
61. **Hall JM, McDonnell DP** 1999 The estrogen receptor β-isoform (ERβ) of the human estrogen receptor modulates ERα transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinology 140:5566–5578
62. **Delaunay F, Pettersson K, Tujague M, Gustafsson J-Å** 2000 Functional differences between the amino-terminal domains of estrogen receptors α and β. Mol Pharmacol 58:584–590
63. **Métivier R, Stark A, Flouriot G, Hübner MR, Brand H, Penot G, Manu D, Denger S, Reid G, Kos M, Russell RB, Kah O, Pakdel F, Gannon F** 2002 A dynamic structural model for estrogen receptor-α activation by ligands, emphasizing the role of interactions between distant A and E domains. Mol Cell 10:1019–1032
64. **Beekman JM, Allan GF, Tsai SY, Tsai M-J, O'Malley BW** 1993 Transcriptional activation by the estrogen receptor requires a conformational change in the ligand binding domain. Mol Endocrinol 7:1266–1274
65. **Fritsch M, Leary CM, Furlow JD, Ahrens H, Schuh TJ, Mueller GC, Gorski J** 1992 A ligand-induced conformational change in the estrogen receptor is localized in the steroid binding domain. Biochemistry 31:5303–5311
66. **McKenna NJ, Lanz RB, O'Malley BW** 1999 Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20:321–344
67. **Robyr D, Wolffe AP, Wahli W** 2000 Nuclear hormone receptor coregulators in action: diversity for shared tasks. Mol Endocrinol 14:329–347
68. **Hermanson O, Glass CK, Rosenfeld MG** 2002 Nuclear receptor coregulators: multiple modes of modification. Trends Endocrinol Metab 13:55–60
69. **McKenna NJ, O'Malley BW** 2002 Combinatorial control of gene expression by nuclear receptors and coregulators. Cell 108:465–474
70. **Jordan VC** 1984 Biochemical pharmacology of antiestrogen action. Pharmacol Rev 36:245–276
71. **Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson J** 1997 Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138:863–870
72. **Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson J-Å** 1998 Interaction of

estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139:4252–4263.
73. **Berry M, Metzger D, Chambon P** 1990 Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. EMBO J 9:2811–2812
74. **Webster NJG, Green S, Jin J-R, Chambon P** 1988 The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. Cell 54:199–207
75. **Tora L, White JH, Brou C, Tasset DM, Webster NJG, Scheer E, Chambon P** 1989 The human estrogen receptor has two independent nonacidic transcriptional activation functions. Cell 59:477–487
76. **Tzukerman MT, Esty A, Santiso-Mere D, Danielian P, Parker MG, Stein RB, Pike JW, McDonnell DP** 1994 Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. Mol Endocrinol 8:21–30
77. **McDonnell DP, Clemm DL, Hermann T, Goldman ME, Pike JW** 1995 Analysis of estrogen receptor function *in vitro* reveals three distinct classes of antiestrogens. Mol Endocrinol 9:659–669
78. **Watanabe T, Inoue S, Ogawa S, Ishii Y, Hiroi H, Ikeda K, Orimo A, Muramatsu M** 1997 Agonistic effect of tamoxifen is dependent on cell type, ERE-promoter context, and estrogen receptor subtype: functional difference between estrogen receptors α and β. Biochem Biophys Res Commun 236:140–145
79. **Jones PS, Parrott E, White IN** 1999 Activation of transcription by estrogen receptor α and β is cell type- and promoter-dependent. J Biol Chem 274:32008–32014
80. **Kushner PJ, Agard DA, Greene GL, Scanlan TS, Shiau AK, Uht RM, Webb P** 2000 Estrogen receptor pathways to AP-1. J Steroid Biochem Mol Biol 74:311–317
81. **Safe S** 2001 Transcriptional activation of genes by 17β-estradiol through estrogen receptor-Sp1 interactions. Vitam Horm 62:231–252
82. **Martin PM, Berthois Y, Jensen EV** 1988 Binding of anti-estrogens exposes an occult antigenic determinant in the human estrogen receptor. Proc Natl Acad Sci USA 85:2533–2537
83. **Allan GF, Leng XH, Tsai SY, Weigel NL, Edwards DP, Tsai M-J, O'Malley BW** 1992 Hormone and antihormone induce distinct conformational changes which are central to steroid receptor activation. J Biol Chem 267:19513–19520
84. **Kuil CW, Berrevoets CA, Mulder E** 1995 Ligand-induced conformational alterations of the androgen receptor analyzed by limited trypsinization. Studies on the mechanism of antiandrogen action. J Biol Chem 270:27569–27576
85. **Modarress KJ, Opoku J, Xu M, Sarlis NJ, Simons Jr SS** 1997 Steroid-induced conformational changes at ends of the hormone-binding domain in the rat glucocorticoid receptor are independent of agonist *versus* antagonist activity. J Biol Chem 272:23986–23994
86. **Berger J, Bailey P, Biswas C, Cullinan CA, Doebber TW, Hayes NS, Saperstein R, Smith RG, Leibowitz MD** 1996 Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-γ: binding and activation correlate with antidiabetic actions in db/db mice. Endocrinology 137:4189–4195
87. **Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL** 1998 The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell 95:927–937
88. **Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson J-Å, Carlquist M** 1997 Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389:753–758
89. **Pike AC, Brzozowski AM, Walton J, Hubbard RE, Thorsell AG, Li Y-L, Gustafsson J-Å, Carlquist M** 2001 Structural insights into the mode of action of a pure antiestrogen. Structure 9:145–153
90. **Fawell SE, White R, Hoare S, Sydenham M, Page M, Parker MG** 1990 Inhibition of estrogen receptor-DNA binding by the "pure" antiestrogen ICI 164, 384 appears to be mediated by impaired receptor dimerization. Proc Natl Acad Sci USA 87:6883–6887
91. **Tamrazi A, Carlson KE, Daniels JR, Hurth KM, Katzenellenbogen JA** 2002 Estrogen receptor dimerization: ligand binding regulates dimer affinity and dimer dissociation rate. Mol Endocrinol 16:2706–2719
92. **Dauvois S, Danielian S, White R, Parker MG** 1992 Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. Proc Natl Acad Sci USA 89:4037–4041
93. **Htun H, Holth LT, Walker D, Davie JR, Hager GL** 1999 Direct visualization of the human estrogen receptor α reveals a role for ligand in the nuclear distribution of the receptor. Mol Biol Cell 10:471–486
94. **Dauvois S, White R, Parker MG** 1993 The antiestrogen ICI 182,780 disrupts estrogen receptor nucleocytoplasmic shuttling. J Cell Sci 106:1377–1388
95. **Stenoien DL, Patel K, Mancini MG, Dutertre M, Smith CL, O'Malley BW, Mancini M** 2001 FRAP reveals that mobility of oestrogen receptor-a is ligand- and proteasome-dependent. Nat Cell Biol 3:15–23
96. **Stenoien DL, Mancini MG, Patel K, Allegretto EA, Smith CL, Mancini MA** 2000 Subnuclear trafficking of estrogen receptor-α and steroid receptor coactivator-1. Mol Endocrinol 14:518–534
97. **McDonnell DP, Chang C-Y, Norris JD** 2000 Development of peptide antagonists that target estrogen receptor-cofactor interactions. J Steroid Biochem Mol Biol 74:327–335
98. **Chang C-Y, Norris JD, Gron H, Paige LA, Hamilton PT, Kenan DJ, Fowlkes D, McDonnell DP** 1999 Dissection of the LXXLL nuclear receptor-coactivator interaction motif using combinatorial peptide libraries: discovery of peptide antagonists of estrogen receptors α and β. Mol Cell Biol 19:8226–8239
99. **Norris JD, Paige LA, Christensen DJ, Chang CY, Huacani MR, Fan D, Hamilton PT, Fowlkes DM, McDonnell DP** 1999 Peptide antagonists of the human estrogen receptor. Science 285:744–746
100. **Chang C-Y, McDonnell DP** 2002 Evaluation of ligand-dependent changes in AR structure using peptide probes. Mol Endocrinol 16:647–660
101. **Baniahmad A, Leng X, Burris TP, Tsai SY, Tsai M-J, O'Malley BW** 1995 The τ4 activation domain of the thyroid hormone receptor is required to release a corepressor(s) necessary for transcriptional silencing. Mol Cell Biol 15:76–86
102. **Vegeto E, Allan GF, Schrader WT, Tsai M-J, McDonnell DP, O'Malley BW** 1992 The mechanism of RU486 antagonism is dependent on the conformation of the carboxy-terminal tail of the human progesterone receptor. Cell 69:703–713
103. **Halachmi S, Marden E, Martin G, MacKay H, Abbondanza C, Brown M** 1994 Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. Science 264:1455–1458
104. **Onate SA, Tsai SY, Tsai M-J, O'Malley BW** 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354–1357
105. **Voegel JJ, Heine MJS, Zechel C, Chambon P, Gronemeyer H** 1996 TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. EMBO J 15:3667–3675
106. **Hong H, Kohli K, Garabedian MJ, Stallcup MR** 1997 GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid, and vitamin D receptors. Mol Cell Biol 17:2735–2744
107. **Li H, Gomes PJ, Chen JD** 1997 RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. Proc Natl Acad Sci USA 94:8479–8484
108. **Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, Evans RM** 1997 Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell 90:569–580
109. **Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin S-C, Heyman RA, Rose DW, Glass CK, Rosenfeld MG** 1996 A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85:403–414
110. **Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK, Rosenfeld MG** 1997 The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. Nature 387:677–684
111. **Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS** 1997 AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277:965–968
112. **Takeshita A, Yen PM, Misiti S, Cardona GR, Liu Y, Chin WW** 1996

Molecular cloning and properties of a full-length putative thyroid hormone receptor coactivator. Endocrinology 137:3594–3597
113. **Heery DM, Kalkhoven E, Hoare S, Parker MG** 1997 A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 387:733–736
114. **McInerney EM, Rose DW, Flynn SE, Westin S, Mullen T-M, Krones A, Inostroza J, Torchia J, Nolte RT, Assa-Munt N, Milburn MV, Glass CK, Rosenfeld MG** 1998 Determinants of coactivator LXXLL motif specificity in nuclear receptor transcriptional activation. Genes Dev 12:3357–3368
115. **Mak HY, Hoare S, Henttu PMA, Parker MG** 1999 Molecular determinants of the estrogen receptor-coactivator interface. Mol Cell Biol 19:3895–3903
116. **Danielian PS, White R, Lees JA, Parker MG** 1992 Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. EMBO J 11:1025–1033
117. **Henttu PM, Kalkhoven E, Parker MG** 1997 AF-2 activity and recruitment of steroid receptor coactivator 1 to the estrogen receptor depend on a lysine residue conserved in nuclear receptors. Mol Cell Biol 17:1832–1839
118. **Webb P, Nguyen P, Shinsako J, Anderson C, Feng W, Nguyen MP, Chen D, Huang S-M, Subramanian S, McKinerney E, Katzenellenbogen BS, Stallcup MR, Kushner PJ** 1998 Estrogen receptor activation function 1 works by binding p160 coactivator proteins. Mol Endocrinol 12:1605–1618
119. **Lavinsky RM, Jepsen K, Heinzel T, Torchia J, Mullen T-M, Schiff R, Del-Rio AL, Ricote M, Ngo S, Gemsch J, Hilsenbeck SG, Osborne CK, Glass CK, Rosenfeld MG, Rose DW** 1998 Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. Proc Natl Acad Sci USA 95:2920–2925
120. **Kobayashi Y, Kitamoto T, Masuhiro Y, Watanabe M, Kase T, Metzger D, Yanagisawa J, Kato S** 2000 p300 Mediates functional synergism between AF-1 and AF-2 of estrogen receptor α and β by interacting directly with the N-terminal A/B domains. J Biol Chem 275:15645–15651
121. **Gill G, Ptashne M** 1988 Negative effect of the transcriptional activator GAL4. Nature 334:721–724
122. **Meyer ME, Gronemeyer H, Turcotte B, Bocquel M-T, Tasset D, Chambon P** 1989 Steroid hormone receptors compete for factors that mediate their enhancer function. Cell 57:433–442
123. **Cavailles V, Dauvois S, Danielian PS, Parker MG** 1994 Interaction of proteins with transcriptionally active estrogen receptors. Proc Natl Acad Sci USA 91:10009–10013
124. **Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, Tsai SY, Tsai M-J, O'Malley BW** 1999 A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. Cell 97:17–27
125. **Chen D, Huang S-M, Stallcup MR** 2000 Synergistic, p160 coactivator-dependent enhancement of estrogen receptor function by CARM1 and p300. J Biol Chem 275:40810–40816
126. **Chen D, Ma H, Hong H, Koh SS, Huang SM, Schurter BT, Aswad DW, Stallcup MR** 1999 Regulation of transcription by a protein methyltransferase. Science 284:2174–2177
127. **Nawaz Z, Lonard DM, Smith CL, Lev-Lehman E, Tsai SY, Tsai M-J, O'Malley BW** 1999 The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily. Mol Cell Biol 19:1182–1189
128. **Imhof MO, McDonnell DP** 1996 Yeast RSP5 and its human homolog hRPF1 potentiate hormone-dependent activation of transcription by human progesterone and glucocorticoid receptors. Mol Cell Biol 16:2594–2605
129. **Auboeuf D, Hönig A, Berget SM, O'Malley BW** 2002 Coordinate regulation of transcription and splicing by steroid receptor coregulators. Science 298:416–419
130. **Monsalve M, Wu Z, Adelmant G, Puigserver P, Fan M, Spiegelman BM** 2000 Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1. Mol Cell 6:307–316
131. **Lonard DM, Nawaz Z, Smith CL, O'Malley BW** 2000 The 26S proteasome is required for estrogen receptor-α and coactivator turnover and for efficient estrogen receptor-α transactivation. Mol Cell 5:939–948
132. **Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M** 2000 Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 103:843–852
133. **Liu Z, Wong J, Tsai SY, Tsai M-J, O'Malley BW** 2001 Sequential recruitment of steroid receptor coactivator-1 (SRC-1) and p300 enhances progesterone receptor-dependent initiation and reinitiation of transcription from chromatin. Proc Natl Acad Sci USA 98:12426–12431
134. **Burakov D, Crofts LA, Chang C-PB, Freedman LP** 2002 Reciprocal recruitment of DRIP/mediator and p160 coactivator complexes *in* vivo by estrogen receptor. J Biol Chem 277:14359–14362
135. **McNally JG, Müller WG, Walker D, Wolford R, Hager GL** 2000 The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. Science 287:1262–1265
136. **Perissi V, Staszewski LM, McInerney EM, Kurokawa R, Krones A, Rose DW, Lambert MH, Milburn MV, Glass CK, Rosenfeld MG** 1999 Molecular determinants of nuclear receptor-corepressor interaction. Genes Dev 13:3198–3208
137. **Chen D, Umesono K, Evans RM** 1996 SMRT isoforms mediate repression and anti-repression of nuclear receptor heterodimers. Proc Natl Acad Sci USA 93:7567–7571
138. **Kurokawa R, Soderstrom M, Horlein A, Halachmi S, Brown M, Rosenfeld MG, Glass CK** 1995 Polarity-specific activities of retinoic acid receptors determined by a co-repressor. Nature 377:451–454
139. **Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamel Y, Soderstrom M, Glass CK, Rosenfeld MG** 1995 Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377:397–404
140. **Leng X, Tsai SY, O'Malley BW, Tsai M-J** 1993 Ligand-dependent conformational changes in thyroid hormone and retinoic acid receptors are potentially enhanced by heterodimerization with retinoic X receptor. J Steroid Biochem Mol Biol 46:643–661
141. **Schulman IG, Juguilon H, Evans RM** 1996 Activation and repression by nuclear hormone receptors: hormone modulates an equilibrium between active and repressive states. Mol Cell Biol 16:3807–3813
142. **Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L, Horwitz KB** 1997 The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. Mol Endocrinol 11:693–705
143. **Smith CL, Nawaz Z, O'Malley BW** 1997 Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. Mol Endocrinol 11:657–666
144. **Dotzlaw H, Moehren U, Mink S, Cato ACB, Lluhi JAI, Baniahmad A** 2002 The amino terminus of the human AR is target for corepressor action and antihormone agonism. Mol Endocrinol 16:661–673
145. **Schulz M, Eggert M, Baniahmad A, Dostert A, Heinzel T, Renkawitz R** 2002 RU486-induced glucocorticoid receptor agonism is controlled by the receptor N terminus and by corepressor binding. J Biol Chem 277:26238–26243
146. **Shang Y, Brown M** 2002 Molecular determinants for the tissue specificity of SERMs. Science 295:2465–2468
147. **Huang H-J, Norris JD, McDonnell DP** 2002 Identification of a negative regulatory surface within estrogen receptor α provides evidence in support of a role for corepressors in regulating cellular responses to agonists and antagonists. Mol Endocrinol 16:1778–1792
148. **Jepsen K, Hermanson O, Onami TM, Gleiberman AS, Lunyak V, McEvilly RJ, Kurokawa R, Kumar V, Liu F, Seto E, Hedrick SM, Mandel G, Glass CK, Rose DW, Rosenfeld MG** 2000 Combinatorial roles of the nuclear receptor corepressor in transcription and development. Cell 102:753–763
149. **Rajendran RR, Nye AC, Frasor J, Balsara RD, Martini PGV, Katzenellenbogen BS** 2003 Regulation of nuclear receptor transcriptional activity by a novel DEAD box RNA helicase (DP97). J Biol Chem 278:4628–4638
150. **Fernandes I, Bastien Y, Wai T, Nygard K, Lin R, Cormier O, Lee HS, Eng F, Bertos NR, Pelletier N, Mader S, Han VKM, Yang X-J, White JH** 2003 Ligand-dependent nuclear receptor corepressor

LCoR functions by histone deacetylase-dependent and -independent mechanisms. Mol Cell 11:139–150
151. **Sohn Y-C, Kim S-W, Lee S, Kong Y-Y, Na DS, Lee S-K, Lee JW** 2003 Dynamic inhibition of nuclear receptor activation by corepressor binding. Mol Endocrinol 17:366–372
152. **Liao G, Chen L-Y, Zhang A, Godavarthy A, Xia F, Ghosh JC, Li H, Chen JD** 2003 Regulation of androgen receptor activity by the nuclear receptor corepressor SMRT. J Biol Chem 278:5052–5061
153. **Wagner BL, Norris JD, Knotts TA, Weigel NL, McDonnell DP** 1998 The nuclear corepressors NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor. Mol Cell Biol 18: 1369–1378
154. **Laherty CD, Billin AN, Lavinsky RM, Yochum GS, Bush AC, Sun J-M, Mullen T-M, Davie JR, Rose DW, Glass CK, Rosenfeld MG, Ayer DE, Eisenman RN** 1998 SAP30, a component of the mSin3 corepressor complex involved in N-CoR-mediated repression by specific transcription factors. Mol Cell 2:33–42
155. **Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, Schreiber SL, Evans RM** 1997 Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell 89:373–380
156. **Heinzel T, Lavinsky RM, Mullen T-M, Torchia J, Yang W-M, Brard G, Ngo SD, Davie JR, Seto E, Eisenman RN, Rose DW, Glass CK, Rosenfeld MG** 1997 A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature 387:43–48
157. **Shi Y, Downes M, Xie W, Kao HY, Ordentlich P, Tsai C-C, Hon M, Evans RM** 2001 Sharp, an inducible cofactor that integrates nuclear receptor repression and activation. Genes Dev 15:1140–1151
158. **Mazumdar A, Wang R-A, Mishra SK, Adam L, Bagheri-Yarmand R, Mandal M, Vadlamudi RK, Kumar R** 2001 Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor. Nat Cell Biol 3:30–37
159. **Windahl SH, Treuter E, Ford J, Zilliacus J, Gustafsson J-A, McEwan IJ** 1999 The nuclear-receptor interacting protein (RIP) 140 binds to the human glucocorticoid receptor and modulates hormone-dependent transactivation. J Steroid Biochem Mol Biol 71: 93–102
160. **Treuter E, Albrektsen T, Johansson L, Leers J, Gustafsson J-A** 1998 A regulatory role for RIP140 in nuclear receptor activation. Mol Endocrinol 12:864–881
161. **Delage-Mourroux R, Martini PGV, Choi I, Kraichely DM, Hoeksema J, Katzenellenbogen BS** 2000 Analysis of estrogen receptor interaction with a repressor of estrogen receptor activity (REA) and the regulation of estrogen receptor transcriptional activity by REA. J Biol Chem 275:35848–35856
162. **Montano MM, Ekena K, Delage-Mourroux R, Chang W, Martini P, Katzenellenbogen BS** 1999 An estrogen receptor-selective coregulator that potentiates the effectiveness of antiestrogens and represses the activity of estrogens. Proc Natl Acad Sci USA 96: 6947–6952
163. **Norris JD, Fan D, Sherk A, McDonnell DP** 2002 A negative coregulator for the human ER. Mol Endocrinol 16:459–468
164. **Zhang H, Thomsen JS, Johansson L, Gustafsson J-Å, Treuter E** 2000 DAX-1 functions as an LXXLL-containing corepressor for activated estrogen receptors. J Biol Chem 275:39855–39859
165. **Liu Z, Auboeuf D, Wong J, Chen JD, Tsai SY, Tsai M-J, O'Malley BW** 2002 Coactivator/corepressor ratios modulate PR-mediated transcription by the selective receptor modulator RU486. Proc Natl Acad Sci USA 99:7940–7944
166. **Szapary D, Huang Y, Simons Jr SS** 1999 Opposing effects of corepressor and coactivators in determining the dose-response curve of agonists, and residual agonist activity of antagonists, for glucocorticoid receptor-regulated gene expression. Mol Endocrinol 13:2108–2121
167. **He Y, Szapary D, Simons Jr SS** 2002 Modulation of induction properties of glucocorticoid receptor-agonist and -antagonist complexes by coactivators involves binding to receptors but is independent of ability of coactivators to augment transactivation. J Biol Chem 277:49256–49266
168. **Segars JH, Driggers PH** 2002 Estrogen action and cytoplasmic signaling cascades. I. Membrane-associated signaling complexes. Trends Endocrinol Metab 13:349–354
169. **Driggers PH, Segars JH** 2002 Estrogen action and cytoplasmic signaling pathways. II. The role of growth factors and phosphorylation in estrogen signaling. Trends Endocrinol Metab 13:422–427
170. **Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson J-Å** 2001 Mechanisms of estrogen action. Physiol Rev 81:1535–1565
171. **McInerney EM, Weis KE, Sun J, Mosselman S, Katzenellenbogen BS** 1998 Transcription activation by the human estrogen receptor subtype β (ERβ) studied with ERβ and ERα receptor chimeras. Endocrinology 139:4513–4522
172. **Cowley SM, Parker MG** 1999 A comparison of transcriptional activation by ERα and ERβ. J Steroid Biochem Mol Biol 69:165–175
173. **Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J-Å, Nilsson S** 1998 Differential response of estrogen receptor α and estrogen receptor β to partial estrogen agonists/antagonists. Mol Pharmacol 54:105–112
174. **Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ, Scanlan TS** 1997 Differential ligand activation of estrogen receptors ERα and ERβ at AP-1 sites. Science 277:1508–1510
175. **Wärnmark A, Almlöf T, Leers J, Gustafsson J-Å, Treuter E** 2001 Differential recruitment of the mammalian mediator subunit TRAP220 by estrogen receptors ERα and ERβ. J Biol Chem 276: 23397–23404
176. **Kressler D, Schreiber SN, Knutti D, Kralli A** 2002 The PGC-1-related protein PERC is a selective coactivator of estrogen receptor α. J Biol Chem 277:13918–13925
177. **Endoh H, Maruyama K, Masuhiro Y, Kobayashi Y, Goto M, Tai H, Yanagisawa J, Metzger D, Hashimoto S, Kato S** 1999 Purification and identification of p68 RNA helicase acting as a transcriptional coactivator specific for the activation function 1 of human estrogen receptor α. Mol Cell Biol 19:5363–5372
178. **Watanabe M, Yanagisawa J, Kitagawa H, Takeyama K-I, Ogawa S, Arao Y, Suzawa M, Kobayashi Y, Yano T, Yoshikawa H, Masuhiro Y, Kato S** 2001 A subfamily of RNA-binding DEAD-box proteins acts as an estrogen receptor α coactivator through the N-terminal activation domain (AF-1) with an RNA coactivator, SRA. EMBO J 20:1341–1352
179. **Wu X, Li H, Chen JD** 2001 The human homologue of the yeast DNA repair and TFIIH regulator MMS19 is an AF-1-specific coactivator of estrogen receptor. J Biol Chem 276:23962–23968
180. **Kraichely DM, Sun J, Katzenellenbogen JA, Katzenellenbogen BS** 2000 Conformational changes and coactivator recruitment by novel ligands for estrogen receptor-α and estrogen receptor-β: correlations with biological character and distinct differences among SRC coactivator family members. Endocrinology 141:3534–3545
181. **Wong C-W, Komm B, Cheskis BJ** 2001 Structure-Function evaluation of ER α and β interplay with SRC family coactivators. ER selective ligands. Biochemistry 40:6756–6765
182. **Cheskis BJ, McKenna NJ, Wong C-W, Wong J, Komm B, Lyttle CR, O'Malley BW** 2003 Hierarchical affinities and a bipartite interaction model for estrogen receptor isoforms and full-length steroid receptor coactivator (SRC/p160) family members. J Biol Chem 278:13271–13277
183. **Bramlett KS, Wu Y, Burris TP** 2001 Ligands specify coactivator nuclear receptor (NR) box affinity for estrogen receptor subtypes. Mol Endocrinol 15:909–922
184. **Bramlett KS, Burris TP** 2002 Effects of selective estrogen receptor modulators (SERMs) on coactivator nuclear receptor (NR) box binding to estrogen receptors. Mol Gene Metab 76:225–233
185. **Routledge EJ, White R, Parker MG, Sumpter JP** 2000 Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER) α and ERβ. J Biol Chem 275:35986–35993
186. **Yudt MR, Cidlowski JA** 2001 Molecular identification and characterization of A and B forms of the glucocorticoid receptor. Mol Endocrinol 15:1093–1103
187. **Yudt MR, Cidlowski JA** 2002 The glucocorticoid receptor: coding a diversity of proteins and responses through a single gene. Mol Endocrinol 16:1719–1726
188. **Conneely OM, Maxwell BL, Toft DO, Schrader WT, O'Malley BW** 1987 The A and B forms of the chicken progesterone receptor

arise by alternate initiation of translation of a unique mRNA. Biochem Biophys Res Commun 149:493–501
189. **Kastner P, Krust A, Turcotte B, Strupp U, Tora L, Gronemeyer H, Chambon P** 1990 Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J 9:1603–1614
190. **Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP** 1993 Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. Mol Endocrinol 7:1244–1255
191. **McDonnell DP, Goldman ME** 1994 RU486 exerts antiestrogenic activities through a novel progesterone receptor A form-mediated mechanism. J Biol Chem 269:11945–11949
192. **Tung L, Mohamed MK, Hoeffler JP, Takimoto GS, Horwitz KB** 1993 Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. Mol Endocrinol 7:1256–1265
193. **Giangrande PH, Pollio G, McDonnell DP** 1997 Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. J Biol Chem 272:32889–32900
194. **Giangrande PH, Kimbrel EA, Edwards DP, McDonnell DP** 2000 The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. Mol Cell Biol 20:3102–3115
195. **Li X, Wong J, Tsai SY, Tsai M-J, O'Malley BW** 2003 Progesterone and glucocorticoid receptors recruit distinct coactivator complexes and promote distinct patterns of local chromatin modification. Mol Cell Biol 23:3763–3773
196. **Klinge CM** 2001 Estrogen receptor interaction with estrogen response elements. Nucleic Acids Res 29:2905–2919
197. **Loven MA, Wood JR, Nardulli AM** 2001 Interaction of estrogen receptors α and β with estrogen response elements. Mol Cell Endocrinol 181:151–163
198. **Wood JR, Likhite VS, Loven MA, Nardulli AM** 2001 Allosteric modulation of estrogen receptor conformation by different estrogen response elements. Mol Endocrinol 15:1114–1126
199. **Loven MA, Likhite VS, Choi I, Nardulli AM** 2001 Estrogen response elements alter coactivator recruitment through allosteric modulation of estrogen receptor β conformation. J Biol Chem 276: 45282–45288
200. **Yi P, Driscoll MD, Huang J, Bhagat S, Hill R, Bambara RA, Muyan M** 2002 The effects of estrogen-responsive element- and ligand-induced structural changes on the recruitment of cofactors and transcriptional responses by ERα and ERβ. Mol Endocrinol 16:674–693
201. **Lefstin JA, Yamamoto KR** 1998 Allosteric effects of DNA on transcriptional regulators. Nature 392:885–888
202. **Ikeda M, Wilcox EC, Chin WW** 1996 Different DNA elements can modulate the conformation of thyroid hormone receptor heterodimer and its transcriptional activity. J Biol Chem 271:23096–23104
203. **Hall JM, McDonnell DP, Korach KS** 2002 Allosteric regulation of estrogen receptor structure, function, and coactivator recruitment by different estrogen response elements. Mol Endocrinol 16:469–486
204. **Barkhem T, Haldosén L-A, Gustafsson J-Å, Nilsson S** 2002 Transcriptional synergism on the pS2 gene promoter between a p160 coactivator and estrogen receptor-α depends on the coactivator subtype, the type of estrogen response element, and the promoter context. Mol Endocrinol 16:2571–2581
205. **Berry M, Nunez A-M, Chambon P** 1989 Estrogen-responsive element of the human pS2 gene is an imperfectly palindromic sequence. Proc Natl Acad Sci USA 86:1218–1222
206. **Cavarretta ITR, Mukopadhyay R, Lonard DM, Cowsert LM, Bennett CF, O'Malley BW, Smith CL** 2002 Reduction of coactivator expression by antisense oligodeoxynucleotides inhibits ERα transcriptional activity and MCF-7 proliferation. Mol Endocrinol 16: 253–270
207. **Dubik D, Shiu RP** 1992 Mechanism of estrogen activation of c-myc oncogene expression. Oncogene 7:1587–1594
208. **Augereau P, Miralles F, Cavailles V, Gaudelet C, Parker M, Rochefort H** 1994 Characterization of the proximal estrogen-responsive element of human cathepsin D gene. Mol Endocrinol 8:693–703
209. **Lannigan DA** 2003 Estrogen receptor phosphorylation. Steroids 68:1–9
210. **Weigel NL** 1996 Steroid hormone receptors and their regulation by phosphorylation. Biochem J 319:657–667
211. **Gioeli D, Ficarro SB, Kwiek JJ, Aaronson D, Hancock M, Catling AD, White FM, Christian RE, Settlage RE, Shabanowitz J, Hunt DF, Weber MJ** 2002 Androgen receptor phosphorylation. Regulation and identification of the phosphorylation sites. J Biol Chem 277:29304–29314
212. **Coleman KM, Smith CL** 2001 Intracellular signaling pathways: nongenomic actions of estrogens and ligand-independent activation of estrogen receptors. Front Biosci 6:D1379–D1391
213. **Dutertre M, Smith CL** 2003 Ligand-independent interactions of p160/SRC coactivators and CBP with estrogen receptor-α: regulation by phosphorylation sites in the A/B region depends on other receptor domains. Mol Endocrinol 17:1296–1314
214. **Tremblay A, Tremblay GB, Labrie F, Giguère V** 1999 Ligand-independent recruitment of SRC-1 to estrogen receptor β through phosphorylation of activation function AF-1. Mol Cell 3:513–519
215. **Tremblay A, Giguère V** 2001 Contribution of steroid receptor coactivator-1 and CREB binding protein in ligand-independent activity of estrogen receptor β. J Steroid Biochem Mol Biol 77:19–27
216. **Rowan BG, Weigel NL, O'Malley BW** 2000 Phosphorylation of steroid receptor coactivator-1. Identification of the phosphorylation sites and phosphorylation through the mitogen-activated protein kinase pathway. J Biol Chem 275:4475–4483
217. **Rowan BG, Garrison N, Weigel NL, O'Malley BW** 2000 8-Bromocyclic AMP induces phosphorylation of two sites in SRC-1 that facilitate ligand-independent activation of the chicken progesterone receptor and are critical for functional cooperation between SRC-1 and CREB binding protein. Mol Cell Biol 20:8720–8730
218. **Ueda T, Mawji NR, Bruchovsky N, Sadar MD** 2002 Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. J Biol Chem 277:38087–38094
219. **Coleman KM, Dutertre M, El-Gharbawy A, Rowan BG, Weigel NL, Smith CL** 2003 Mechanistic differences in the activation of estrogen receptor-α (ERα)- and ERβ-dependent gene expression by cAMP signaling pathway(s). J Biol Chem 278:12834–12845
220. **Lopez GN, Turck CW, Schaufele F, Stallcup MR, Kushner PJ** 2001 Growth factors signal to steroid receptors through mitogen-activated protein kinase regulation of p160 co-activator activity. J Biol Chem 276:22177–22182
221. **Font de Mora JF, Brown M** 2000 AIB1 is a conduit for kinase-mediated growth factor signaling to the estrogen receptor. Mol Cell Biol 20:5041–5047
222. **Wu R-C, Qin J, Hashimoto Y, Wong J, Xu J, Tsai SY, Tsai M-J, O'Malley BW** 2002 Regulation of SRC-3 (pCIP/ACTR/AIB-1/RAC-3/TRAM-1) coactivator activity by IκB kinase. Mol Cell Biol 22:3549–3561
223. **Qutob MS, Bhattacharjee RN, Pollari E, Yee SP, Torchia J** 2002 Microtubule-dependent subcellular redistribution of the transcriptional coactivator p/CIP. Mol Cell Biol 22:6611–6626
224. **Ko L, Cardona GR, Chin WW** 2000 Thyroid hormone receptor-binding protein, an LXXLL motif-containing protein, functions as a general coactivator. Proc Natl Acad Sci USA 97:6212–6217
225. **Iwasaki T, Chin WW, Ko L** 2001 Identification and characterization of RRM-containing coactivator activator (CoAA) as TRBP-interacting protein, and its splice variant as a coactivator modulator (CoAM). J Biol Chem 276:33375–33383
226. **Caira F, Antonson P, Pelto-Huikko M, Treuter E, Gustafsson J-Å** 2000 Cloning and characterization of RAP250, a novel nuclear receptor coactivator. J Biol Chem 275:5308–5317
227. **Lee S-K, Anzick SL, Choi J-E, Bubendorf L, Guan X-Y, Jung Y-K, Kallioniemi OP, Kononen J, Trent JM, Azorsa D, Jhun B-H, Cheong JH, Lee YC, Meltzer PS, Lee JW** 1999 A nuclear factor, ASC-2, as a cancer-amplified transcriptional coactivator essential for ligand-dependent transactivation by nuclear receptors *in vivo.* J Biol Chem 274:34283–34293
228. **Ko L, Cardona GR, Iwasaki T, Bramlett KS, Burris TP, Chin WW**

2002 Ser-884 adjacent to the LXXLL motif of coactivator TRBP defines selectivity for ERs and TRs. Mol Endocrinol 16:128–140
229. **Wang X, Yang Y, Guo X, Sampson ER, Hsu CL, Tsai M-Y, Yeh S, Wu G, Guo Y, Chang C** 2002 Suppression of androgen receptor transactivation by Pyk2 via interaction and phosphorylation of the ARA55 coregulator. J Biol Chem 277:15426–15431
230. **Yuan LW, Soh J-W, Weinstein IB** 2002 Inhibition of histone acetyltransferase function of p300 by PKCδ. Biochim Biophys Acta 1592: 205–211
231. **Yang W, Hong YH, Shen X-Q, Frankowski C, Camp HS, Leff T** 2001 Regulation of transcription by AMP-activated protein kinase. Phosphorylation of p300 blocks its interaction with nuclear receptors. J Biol Chem 276:38341–38344
232. **See RH, Calvo D, Shi Y, Kawa H, Luke MP-S, Yuan Z, Shi Y** 2001 Stimulation of p300-mediated transcription by the kinase MEKK1. J Biol Chem 276:16310–16317
233. **Liu Y-Z, Chrivia JC, Latchman DS** 1998 Nerve growth factor upregulates the transcriptional activity of CBP through activation of the $p42/p44^{MAPK}$ cascade. J Biol Chem 273:32400–32407
234. **Janknecht R, Nordheim A** 1996 MAP kinase-dependent transcriptional coactivation by Elk-1 and its cofactor CBP. Biochem Biophys Res Commun 228:831–837
235. **Hong S-H, Privalsky ML** 2000 The SMRT corepressor is regulated by a MEK-1 kinase pathway: inhibition of corepressor function is associated with SMRT phosphorylation and nuclear export. Mol Cell Biol 20:6612–6625
236. **Knutti D, Kressler D, Kralli A** 2001 Regulation of the transcriptional coactivator PGC-1 via MAPK-sensitive interaction with a repressor. Proc Natl Acad Sci USA 98:9713–9718
237. **Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, Krauss S, Mootha VK, Lowell BB, Spiegelman BM** 2001 Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARγ coactivator-1. Mol Cell 8:971–982
238. **Ichida M, Nemoto S, Finkel T** 2002 Identification of a specific molecular repressor of the peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α). J Biol Chem 277:50991–50995
239. **Espinosa L, Inglés-Esteve J, Robert-Moreno A, Bigas A** 2003 IκBα and p65 regulate the cytoplasmic shuttling of nuclear corepressors: cross-talk between notch and NFκB pathways. Mol Biol Cell 14: 491–502
240. **Zilliacus J, Holter E, Wakui H, Tazawa H, Treuter E, Gustafsson J-Å** 2001 Regulation of glucocorticoid receptor activity by 14-3-3-dependent intracellular relocalization of the corepressor RIP140. Mol Endocrinol 15:501–511
241. **Wijayaratne AL, McDonnell DP** 2001 The human estrogen receptor-α is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J Biol Chem 276:35684–35692
242. **Lange CA, Shen T, Horwitz KB** 2000 Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. Proc Natl Acad Sci USA 97:1032–1037
243. **Wallace JD, Cidlowski JA** 2001 Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. J Biol Chem 276:42714–42721
244. **Hauser S, Adelmant G, Sarraf P, Wright HM, Mueller E, Spiegelman BM** 2000 Degradation of the peroxisome proliferator-activated receptor γ is linked to ligand-dependent activation. J Biol Chem 275:18527–18533
245. **Voges D, Zwickl P, Baumeister W** 1999 The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu Rev Biochem 68:1015–1068
246. **Baumeister W, Walz J, Zühl F, Seemüller E** 1998 The proteasome: paradigm of a self-compartmentalizing protease. Cell 92:367–380
247. **Lonard DM, Smith CL** 2002 Molecular perspectives on selective estrogen receptor modulators (SERMs): progress in understanding their tissue-specific agonist and antagonist actions. Steroids 67: 15–24
248. **Lin H-K, Altuwaijri S, Lin W-J, Kan P-Y, Collins LL, Chang C** 2002 Proteasome activity is required for androgen receptor transcriptional activity via regulation of androgen receptor nuclear translocation and interaction with coregulators in prostate cancer cells. J Biol Chem 277:36570–36576
249. **Deroo BJ, Rentsch C, Sampath S, Young J, DeFranco DB, Archer TK** 2002 Proteasomal inhibition enhances glucocorticoid receptor transactivation and alters its subnuclear trafficking. Mol Cell Biol 22:4113–4123
250. **Deroo BJ, Archer TK** 2002 Proteasome inhibitors reduce luciferase and β-galactosidase activity in tissue culture cells. J Biol Chem 277:20120–20123
251. **Wang X, Pongrac JL, DeFranco DB** 2002 Glucocorticoid receptors in hippocampal neurons that do not engage proteasomes escape from hormone-dependent down-regulation but maintain transactivation activity. Mol Endocrinol 16:1987–1998
252. **Reid G, Hübner MR, Métivier R, Brand H, Denger S, Manu D, Beaudouin J, Ellenberg J, Gannon F** 2003 Cyclic, proteasome-mediated turnover of unliganded and liganded ERα on responsive promoters is an integral feature of estrogen signaling. Mol Cell 11:695–707
253. **Yan F, Gao X, Lonard DM, Nawaz Z** 2003 Specific ubiquitin-conjugating enzymes promote degradation of specific nuclear receptor coactivators. Mol Endocrinol 17:1315–1331
254. **Kang Z, Pirskanen A, Janne OA, Palvimo JJ** 2002 Involvement of proteasome in the dynamic assembly of the androgen receptor transcription complex. J Biol Chem 277:48366–48371
255. **Kotaja N, Karvonen U, Jänne OA, Palvimo JJ** 2002 PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. Mol Cell Biol 22:5222–5234
256. **Chauchereau A, Amazit L, Quesne M, Guiochon-Mantel A, Milgrom E** 2003 Sumoylation of the progesterone receptor and of the steroid receptor coactivator SRC-1. J Biol Chem 278:12335–12343
257. **Muller S, Hoege C, Pyrowolakis G, Jentsch S** 2001 SUMO, ubiquitin's mysterious cousin. Nat Rev Mol Cell Biol 2:202–210
258. **Melchior F** 2000 SUMO–nonclassical ubiquitin. Annu Rev Cell Dev Biol 16:591–626
259. **Yeh ETH, Gong L, Kamitani T** 2000 Ubiquitin-like proteins: new wines in new bottles. Gene 248:1–14
260. **Kotaja N, Karvonen U, Jänne OA, Palvimo JJ** 2002 The nuclear receptor interaction domain of GRIP1 is modulated by covalent attachment of SUMO-1. J Biol Chem 277:30283–30288
261. **vom Baur E, Zechel C, Heery D, Heine MJS, Garnier JM, Vivat V, Le Douarin B, Gronemeyer H, Chambon P, Losson R** 1996 Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. EMBO J 15:110–124
262. **Fan S, Wang J, Yuan R, Ma Y, Meng Q, Erdos MR, Pestell RG, Yuan F, Auborn KJ, Goldberg ID, Rosen EM** 1999 BRCA1 inhibition of estrogen receptor signaling in transfected cells. Science 284:1354–1356
263. **Fan S, Ma YX, Wang C, Yuan RQ, Meng Q, Wang JA, Erdos M, Goldberg ID, Webb P, Kushner PJ, Pestell RG, Rosen EM** 2001 Role of direct interaction in BRCA1 inhibition of estrogen receptor activity. Oncogene 20:77–87
264. **Fan S, Ma YX, Wang C, Yuan R-Q, Meng Q, Wang J-A, Erdos M, Goldberg ID, Webb P, Kushner PJ, Pestell RG, Rosen EM** 2002 p300 modulates the BRCA1 inhibition of estrogen receptor activity. Cancer Res 62:141–151
265. **Zhang J, Guenther MG, Carthew RW, Lazar MA** 1998 Proteasomal regulation of nuclear receptor corepressor-mediated repression. Genes Dev 12:1775–1780
266. **Ordentlich P, Downes M, Xie W, Genin A, Spinner NB** 1999 Unique forms of human and mouse nuclear receptor corepressor SMRT. Proc Natl Acad Sci USA 96:2639–2644
267. **Della NG, Senior PV, Bowtell DD** 1993 Isolation and characterisation of murine homologues of the Drosophila seven in absentia gene (sina). Development 117:1333–1343
268. **Koh SS, Chen D, Lee YH, Stallcup MR** 2001 Synergistic enhancement of nuclear receptor function by p160 coactivators and two coactivators with protein methyltransferase activities. J Biol Chem 276:1089–1098
269. **Ma H, Baumann CT, Li H, Strahl BD, Rice R, Jelinek MA, Aswad DW, Allis CD, Hager GL, Stallcup MR** 2001 Hormone-dependent, CARM1-directed, arginine-specific methylation of histone H3 on a steroid-regulated promoter. Curr Biol 11:1981–1985
270. **Bauer U-M, Daujat S, Nielsen SJ, Nightingale K, Kouzarides T**

2001 Methylation at arginine 17 of histone H3 is linked to gene activation. EMBO Rep 31:39–44
271. **Xu W, Chen H, Du K, Asahara H, Tini M, Emerson BM, Montminy M, Evans RM** 2001 A transcriptional switch mediated by cofactor methylation. Science 294:2507–2511
272. **Lee Y-H, Koh SS, Zhang X, Cheng X, Stallcup MR** 2002 Synergy among nuclear receptor coactivators: selective requirement for protein methyltransferase and acetyltransferase activities. Mol Cell Biol 22:3621–3632
273. **Chevillard-Briet M, Trouche D, Vandel L** 2002 Control of CBP co-activating activity by arginine methylation. EMBO J 21:5457–5466
274. **Chen H, Lin RJ, Xie W, Wilpitz D, Evans RM** 1999 Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. Cell 98:675–686
275. **Wang C, Fu M, Angeletti RH, Siconolfi-Baez L, Reutens AT, Albanese C, Lisanti MP, Katzenellenbogen BS, Kato S, Hopp T, Fuqua SAW, Lopez GN, Kushner PJ, Pestell RG** 2001 Direct acetylation of the estrogen receptor α hinge region by p300 regulates transactivation and hormone sensitivity. J Biol Chem 6:18375–18383
276. **Loven MA, Muster N, Yates JR, Nardulli AM** 2003 A novel estrogen receptor α-associated protein, template-activating factor 1β, inhibits acetylation and transactivation. Mol Endocrinol 17:67–78
277. **Kadener S, Cramer P, Nogues G, Cazalla D, de la Mata M, Fededa JP, Werbajh SE, Srebrow A, Kornblihtt AR** 2001 Antagonistic effects of T-Ag and VP16 reveal a role for RNA pol II elongation on alternative splicing. EMBO J 20:5759–5768
278. **Hönig A, Auboeuf D, Parker MM, O'Malley BW, Berget SM** 2002 Regulation of alternative splicing by the ATP-dependent DEAD-box RNA helicase p72. Mol Cell Biol 22:5698–5707
279. **Liu Z-R** 2002 p68 RNA helicase is an essential human splicing factor that acts at the U1 snRNA-5′ splice site duplex. Mol Cell Biol 22:5443–5450
280. **Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley BW, Spiegelman BM** 1999 Activation of PPARγ coactivator-1 through transcription factor docking. Science 286:1368–1371
281. **Slagsvold T, Kraus I, Fronsdal K, Saatcioglu F** 2001 DNA binding-independent transcriptional activation by the androgen receptor through triggering of coactivators. J Biol Chem 276:31030–31036
282. **Ko L, Cardona GR, Henrion-Caude A, Chin WW** 2002 Identification and characterization of a tissue-specific coactivator, GT198, that interacts with the DNA-binding domains of nuclear receptors. Mol Cell Biol 22:357–369
283. **Knutti D, Kaul A, Kralli A** 2000 A tissue-specific coactivator of steroid receptors, identified in a functional genetic screen. Mol Cell Biol 20:2411–2422
284. **Muller JM, Isele U, Metzger E, Rempel A, Moser M, Pscherer A, Breyer T, Holubarsch C, Buettner R, Schule R** 2000 FHL2, a novel tissue-specific coactivator of the androgen receptor. EMBO J 19: 359–369
285. **Misiti S, Schomburg L, Yen PM, Chin WW** 1998 Expression and hormonal regulation of coactivator and corepressor genes. Endocrinology 139:2493–2500
286. **Eckner R, Ewen ME, Newsome D, Gerdes M, DeCaprio JA, Lawrence JB, Livingston DM** 1994 Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev 8:869–884
287. **Xu J, Liao L, Ning G, Yoshida-Komiya H, Deng C, O'Malley BW** 2000 The steroid receptor coactivator SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. Proc Natl Acad Sci USA 97:6379–6384
288. **Li H, Chen JD** 1998 The receptor-associated coactivator 3 activates transcription through CREB-binding protein recruitment and autoregulation. J Biol Chem 273:5948–5954
289. **Apostolakis EM, Ramamurphy M, Zhou D, Onate S, O'Malley BW** 2002 Acute disruption of select steroid receptor coactivators prevents reproductive behavior in rats and unmasks genetic adaptation in knockout mice. Mol Endocrinol 16:1511–1523
290. **Gregory CW, Wilson EM, Apparao KBC, Lininger RA, Meyer WR, Kowalik A, Fritz MA, Lessey BA** 2002 Steroid receptor coactivator expression throughout the menstrual cycle in normal and abnormal endometrium. J Clin Endocrinol Metab 87:2960–2966
291. **Nephew KP, Ray S, Hlaing M, Ahluwalia A, Wu SD, Long X, Hyder SM, Bigsby RM** 2000 Expression of estrogen receptor coactivators in the rat uterus. Biol Reprod 63:361–367
292. **Shiozawa T, Shih H-C, Miyamoto T, Feng Y-Z, Uchikawa J, Itoh K, Konishi I** 2003 Cyclic changes in the expression of steroid receptor coactivators and corepressors in the normal human endometrium. J Clin Endocrinol Metab 88:871–878
293. **Lauritsen KJ, List H-J, Reiter R, Wellstein A, Riegel AT** 2002 A role for TGF-β in estrogen and retinoid mediated regulation of the nuclear receptor coactivator AIB1 in MCF-7 breast cancer cells. Oncogene 21:7147–7155
294. **Thenot S, Charpin M, Bonnet S, Cavailles V** 1999 Estrogen receptor cofactors expression in breast and endometrial human cancer cells. Mol Cell Endocrinol 156:85–93
295. **Akiyama N, Matsuo Y, Sai H, Noda M, Kizaka-Kondoh S** 2000 Identification of a series of transforming growth factor β-responsive genes by retrovirus-mediated gene trap screening. Mol Cell Biol 20:3266–3273
296. **Borud B, Hoang T, Bakke M, Jacob AL, Lund J, Mellgren G** 2002 The nuclear receptor coactivators p300/CBP/cointegrator-associated protein (p/CIP) and transcription intermediary factor 2 (TIF2) differentially regulate PKA-stimulated transcriptional activity of steroidogenic factor 1. Mol Endocrinol 16:757–773
297. **Li Q, Su A, Chen J, Lefebvre YA, Haché RJG** 2002 Attenuation of glucocorticoid signaling through targeted degradation of p300 via the 26S proteasome pathway. Mol Endocrinol 16:2819–2827
298. **Bautista S, Valles H, Walker RL, Anzick S, Zeillinger R, Meltzer P, Theillet C** 1998 In breast cancer, amplification of the steroid receptor coactivator gene AIB1 is correlated with estrogen and progesterone receptor positivity. Clin Cancer Res 4:2925–2929
299. **Murphy LC, Simon SL, Parkes A, Leygue E, Dotzlaw H, Snell L, Troup S, Adeyinka A, Watson PH** 2000 Altered expression of estrogen receptor coregulators during human breast tumorigenesis. Cancer Res 60:6266–6271
300. **Leygue E, Dotzlaw H, Watson PH, Murphy LC** 1999 Expression of the steroid receptor RNA activator in human breast tumors. Cancer Res 59:4190–4193
301. **Bouras T, Southey MC, Venter DJ** 2001 Overexpression of the steroid receptor coactivator AIB1 in breast cancer correlates with the absence of estrogen and progesterone receptors and positivity for p53 and HER2/*neu*1. Cancer Res 61:903–907
302. **Osborne CK, Bardou V, Hopp TA, Chamness GC, Hilsenbeck SG, Fuqua SAW, Wong J, Allred DC, Clark GM, Schiff R** 2003 Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. J Natl Cancer Inst 95:353–361
303. **Girault I, Lerebours R, Amarir S, Tozlu S, Tubiana-Hulin M, Lidereau R, Bièche I** 2003 Expression analysis of estrogen receptor α coregulators in breast carcinoma: evidence that *NCOR1* expression is predictive of the response to tamoxifen. Clin Cancer Res 9:1259–1266
304. **Balen A** 2001 Polycystic ovary syndrome and cancer. Hum Reprod Update 7:522–555
305. **Kumar R, Wang R-A, Mazumdar A, Talukder AH, Mandal M, Yang Z, Bagheri-Yarmand R, Sahin A, Hortobagyi G, Adam L, Barnes CJ, Vadlamudi RK** 2002 A naturally occurring MTA1 variant sequesters oestrogen receptor-α in the cytoplasm. Nature 418: 654–657
306. **Kurebayashi J, Otsuki T, Kunisue H, Tanaka K, Yamamoto S, Sonoo H** 2000 Expression levels of estrogen receptor-α, estrogen receptor-β, coactivators, and corepressors in breast cancer. Clin Cancer Res 6:512–518
307. **Murphy LC, Leygue E, Niu Y, Snell L, Ho SM, Watson PH** 2002 Relationship of coregulator and oestrogen receptor isoform expression to *de novo* tamoxifen resistance in human breast cancer. Br J Cancer 87:1411–1416
308. **Gregory CW, He B, Johnson RT, Ford OH, Mohler JL, French FS, Wilson EM** 2001 A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. Cancer Res 61:4315–4319
309. **Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai M-J, O'Malley BW** 1998

Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science 279:1922–1925

310. **Gehin M, Mark M, Dennefeld C, Dierich A, Gronemeyer H, Chambon P** 2002 The function of TIF2/GRIP1 in mouse reproduction is distinct from those of SRC-1 and p/CIP. Mol Cell Biol 22:5923–5937
311. **Wang Z, Rose DW, Hermanson O, Liu F, Herman T, Wu W, Szeto D, Gleiberman A, Krones A, Pratt K, Rosenfeld R, Glass CK, Rosenfeld MG** 2000 Regulation of somatic growth by the p160 coactivator p/CIP. Proc Natl Acad Sci USA 97:13549–13554
312. **Antonson P, Schuster GU, Wang L, Rozell B, Holter E, Flodby P, Treuter E, Holmgren L, Gustafsson J-A** 2003 Inactivation of the nuclear receptor coactivator RAP250 in mice results in placental vascular dysfunction. Mol Cell Biol 23:1260–1268.
313. **Jiang YH, Armstrong D, Albrecht U, Atkins CM, Noebels JL, Eichele G, Sweatt JD, Beaudet AL** 1998 Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. Neuron 21:799–811
314. **White R, Leonardsson G, Rosewell I, Jacobs MA, Milligan S, Parker M** 2000 The nuclear receptor co-repressor Nrip1 (RIP140) is essential for female fertility. Nat Med 6:1368–1374
315. **Smith CL, DeVera DG, Lamb DJ, Nawaz Z, Jiang Y-H, Beaudet AL, O'Malley BW** 2002 Genetic ablation of the steroid receptor coactivator-ubiquitin ligase, E6-AP, results in tissue-selective steroid hormone resistance and defects in reproduction. Mol Cell Biol 22:525–535
316. **Picard F, Géhin M, Annicotte J-S, Rocchi S, Champy M-F, O'Malley BW, Chambon P, Auwerx J** 2002 SRC-1 and TIF2 control energy balance between white and brown adipose tissues. Cell 111:931–941
317. **Weiss RE, Xu J, Ning G, Pohlenz J, O'Malley BW, Refetoff S** 1999 Mice deficient in the steroid receptor co-activator 1 (SRC-1) are resistant to thyroid hormone. EMBO J 18:1900–1904
318. **Takeuchi Y, Murata Y, Sadow P, Hayashi Y, Seo H, Xu J, O'Malley BW, Weiss RE, Refetoff S** 2002 Steroid receptor coactivator-1 deficiency causes variable alterations in the modulation of T_3-regulated transcription of genes *in vivo*. Endocrinology 143:1346–1352
319. **Sadow PM, Chassande O, Gauthier K, Samarut J, Xu J, O'Malley BW, Weiss RE** 2003 Specificity of thyroid hormone receptor subtype and steroid receptor coactivator-1 on thyroid hormone action. Am J Physiol 284:E36–E46
320. **Weiss RE, Gehin M, Xu J, Sadow PM, O'Malley BW, Chambon P, Refetoff S** 2002 Thyroid function in mice with compound heterozygous and homozygous disruptions of SRC-1 and TIF-2 coactivators: evidence for haploinsufficiency. Endocrinology 143:1554–1557
321. **Tanaka Y, Naruse I, Maekawa T, Masuya H, Shiroishi T, Ishii S** 1997 Abnormal skeletal patterning in embryos lacking a single Cbp allele: a partial similarity with Rubinstein-Taybi syndrome. Proc Natl Acad Sci USA 94:10215–10220
322. **Tanaka Y, Naruse I, Hongo T, Xu M, Nakahata T, Maekawa T, Ishii S** 2000 Extensive brain hemorrhage and embryonic lethality in a mouse null mutant of CREB-binding protein. Mech Dev 95: 133–145
323. **Yao TP, Oh SP, Fuchs M, Zhou ND, Ch'ng LE, Newsome D, Bronson RT, Li E, Livingston DM, Eckner R** 1998 Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. Cell 93:361–372
324. **Ito M, Yuan C-X, Okano HJ, Darnell RB, Roeder RG** 2000 Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development and thyroid hormone action. Mol Cell 5:683–693
325. **Goodman RH, Smolik S** 2000 CBP/p300 in cell growth, transformation, and development. Genes Dev 14:1553–1577
326. **Ito M, Roeder RG** 2001 The TRAP/SMCC/mediator complex and thyroid hormone receptor function. Trends Endocrinol Metab 12: 127–134
327. **Kung AL, Rebel VI, Bronson RT, Ch'ng L-E, Sieff CA, Livingston DM, Yao T-P** 2000 Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. Genes Dev 14:272–277
328. **Lyttle CR,** Novel SERMs for hormone therapy. Proc 50th Annual Meeting of the Society for Gyneacological Investigation, Washington, DC, 2003, p 8 (Abstract)
329. **Fu M, Wang C, Reutens AT, Wang J, Angeletti RH, Siconolfi-Baez L, Ogryzko V, Avantaggiati ML, Pestell RG** 2000 p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. J Biol Chem 275:20853–20860
330. **Pazin MJ, Kadonaga JT** 1997 What's up and down with histone deacetylation and transcription? Cell 89:325–328
331. **Chen D, Riedl T, Washbrook E, Pace PE, Coombes RC, Egly J-M, Ali S** 2000 Activation of estrogen receptor α by S118 phosphorylation involves a ligand-dependent interaction with TFIIH and participation of CDK7. Mol Cell 6:127–137
332. **Bastien J, Adam-Stitah S, Riedl T, Egly J-M, Chambon P, Rochette-Egly C** 2000 TFIIH interacts with the retinoic acid receptor γ and phosphorylates its AF-1-activating domain through cdk7. J Biol Chem 275:21896–21904
333. **Fan M, Bigsby RM, Nephew KP** 2003 The NEDD8 pathway is required for proteasome-mediated degradation of human estrogen receptor (ER)-α and essential for the antiproliferative activity of ICI 182,780 in ERα-positive breast cancer cells. Mol Endocrinol 17:356–365
334. **Fan M, Long X, Bailey JA, Reed CA, Osborne E, Gize EA, Kirk EA, Bigsby RM, Nephew KP** 2002 The activating enzyme of NEDD8 inhibits steroid receptor function. Mol Endocrinol 16:315–330
335. **Ma Z-Q, Liu Z, Ngan ESW, Tsai SY** 2001 Cdc25B functions as a novel coactivator for the steroid receptors. Mol Cell Biol 21:8056–8067
336. **Poukka H, Karvonen U, Janne OA, Palvimo JJ** 2003 Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). Proc Natl Acad of Sci USA 97:14145–14150
337. **Grossman SR, Deato ME, Brignone C, Chan HM, Kung AL, Tagami H, Nakatani Y, Livingston DM** 2003 Polyubiquitination of p53 by a ubiquitin ligase activity of p300. Science 300:342–344

Endocrine Reviews **is published bimonthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**