Review

Estrogen receptor transcription and transactivation Estrogen receptor alpha and estrogen receptor beta: regulation by selective estrogen receptor modulators and importance in breast cancer

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Abstract

Estrogens display intriguing tissue-selective action that is of great biomedical importance in the development of optimal therapeutics for the prevention and treatment of breast cancer, for menopausal hormone replacement, and for fertility regulation. Certain compounds that act through the estrogen receptor (ER), now referred to as selective estrogen receptor modulators (SERMs), can demonstrate remarkable differences in activity in the various estrogen target tissues, functioning as agonists in some tissues but as antagonists in others. Recent advances elucidating the tripartite nature of the biochemical and molecular actions of estrogens provide a good basis for understanding these tissue-selective actions. As discussed in this thematic review, the development of optimal SERMs should now be viewed in the context of two estrogen receptor subtypes, ERα and ERβ, that have differing affinities and responsiveness to various SERMs, and differing tissue distribution and effectiveness at various gene regulatory sites. Cellular, biochemical, and structural approaches have also shown that the nature of the ligand affects the conformation assumed by the ER–ligand complex, thereby regulating its state of phosphorylation and the recruitment of different coregulator proteins. Growth factors and protein kinases that control the phosphorylation state of the complex also regulate the bioactivity of the ER. These interactions and changes determine the magnitude of the transcriptional response and the potency of different SERMs. As these critical components are becoming increasingly well defined, they provide a sound basis for the development of novel SERMs with optimal profiles of tissue selectivity as medical therapeutic agents.

Keywords: coactivators, corepressors, estrogen receptor, ligands for estrogen receptors, selective estrogen receptor modulators

Introduction

The pharmacology of various estrogens is intriguing. While many compounds are able to bind to the estrogen receptor (ER), they can differ markedly in their stimulatory and/or inhibitory effects. In addition, certain compounds, now referred to as selective estrogen receptor modulators (SERMs) [1,2], can demonstrate remarkable differences in efficacy in the various tissues in which estrogens act,

EGF = epidermal growth factor; ER = estrogen receptor; ERE = estrogen response element; MAP = mitogen-activated protein; NHE-RF = sodium–hydrogen exchanger regulatory factor; SERM = selective estrogen receptor modulator; TGFβ3 = transforming growth factor β3.

functioning as agonists in some tissues but as antagonists in others. Such tissue-selective action is of great biomedical importance in the prevention and treatment of breast cancer, in menopausal hormone replacement, and in fertility regulation.

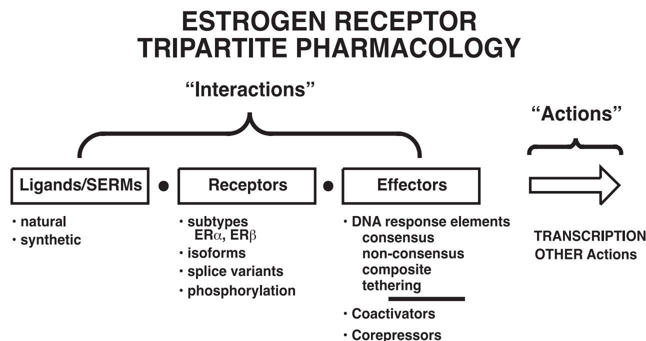
Originally termed an antiestrogen but now more properly designated as a SERM, tamoxifen is the most widely used agent in the treatment of breast cancer. In addition to its well documented effectiveness in the treatment of hormone-responsive breast cancer, there has been great excitement generated by the findings that tamoxifen [3••], as well as the related SERM raloxifene [4], are effective in preventing breast cancer in women at high risk for the disease. Despite these exciting new findings, it was also noted in the National Cancer Institute-sponsored Prevention Trial [3••] that tamoxifen was not a perfect SERM because there was increased incidence of endometrial cancer and venous thromboembolism. These findings highlight the importance of developing more optimal SERMs, particularly if these agents are to be used for breast cancer prevention and menopausal hormone replacement, where large numbers of healthy women would receive treatment for an extended period of time. An ideal SERM for these applications would be one that has no stimulatory action in the breast and uterus, and one that would block estrogen action at these sites, yet would act as an estrogen agonist in bone, liver, and the cardiovascular and central nervous systems.

Tripartite receptor pharmacology: a framework for understanding the tissue-selective actions of estrogens

Classical concepts in pharmacology cannot readily explain tissue selectivity in the actions of estrogens. However, recent advances in the molecular and cellular interactions of nuclear hormone receptors provide, for the first time, a view of many of the critical components that mediate the action of estrogens at the molecular level. These new findings provide a rich context within which one can begin to understand the unique properties of SERMs and to devise strategies for enhancing their desirable selective action.

As these findings were emerging a few years ago, we advanced the concept of 'tripartite receptor pharmacology' to provide a conceptual framework for understanding the tissue-selective actions of estrogens and other hormones for nuclear receptors (eg androgens, progestins, corticosteroids, etc), and the underlying molecular pharmacology [5]. The action of a particular estrogen, according to the tripartite receptor pharmacology scheme, is determined by three principal components: first, the structure of the ligand itself; second, the ER subtype or isoform with which the ligand binds to form a ligand–receptor complex of a particular conformation; and, finally, the interaction of this complex with an array of effector components through which the action of the hormone–receptor complex is linked to transcriptional regulation. The most critical effector components include the gene-regulatory DNA site to which the receptor binds (either directly or indirectly), as well as an array of coregulator proteins that determine the magnitude of the transcriptional response and its sensitivity to hormonal regulation (see Fig. 1). The hormone–receptor complex then recruits these coregulators, thereby linking the complex physically and/or functionally to the basal transcription complex and affecting the local chromatin structure.

Figure 1



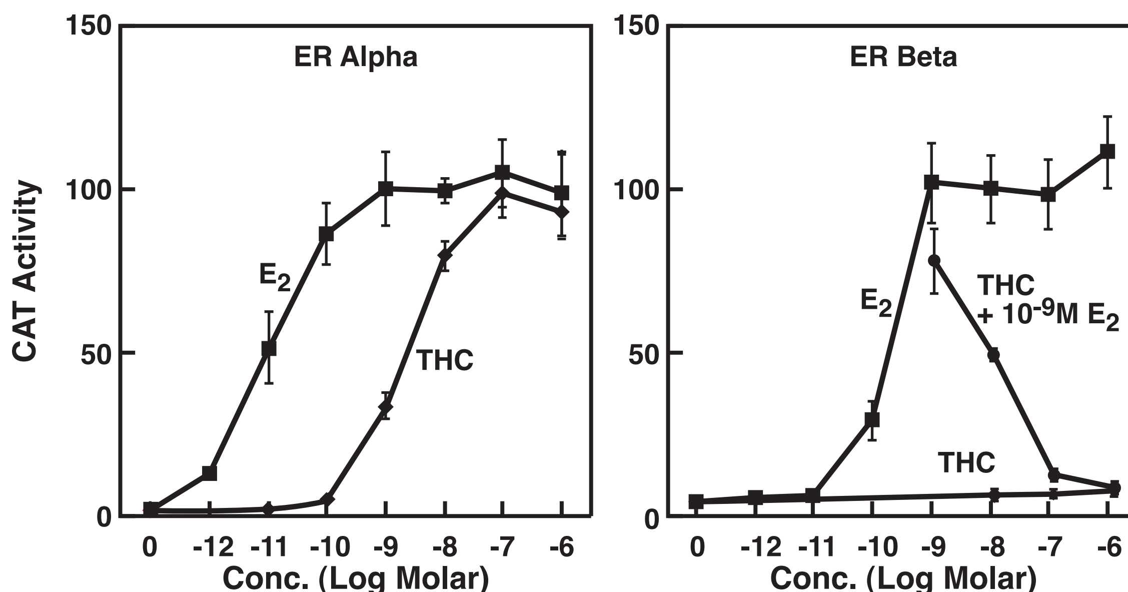
Estrogen receptor tripartite pharmacology. The diagram outlines the three components (ligands, receptors, and effectors) that together determine the magnitude and character of transcriptional and other responses to estrogens in target tissues.

Estrogen receptor alpha and estrogen receptor beta: receptor subtypes that underlie the diversity of responses to estrogens and provide opportunities for the development of novel SERMs

In thinking about the actions of SERMs, the discovery of a second ER gene, estrogen receptor-beta (ERβ), now distinguished from the classical ER (denoted ERα), is of particular importance [6••,7••]. ERα and ERβ differ significantly in their tissue distribution and ligand binding characteristics, as shown in this thematic review [8], thereby affording interesting potential for tissue-selective estrogen action.

While ERα and ERβ have nearly identical DNA-binding domains, these receptor subtypes have only 56% amino acid identity in their hormone binding domains, and they differ even more markedly (only 21% amino acid identity) in their N-terminal activation function 1 regions. These differences suggest that it should be possible to identify ligands that will have different levels of potency or efficacy through the two ER subtypes, which would allow selective stimulation of diverse estrogen-regulated genes. Indeed, initial screening of known ER ligands showed that certain

Figure 2



Transcription activation assays demonstrating that a tetrahydrochrysene (THC) ligand is an agonist on ERα and an antagonist on ERβ. Transfection assays were conducted in human endometrial cancer cells using an estrogen-responsive reporter gene and either ERα or ERβ [14••].

steroidal compounds exhibited moderate affinity and potency preference for ERα, whereas certain phytoestrogens and androgen-derived diols had moderate preference for ERβ [9•]. *In vivo* studies have indeed shown that, compared with estradiol, the soy phytoestrogen genestein is more effective in providing vascular protection, presumably mediated through ERβ, than uterine stimulation, presumably mediated through ERα [10•]. SERMs such as hydroxytamoxifen and raloxifene that are partial agonists on ERα [11•] were found to be complete antagonists on ERβ [12,13]. Studies utilizing chimeric ER subtypes, in which the activation function 1 regions were exchanged, indicate that the agonism of these SERMs tracks with the activation function 1 of ERα [12].

We have shown that it is possible to develop compounds of novel structure that can show remarkably high potency and/or efficacy selectivity on ERα and ERβ. For example, we found that a triaryl pyrazole, which had nearly a 500-fold binding affinity preference for ERα, could fully activate genes through ERα at 1 nM, whereas there was no gene activation through ERβ, even at 1 μM [14••,15]. We have also developed a series of substituted tetrahydrochrysenes that were full agonists on ERα but were complete antagonists on ERβ (see Fig. 2) [14••,16•]. Examinations with these compounds demonstrated that minor changes in the size and stereochemistry of the ligand substituents dramatically affected their activity as ERβ agonists or antagonists [16•]. These compounds are used to help define the respective biological roles of ERα and ERβ in the actions of estrogens in different target tissues. They are also being used to study, by X-ray crystallography, the ligand-induced conformation of the ER subtypes that mediate agonist versus antagonist activity. Genistein, which is more potent in activation of ERβ than ERα, curiously induces a conformation of helix-12 in ERβ that is considered to resemble an antagonist complex more than an agonist complex [17••]. These investigations further substantiate the observation that all agonists (or antagonists) do not contact the identical set of amino acids within the binding pocket of the receptor, nor induce identical receptor conformations [18,19•]. This is consistent with prior observations of differences in ligand–receptor proteolysis profiles [20•,21,22], as well as more recent studies using phage display of peptide probes, through which differences in the conformation of ERα and ERβ complexes with agonists and antagonists can be distinguished [23••,24•,25].

There have been several active programs directed at the development of new SERMs, and a number of analogs of tamoxifen, raloxifene and other nonsteroidal ER ligands [2,26,27] that appear to have favorable tissue-selective character have been described in the recent literature. The extent to which these new-generation SERMs act through ERα and ERβ, and the degree to which they provide substantial improvements over estrogens and antiestrogens currently in use in hormone replacement and in breast cancer prevention and treatment, will require careful evaluation. Likewise, studies on steroidal estrogens used in

hormone replacement have shown that some B-ring unsaturated compounds have distinct tissue-selective actions, being more efficacious in vasomotor, neuroendocrine and bone preservation parameters than in other peripheral actions of estrogens [28•]. The underlying bases for the tissue selectivity of these agents may be multifactorial, as discussed in this thematic review.

Effector components I: the nature of the gene DNA response element through which the estrogen receptor regulates transcription

Although the DNA binding domains of ERα and ERβ are nearly identical, there is considerable documentation that these receptor subtypes differ markedly in their abilities to activate different estrogen-responsive genes. This clearly highlights the fact that multiple regions of the receptor protein determine the specificity of gene activation [11•]. The fact that there are distinctly different modes of ER interaction with gene regulatory sites is of note in this regard. These different modes include direct binding of the receptor to estrogen response elements (EREs). These elements may be consensus or, more commonly, nonconsensus and may exist as single or multiple full or half sites; they may also be composite sites, consisting of EREs flanked by response elements for other transcription factors (such as Sp1), which themselves may or may not be occupied by their respective transactivating factors. It is interesting to note that there are differences in the affinities with which ERα and ERβ bind to the various EREs present in several estrogen-responsive genes (c-*fos*, c-*jun*, pS2, cathepsin D, choline acetyltransferase), measured by electrophoretic mobility gel shift assays, despite the near identity of the DNA-binding domains of the two receptors [29]. Studies showing that the DNA gene site itself also has an allosteric effect on the conformation of the ER monitored by protease digestion and immunoreactivity are relevant to this fact [30,31].

In an alternate manner, ER may interact with DNA indirectly through tethering to other DNA-bound transcription factors, as appears to be the case with the interaction of the ERs at AP1 sites, where the receptor is tethered through the Fos/Jun complex [32••,33]. Interestingly, the ERs also activate the quinone reductase gene [34••,35] and the transforming growth factor β3 (TGFβ3) gene [36•] through regulatory regions at which they work along with other protein factors.

There are intriguing differences in the pharmacological character of estrogens acting through ERα versus ERβ at these various gene sites. Compounds that are normally agonists or antagonists at ERE sites showed similar agonist or antagonist behavior through ERα at AP1 sites. When acting through ERβ at AP1 sites, however, compounds such as estradiol and diethylstilbestrol were curiously antagonistic, whereas antiestrogens such as hydroxytamoxifen and raloxifene showed strong stimulatory activity [32••]. Antiestrogens also activate the gene for quinone reductase, an antioxidant, detoxifying enzyme, with this stimulation being reversed by estrogens. This behavior is observed through both ERα and ERβ, but the magnitude of stimulation appears to be somewhat greater through ERβ [34••,35]. The upregulation of the quinone reductase gene by antiestrogens may contribute to the beneficial effects of antiestrogens in breast cancer prevention as well as treatment. The TGFβ3 gene in bone cells is also better stimulated by antiestrogen ligands, such as raloxifene, and by some equilin-type estrogens than by estradiol, although the respective roles of ERα and ERβ in this response have not been elucidated [36•].

These and other studies highlight not only the importance of the nature of the gene promoter site itself, but also the cell background (ie whether uterine, breast cancer, bone, or another type of cell) in determining the pharmacology of the hormone–receptor complex. This is due, at least in part, to differences in activity of the receptor activation functions in different cell backgrounds, reflecting differences in the balance and spectrum of coregulator proteins present in different types of cells [37••,38,39•,40]. It is also relevant to note that there is interaction between the two major (N- and C-terminal) activation function-containing regions of the ER, allowing for the synergistic regulation of transcription of many genes [41•,42].

Effector components II: coregulator proteins

The ER works with many other proteins in the regulation of gene expression. These coregulators play several critical roles: they affect the magnitude of gene stimulation or repression ([43–47] and references cited therein); they influence ligand dissociation kinetics [48]; and they alter the dose–response profile to hormone [48,49]. The magnitude of stimulation or repression of receptor transcriptional activity can be considered as first determined by the nature of the ligand, which controls the recruitment of coregulators to the ligand–receptor complex [50••,51••]. The agonist–receptor complex, most notably, recruits the p160 family of coactivators and other proteins, some of which possess histone acetyltransferase activity. Of interest in this regard in breast cancer is the report that AIB1/SRC-3/ACTR is amplified and upregulated in a significant number of breast tumors [52]. Such a change might indicate that these tumors show enhanced sensitivity to estrogens that may have affected tumorigenesis and/or progression of the disease.

The antagonist–receptor complex recruits other coregulators, including an ER-selective repressor of estrogen receptor activity (denoted REA) that enhances the inhibitory potency of antiestrogens [51••], as well as N-CoR and SMRT [53,54••]. The balance between coactivators and corepressors in breast cancers is considered to be an

important determinant of the agonist/antagonist activity of SERMs. There is already evidence that the level of N-CoR is correlated with tamoxifen sensitivity or resistance [54••], and L7/SPA is recruited by the ERα–tamoxifen complex and acts specifically to enhance the agonism by antiestrogen, an effect that is reversed by N-CoR [55•]. There is clear evidence for transcription factor specific requirements for coregulators [56], and mounting evidence for differential recruitment of coregulators by the occupied ERα–receptor and ERβ–receptor complexes, with the nature of the ligand and the nature of the receptor subtype determining the preference for different coregulators [57].

Since ERα and ERβ can also form heterodimers when both are present in the same cell [58,59], these heterodimers could potentially also differ from either homodimer complex in the profile of coregulators that are recruited to a hormone–receptor complex. This may be of importance in some breast cancers. Both ERα and ERβ are present in most breast cancers, although ERα is usually the predominant form [60,61,62•]. There is also evidence for several splice variants and other isoforms of both ERα and ERβ that might also differ in their bioactivity from the wild-type receptor forms [60,63]. Since there is evidence that ERβ can modulate the activity of ERα under some circumstances [64], it is possible that normal breast development as well as breast cancer progression may be accompanied by changes in the ratios of these two receptors [8]. Whether the onset of tamoxifen resistance might be explained by changes in either the levels or bioactivity of these two receptors or changes in its coregulator partners (such as SRCs or REA) is equally important. In the case of ERα, there is evidence for changes in cell signaling pathways that impact on the ER in tamoxifen-resistant breast cancer [65], as well as evidence for the presence of mutations in ERα in a small proportion of tamoxifen-resistant breast cancers [66–68]. The role of ERβ (wild type and variant) in breast cancer and in tamoxifen resistance needs to be investigated further.

The development of tamoxifen resistance limits the effective treatment of hormone-responsive breast cancer with this drug. This has placed a premium on understanding the mechanism by which tamoxifen resistance develops [69–71]. Although many hypotheses have been advanced, it now appears likely that the resistance to antiestrogen therapy most frequently results from a cellular adaptation process. One such process may involve a change in the cellular milieu of coactivators and corepressors (as well as changes in cell signaling pathways; see later) such that they abrogate the tumor growth inhibitory activity of the ER–tamoxifen complex, and/or may even make this complex a growth stimulator (see, for example, [54••,65]).

Very relevant in this regard are the recent studies using phage-displayed peptides in which certain peptides that specifically recognized ER complexes with the active tamoxifen metabolite, hydroxytamoxifen, were found to block selectively the partial agonistic activity of this ligand, without affecting the agonism of estradiol [24•]. This suggests that specific coregulator proteins, distinct from those involved in mediating the agonism of estrogens such as estradiol, are responsible for mediating the agonistic actions of antiestrogens such as tamoxifen. Learning how such factors are regulated in the cell, particularly with prolonged tamoxifen exposure, may lead to a greater understanding of the mechanism of tamoxifen resistance and may open up new approaches for preventing the development of this therapy-limiting cellular adaptation.

Crosstalk between the estrogen receptor and other cell signaling pathways

A considerable number of studies have documented the fact that growth factors (eg epidermal growth factor [EGF], insulin-like growth factor), cAMP and other agents (eg dopamine) can stimulate activity of the ER and also alter the agonist/antagonist balance of SERMs [72,73,74•, 75,76•,77]. There is mounting evidence for changes in growth factor and protein kinase pathways in hormone resistance in breast cancer ([69] and references cited therein). Stimulation of the protein kinase A signaling pathway, in particular, enhanced the agonistic activity of tamoxifen-like antiestrogens, and reduced the antagonistic effectiveness of this and related SERMs; observations that may in part account for the development of tamoxifen resistance by some ER-containing breast cancers [73]. Tamoxifen-resistant breast cancer cells also showed complete insensitivity to growth inhibition by TGFβ and reduced sensitivity to the growth inhibitory effects of retinoic acid, supporting interrelationships among the cell regulatory pathways utilized by these three growth-suppressive agents [65]. The effects of many of these agents are believed to reflect their ability to change the phosphorylation state of ER, as well as that of coregulators and other proteins with which the ER interacts to modulate gene expression. Interestingly, there is considerable evidence for interactions between cAMP and estrogen in regulating growth of the mammary gland and breast cancer cells [78,79].

Several groups have documented enhanced phosphorylation of ER on serine residues upon hormone occupancy as well as upon cell exposure to cAMP and some growth factors. Insulin-like growth factor and EGF stimulation, as well as estrogen stimulation, of ER transcriptional activity are associated with phosphorylation of several serine residues present in the N-terminal activation function 1 region of ERα and ERβ [76•,80–84]. These include, most notably, Ser-118 in ERα (and the equivalent serine in ERβ), a mitogen-activated protein (MAP) kinase site, and Ser 167 in ERα, which appears to be a pp90^{rsk1} site [85]. While growth factor-induced phosphorylation of Ser-118

by MAP kinase is well documented, there is evidence that another kinase may be involved in estrogen-induced phosphorylation of Ser-118. The cAMP-stimulated phosphorylation of ER probably occurs on different residues of the ER [82]. Mutational analyses indicate that these sites play an important role in the transactivation ability of the ER [76•,82,83,85,86].

Crosstalk between the ER and EGF signaling systems has been nicely documented more recently in the ERα knockout mouse, where the mice lose responsiveness to the EGF, as well as to estrogen, in the uterus [87]. Our observations that the sodium–hydrogen exchanger regulatory factor (NHE-RF, also known as EBP50) is upregulated by estrogen suggests that this protein may serve as a link between the ER and some cell signaling pathways [88]. NHE-RF has been shown to interact with ezrin–radixin–moesin cytoskeletal proteins that link actin filaments to the cell membrane, an interaction that may mediate the estrogen-induced changes in cellular architecture ([88] and references cited therein). NHE-RF also interacts through its two PDZ domains with several important receptors, including the beta-adrenergic receptor, the platelet-derived growth factor receptor, and the cystic fibrosis transporter receptor, and may thereby provide a link between ER and these other regulatory pathways.

The issue of whether hormone-dependent phosphorylation of the ER involves tyrosine residues and whether this affects receptor activity has been controversial. Several articles have reported phosphorylation of ERα on tyrosine 537 and provided evidence for the role of this site in regulating hormone binding and DNA binding of the receptor [89,90]. However, other studies involving replacement of this residue with amino acids incapable of being phosphorylated, indicate that phosphorylation at this site is not required for hormone or DNA binding, nor for transcriptional activity of the receptor [21,91–94]. The amino acid substitution studies revealed that substitution of certain amino acids for tyrosine 537 in ERα (and at the corresponding tyrosine in ERβ [95]) produced constitutively active ERs (ie ERs fully active in the absence of hormone). These findings suggest that the nature of the residue at this position, which is at the start of helix-12, may facilitate the shift of this helix into an active conformation and/or allow stabilization of the receptor in its active form [21,91–93].

Aside from the well documented synergistic effects of estrogens and some protein kinase activators and growth factors on gene transcription (see, for example, [96•]), estrogens also exert rapid membrane-initiated effects that are known to impact importantly on cell signaling and may also influence nuclear gene transcription. For example, estrogens increase the overall levels of tyrosine phosphorylation in cells [97], increase intracellular calcium concentration in some cells [98,99], increase the phosphorylation of CREB [100], activate G protein-coupled signaling [101], and rapidly increase MAP kinase activity associated with estrogen stimulation of cell proliferation [99,102••]. Several studies suggest that these effects may be due to ERs present in the membrane that are similar to those that mediate gene transcription in the nucleus [101,103,104•], although other studies indicate a receptor pharmacology and ligand selectivity different from that of the classical nuclear ERs [98,105]. This remains an area of great importance and active investigation.

Conclusion

There have been great advances in our understanding of the biochemical and molecular basis for biomedically important tissue selective actions of estrogens. The development of optimal SERMs for the prevention and treatment of breast cancer, and for hormone replacement therapy and fertility regulation, can now be viewed in the context of two estrogen receptor subtypes, ERα and ERβ, that have differing affinities and responsiveness to various SERMs, and differing tissue distribution and effectiveness at different gene regulatory sites. Cellular, biochemical, and structural approaches have revealed that the nature of the ligand affects the conformation assumed by the ER–ligand complex, thereby regulating its state of phosphorylation and the recruitment of different coactivators and corepressors that determine the magnitude of the transcriptional response and its sensitivity to the SERM. The ER and its ligands do not work in isolation in various estrogen target tissues; the ER also has its bioactivity regulated by growth factors and various protein kinases that regulate its phosphorylation, as well as the state of phosphorylation of coregulator proteins with which it interacts. As these critical components are becoming increasingly well defined, they provide a sound basis for the development of novel SERMs with optimal profiles of tissue selectivity as medical therapeutic agents.

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