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Topic 1.9

Estrogen receptor action through target genes with classical and alternative response elements*

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Abstract: The estrogen receptors alpha and beta (ERα and ERβ) mediate the changes in gene expression from physiological and environmental estrogens. Early studies identified classical estrogen response elements (EREs) in the promoter region of target genes whose expression is regulated by estrogen and to which the ERs bind via their DNA-binding domain (DBD). EREs in the pituitary prolactin promoter, for example, mediate an activation by both ERα and ERβ albeit with different affinities for different ligands. Full activation in most cell types requires the integrity of the activation function 2 (AF-2) in the receptors ligand binding domain (LBD), which is engaged by estrogens and disengaged by tamoxifen, raloxifene, and other antiestrogens. However, in some cells and ERE contexts, the AF-1 in the ERα amino terminal domain (NTD) is sufficient.

We now know that ERs also regulate expression of target genes that do not have EREs, but instead have various kinds of alternative response elements that bind heterologous transcription factors whose activity is regulated by interactions with ERs. Thus, ERα activates genes, including collagenase and cyclin D1, an important mediator of cellular proliferation, by AP-1 and CRE sites, which bind Jun/Fos or Jun/ATF-2 transcription factors. ERα also activates gene expression through GC-rich elements that bind the SP1 transcription factor. Finally, we also know that ERs mediate inhibition of the expression of many genes. In one well-studied instance, ERs counterexpression of genes involved in the inflammatory response by inhibiting the action at tumor necrosis factor response elements (TNF-REs) that bind the NFkappaB transcription factor. ERβ is especially efficient at this inhibition.

ERα activation of AP-1/CRE target genes is of special interest because of the putative role of these target genes in mediating proliferation. The AF-1 and AF-2 functions of ERα are both needed for this activation in most cell types. However, in uterine cells, the AF-1 function is sufficient. Thus, the antiestrogen tamoxifen, which allows AF-1, mimics estrogen and drives activation of AP-1/CRE target genes and proliferation of uterine cells. This estrogen-like action, which can increase the risk of uterine cancer, complicates the use of tamoxifen to prevent breast cancer. Surprisingly, ERβ inhibits AP-1/CRE target genes in the presence of estrogen. When both receptors are present, ERβ efficiently opposes activation by ERα. Moreover, ERβ activates the AP-1/CRE target genes in the presence of antiestrogens especially so-called "complete" antiestrogens raloxifene, and ICI 182, 780.

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We here review the evidence for different kinds of promoter elements that mediate ER action, for the differential ligand preferences of ERα and ERβ at these different elements, and the potential mechanisms by which they are mediated. One attractive strategy for the investigation and comparison of potential environmental estrogens is to assay their activity in cell culture systems using reporter genes with simplified promoter elements. Thus, the findings of complexity in ERα and ERβ activation at different types of response elements needs to be taken into account in the development and interpretation of assays using simplified promoter elements systems.

TWO ESTROGEN RECEPTORS, ALPHA AND BETA, MEDIATE THE ACTION OF ENDOGENOUS AND ENVIRONMENTAL ESTROGENS, INCLUDING THE EFFECTS ON CELLULAR PROLIFERATION AND CANCER INDUCTION

Estrogen has profound effects on the development and function of reproductive organs. Among its other effects, it stimulates proliferation of epithelial cells in the reproductive tract and mammary gland of females and in the prostate of males. In the female tissues, and most likely in the prostate as well, it also plays a role in the development of cancer [for recent reviews, see 12,23,57,63]. This proliferative effect of estrogens is, of course, a primary parameter of concern in the study of environmental estrogens.

Two related proteins, the estrogen receptor alpha (ERα) and beta (ERβ), which function as transcription factors to regulate expression of target genes, carry out and modulate the effects of estrogen [23,43]. Studies of mice with disrupted estrogen receptors indicate that ERα mediates the major proliferative effects of estrogen [10]. Thus, female mice in which ERα has been knocked-out (αERKOs) lack estrogen-provoked proliferation of the uterus, cervix and vagina, and have rudimentary mammary glands [6,10,29]. Male αERKO mice are completely resistant to estrogen-provoked prostate proliferation, hyperplasia, and cancer [47,48]. In contrast, ERβ knock-out (βERKO) female mice have full estrogen responses of the reproductive tract, and males have a full response of the prostate.

There is suggestive evidence that ERβ may modulate the proliferative effects of ERα. Thus, the βERKOs are reported to have exaggerated estrogen responses in the uterus, and to have spontaneous hyperplasia of the prostate, although the latter observation is not without controversy and needs to be confirmed [14,74,75]. Consistent with this, there is a progressive loss of ERβ expression in prostate cancer [18,41]. In mammary gland, ERβ is present in high amounts in normal epithelium and progressively lost in multi-stage carcinogenesis, an observation that suggests a potential loss of an inhibitor of proliferation [49].

ERs REGULATE TRANSCRIPTION AT TWO TYPES OF TARGET GENES, THOSE WITH CLASSICAL ESTROGEN RESPONSE ELEMENTS AND THOSE WITH AP-1/CRE SITES, THE LATTER OF WHICH MAY BE IMPORTANT FOR PROLIFERATIVE EFFECTS

ERs are transcriptional activation proteins that bind estrogen and acquire the ability to stimulate transcription of estrogen-responsive target genes [for review, see 32,37,40]. ERs work through at least two different pathways to affect transcription. In one pathway, the ERs act at target genes that have classical estrogen response elements (EREs) within the promoter region that allow the ER to bind DNA and regulate transcription [33]. Examples of these classical target genes with EREs include the vitellogenin genes of birds and frogs and the mammalian prolactin, pS2, cathepsin D, and lactoferrin genes.

In a second pathway of action, ERs regulate transcription at promoter elements that directly bind heterologous transcription factors. These promoter elements include AP-1 sites that bind Jun/Fos [26], variant cyclic-AMP response elements (CREs) that bind c-Jun/ATF-2 proteins [50,69,72], and Sp1 sites [51,52]. ERs have also been reported to regulate through other sites whose binding proteins have not yet been identified [34–36,79]. Neither AP-1, CRE, or Sp1 sites bind ERs and regulation is presumed

to occur through protein–protein interactions, in which the proteins that do bind these sites somehow allow for ER recruitment.

ER regulation of target genes with AP-1 and CRE sites is typically less dramatic than at EREs, yet such AP-1/CRE targets may be important for proliferative effects of estrogen. Two sorts of evidence suggest this. First many of the target genes that are regulated through AP-1 and CRE elements seem to be involved in proliferation or tissue remodeling. These include, for example, the collagenase gene, other genes for matrix metalloproteinases, and the IGF-1 gene, which are regulated by estrogen through AP-1 sites [65,69,72]. Above all, the cyclin D1 gene, an estrogen-induced gene which has been implicated as a central node for estrogen-mediated growth both of human breast cancer cells in culture [46], and of mammary epithelial cells in vivo [61], does not have an ERE. Instead, estrogen regulation of the cyclin D1 gene has been ascribed to a variant CRE element that binds Jun/ATF-2 and does not bind ERs [1,50]. Second, there is an excellent correlation between the ability of selective estrogen receptor modulators (SERMs) to mimic estrogen and stimulate proliferation and the ability of SERMs to mimic estrogen and activate target genes with AP-1/CRE elements. Thus tamoxifen, but not raloxifene, or ICI 182,780 (hereafter ICI) can mimic estrogen and stimulate uterine (but not mammary) cell growth. In parallel, tamoxifen, but not raloxifene or ICI, activates AP-1/CRE transcription in uterine cells, but not mammary cells [69]. This ability of tamoxifen, but not raloxifene, to activate IGF-1, and c-Myc (another estrogen induced gene without an ERE, [13].) and only in uterine cells has recently been confirmed with the endogenous genes in human endometrial cells [53]. Finally it should be mentioned that in some mammary cancer cells the introduction of ERα inhibits proliferation, and in these cells there is a corresponding inhibition of AP-1 regulated target genes [44].

In addition to AP-1/CRE elements, it appears that GC-rich elements that bind the Sp1 family of transcription factors can also mediate estrogen induction via ERα in the context of some promoters. Sp1 sites contribute to estrogen response in the promoters of a large number of genes both with EREs and with alternative response elements [51,52]. Indeed, GC-rich elements that bind Sp1 contribute to the estrogen induction of cyclin D1 [8]. There is also some evidence that Sp1 sites can by themselves with the appropriate core promoter confer an estrogen response.

ERs also downregulate some target genes with estrogen. One well-studied example are target genes with elements for the NF-κB transcription factors, which include those for TNFα and other cytokines including il-1 and il-6 [2]. This mechanism is believed to underlie some of the anti-inflammatory, and possibly some of the anti-osteoclastogenic activities of estrogen.

ERα AND ERβ HAVE PARALLEL ACTION AT ERE TARGET GENES

ERα and ERβ each activate ERE containing target genes in the presence of estrogen. In most ERE contexts ERβ tends to be a weaker activator than ERα, and the weaker activation is dominant in cells with both receptors, as illustrated in Fig. 1 [11,16,42]. In some contexts, ERβ also requires higher estrogen concentrations for activation than ERα, and thus, the dominant weaker activation by ERβ is exaggerated under conditions where estrogen is limiting [16]. ERβ has preferential binding to xeno-estrogens, most notably to genistein and other phytoestrogens, which bind and activate ERβ at lower concentrations than at ERα [5,25].

How ERs work at EREs is covered in other articles in this topic section (see article by Donald McDonnell et al.), and a simple outline is indicated in Fig. 1b. Estrogen binding to the ER releases it from complexes with chaperone proteins, allowing it to dimerize through the C-terminal ligand-binding domain (LBD), and bind to the ERE via the centrally located DNA-binding domain (DBD). The ERE is itself a palindromic dimer of two half-sites (sequence AGGTCA) separated by three base pairs. Once situated on the ERE, the ER works as a molecular tether for coactivators, which it recruits through two activation functions, AF-1 and AF-2. The AF-1 in the amino terminal domain of ERα is constitutive and relatively weak in most cell types. No AF-1 is found in the somewhat shorter amino terminal

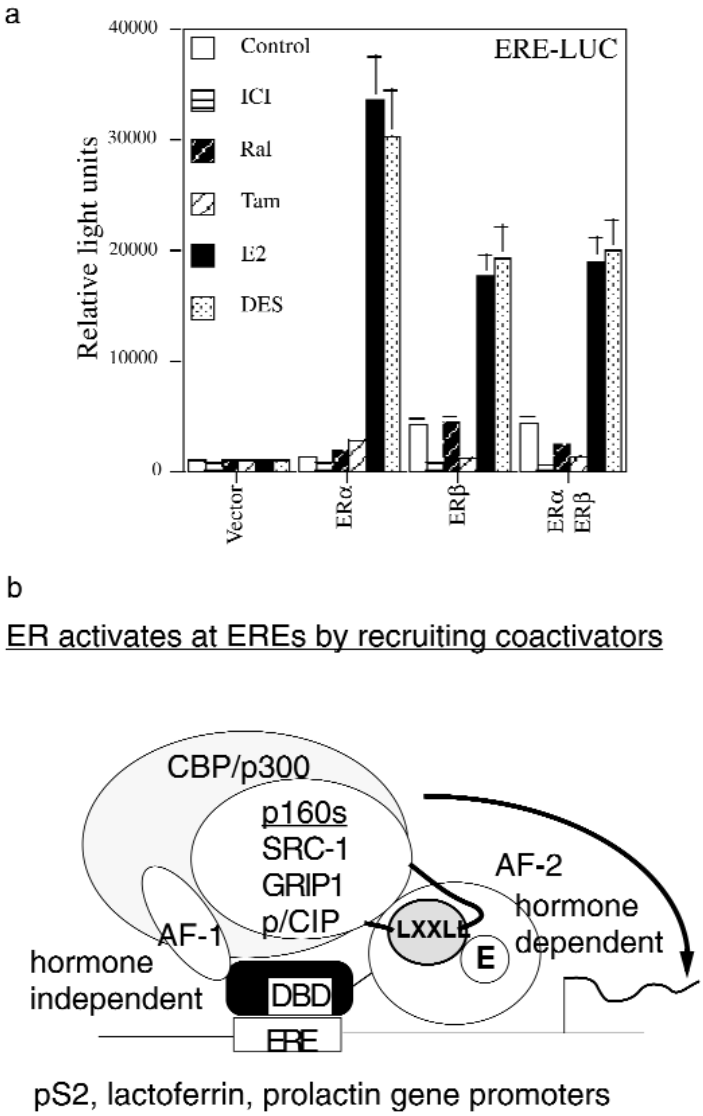

Fig. 1 (a) *ERβ modulates ERα activation at the ERE elements.* Activity of the ERE-II-LUC reporter in HeLa cells transfected with expression vectors for ERα, ERβ, both, or empty vector and treated with ligands as indicated. (b) *How ERs activate at an ERE.* ERs bind to the ERE with their DBD, ERα recruits coactivators with the hormone independent AF-1 and both ERs with their hormone dependent AF-2. The coactivators then stimulate transcription. AF-1 binds both components of the p160/CBP coactivator complex, and AF-2 forms a hydrophobic cleft (stippled) into which NR boxes (LXXLL) of the p160s bind.

domain of ERβ. The powerful and hormone-activated AF-2 function is found in the LBDs of both ERs. AF-2 is active when bound to estrogen, DES or other agonist and is inactive when bound to tamoxifen, raloxifene, ICI, or other antiestrogens.

A family of related proteins SRC-1, GRIP1, p/CIP, here referred to as p160s, mediate both AF-2 activity and a substantial amount of AF-1 activity [71]. The p160s bind to the ER-LBD only when it is liganded to estrogen or other agonist, but not when liganded to antiestrogens (for review, see ref. [56]). p160 Binding to AF-1 is hormone-independent. That p160 proteins mediate AF-1 or AF-2 function is shown in part because increasing their abundance potentiates AF-1 and AF-2, and interfering with them

abolishes AF-1 and AF-2 [38,71]. Other proteins including the CBP/p300 family of coactivators complex with the p160s and also appear to play a role in AF-1 and AF-2 function [9,17,21,24,58]. Antiestrogens block AF-2 because they block recruitment to the ER of the p160-CBP coactivator complex that mediates AF-2 function. Interestingly, AF-1 and AF-2 are modular, and can each be inactivated without inactivating the other. Thus, tamoxifen blocks AF-2 but allows AF-1.

Once recruited to the ERE by ER, the coactivator complex is believed to mediate transcriptional activation by two means. First, the complex remodels chromatin in part through the strong histone acetyl transferase activity (HAT) in CBP that acetylates lysines in the N-terminal tails of histones H3 and H4 [77] (see also [54]). CBP may also make direct contact with components of the transcriptional apparatus. The p160s, in addition to their binding to CBP, bind CARM1/PRMT1 two histone arginine methyltransferases. CARM1/PRMT1 enhances p160 action, possibly by contributing to chromatin remodeling through methylation of arginines in H3 and H4 [60].

Exactly how estrogens allow the binding of p160s, and how antiestrogens block the binding has been revealed by 3D X-ray structures and structure-guided mutational analysis and is shown in Fig. 2 [7,55]. Estrogen is bound within the LBD and allows a hydrophobic cleft made up of helices 3, 5, and 12 to form on the surface of the LBD. The hydrophobic cleft is the docking surface for p160s [15,30]. GRIP1 and other p160 coactivators have multiple nuclear receptor boxes (NR boxes) with the sequence LXXLL. The leucines of the NRboxes project into the hydrophobic cleft to couple the two proteins [55]. Key residues in helix 3, 5, and especially 12, of the ER are needed for both this coupling, for AF-2 function, and for gene activation. Tamoxifen blocks AF-2 function and binding of p160s because it projects from the LBD displacing helix 12. In the tamoxifen structure, helix 12 rotates from its normal position into the hydrophobic cleft, thereby occluding the cleft from coactivators [55]. Notice that in the ta-

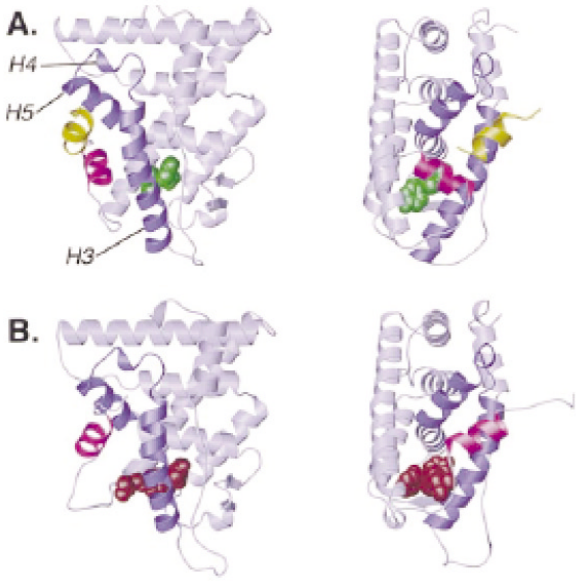

Fig. 2 *How tamoxifen blocks AF-2. Overall structures of the DES-ERα LBD-GRIP1 NR box II peptide complex and of the OHT-ERα LBD complex. (a) Two orthogonal views of the DES-ERα LBD-NR box II peptide.* The coactivator peptide and the LBD are shown as ribbon drawings. The peptide is colored gold, and helix 12 (residues 538–546) is colored magenta. Helices 3, 4, and 5 (labeled H3, H4, and H5, respectively) are colored blue. DES, colored green, is shown in space-filling representation. (b) *Two orthogonal views of the OHT-ERα LBD complex similar to those of the agonist complex in (a).* The LBD is depicted as a ribbon drawing. As in (A), helix 12 (residues 536–544) is colored in magenta, and helices 3, 4, and 5 are colored blue. OHT, in red, is shown in space-filling representation.

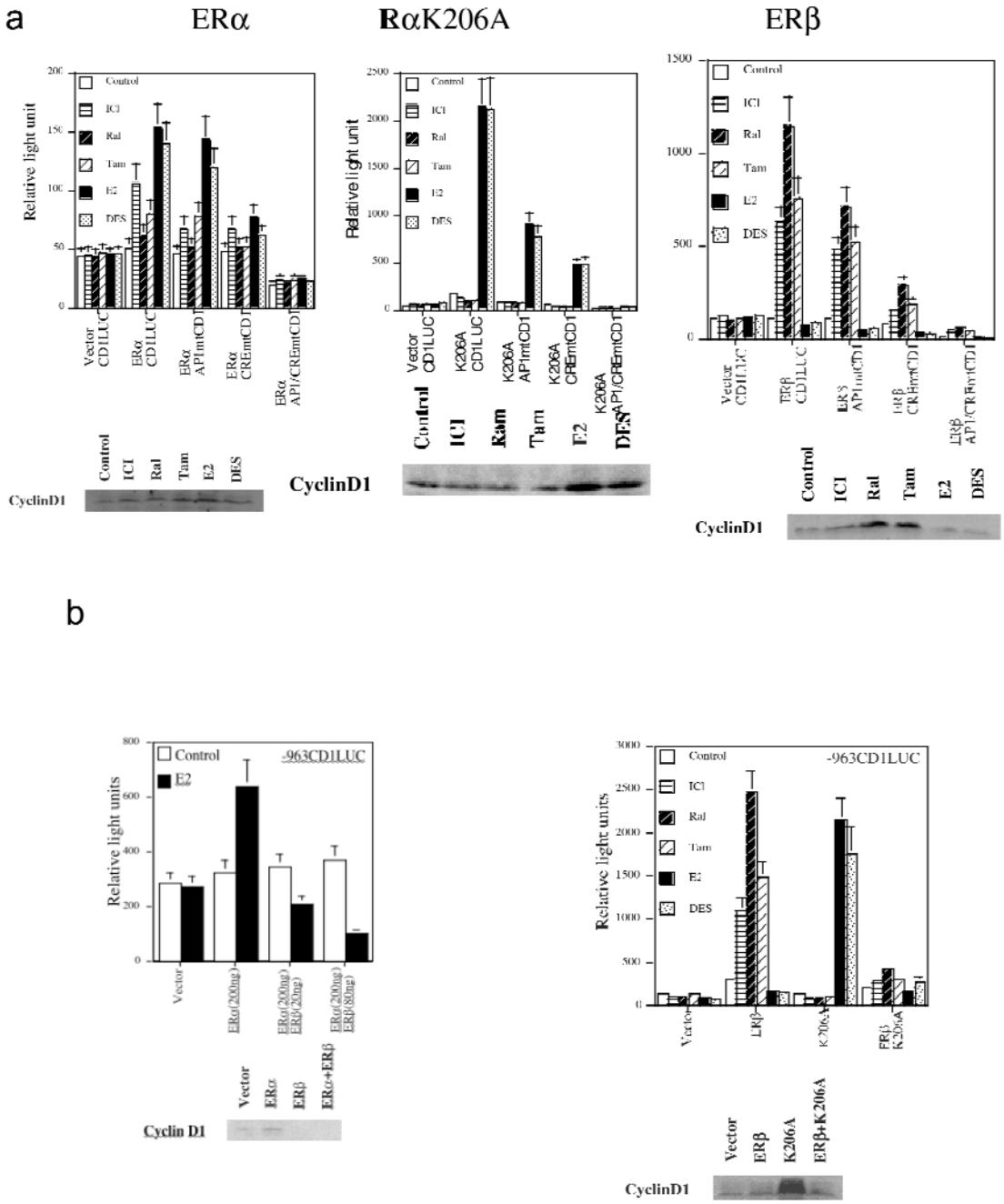

Fig. 3 *Opposing action of ERα and ERβ on the cyclin D1 promoter. (a) Opposite ligand preferences of ERα, the superactive ERαK206A versus ERβ.* The upper panels show the response to various ligands of the cyclin D1 promoter driving luciferase in HeLa cells with the indicated ER (left ERα, middle K206A, right ERβ). Mutants in the AP-1 element, CRE element, or both are also shown. The lower panel shows the response of the endogenous HeLa cell cyclin D1 gene by Westerns. *(b) ERβ inhibits ERα or even K206A activation of cyclin D1 with estrogen.* Upper panels show the response of the cyclin D1 promoter luciferase reporter gene to activated by ERα (on the left) or by K206A (on the right), and then inhibited by ERβ. The lower panels show endogenous HeLa cell cyclin D1 gene expression first activated by ERα or K206A and then inhibited by ERβ.

moxifen structure helix 12 of the receptor is in the space that is occupied by the NR boxes of the coactivator in the structure with the estrogen diethylstilbestrol (DES). In summary then, ERα and ERβ both activate at EREs, and each works by recruiting a CBP-p160 coactivator complex through AF-2.

ERα has a potent AF-1 in the first 109 amino acids that also works by contacting p160s, but through a different surface than AF-2 [70]. ERβ lacks this conventional AF-1 but seems to share with ERα an AF-1 that is inducible by MAP kinase cascades and subsequent phosphorylation in the NTD [22,64,66].

Corepressors also may modulate the actions of ERs especially in the presence of antagonist of AF-2 (antiestrogens). The corepressors N-CoR and SMRT bind directly to the ERα antiestrogen complexes and inhibit AF-1 action [19,27,78]. It is known that N-CoR and SMRT recognize the thyroid and other nuclear receptors through hydrophobic helices (called ID domains) which couple with the hydrophobic cleft in the LBD [68]. The binding is similar to that of coactivators except that the corepressor ID domains displace helix 12 and bind both to the newly exposed surface and the hydrophobic cleft [76]. A structure of the ERs with corepressors has not yet been solved, but genetic analysis suggests a similar mode of binding with key contacts underneath helix 12. Mutations of these contacts (L379) eliminates corepressor binding, whereas eliminating helix 12 increases N-CoR binding and allows all ligands to promote binding. Thus, while the molecular details are still unknown it appears that corepressors modulate ER action, especially AF-1.

ERα AND ERβ HAVE OPPOSING ACTION AT AP-1/CRE TARGET GENES, AND SEPARATE MODELS FOR THEIR ACTION ARE PROPOSED

The action of ERα and ERβ at AP-1 target genes could not be more different from their action at classical EREs. ERα activates AP-1/CRE target genes primarily with estrogen, just as it does at an ERE. In uterine cells ERα can also activate AP-1 targets to with tamoxifen, but not with raloxifene, or ICI [26,69,72]. Estrogen, however, is still the preferred ligand for ERα activation. ERβ, in contrast activates AP-1/CRE target genes with antiestrogens, especially with ICI and raloxifene, and not with estrogen [28,39]. The contrast between the ligand preferences for ERα and ERβ activation of the AP-1/CRE regulated cyclin D1 promoter is illustrated in Fig. 4. ERα also prefers estrogens and ERβ antiestrogens for activating retinoic acid receptor α-1 gene expression. Interestingly, the RARα promoter has no ERE, and the reported ERβ preference requires the integrity of Sp1 sites in the promoter [80].

These contrasting ligand preferences have suggested that ERα and ERβ might have opposing action on the expression of target genes that are regulated via alternative response elements such as AP-1/CRE sites and Sp1 sites [31,39]. This has been confirmed for cyclin D1 gene expression which is under the regulation of AP-1/CRE and Sp1 elements, and an example of whose regulation by different ligands is illustrated. Notice that whereas ERα activates cyclin D1 expression with estrogen, ERβ inhibits with estrogen (and activates only with antiestrogens) [28]. When both receptors are present ERβ efficiently blocks the activation by ERα. The observed opposing effects of ERα and ERβ on cyclin D1 gene expression are consistent with the reports noted above that suggest an inhibitory role for ERβ in estrogen-mediated cellular proliferation, a process in which cyclin D1 appears to play a central role.

Each of the ligands discussed here binds well to both ERα and ERβ. Thus, it is suggested that the dramatic difference in the ligand preference for activation at AP-1/CRE, and possibly Sp1, reflects different modes of action of the two liganded receptors [26]. ERα and ERβ do indeed have very different modes of action at AP-1/CRE [72]. The actions of ERα at AP-1 are primarily mediated by the activation functions AF-1 and AF-2 and their ability to dock to p160 coactivators. When the AF1 and AF-2 surfaces that contact p160 coactivators are disrupted by mutation, ERα cannot activate with estrogen. Increasing the levels of p160s increases the ability of ERα to activate with estrogen, whereas mutation of the p160 coactivator surfaces (the NR boxes and the AF-1 interaction domain) that contact ERα disrupts the ability to enhance ERα activation with estrogen. Moreover, tamoxifen response is completely

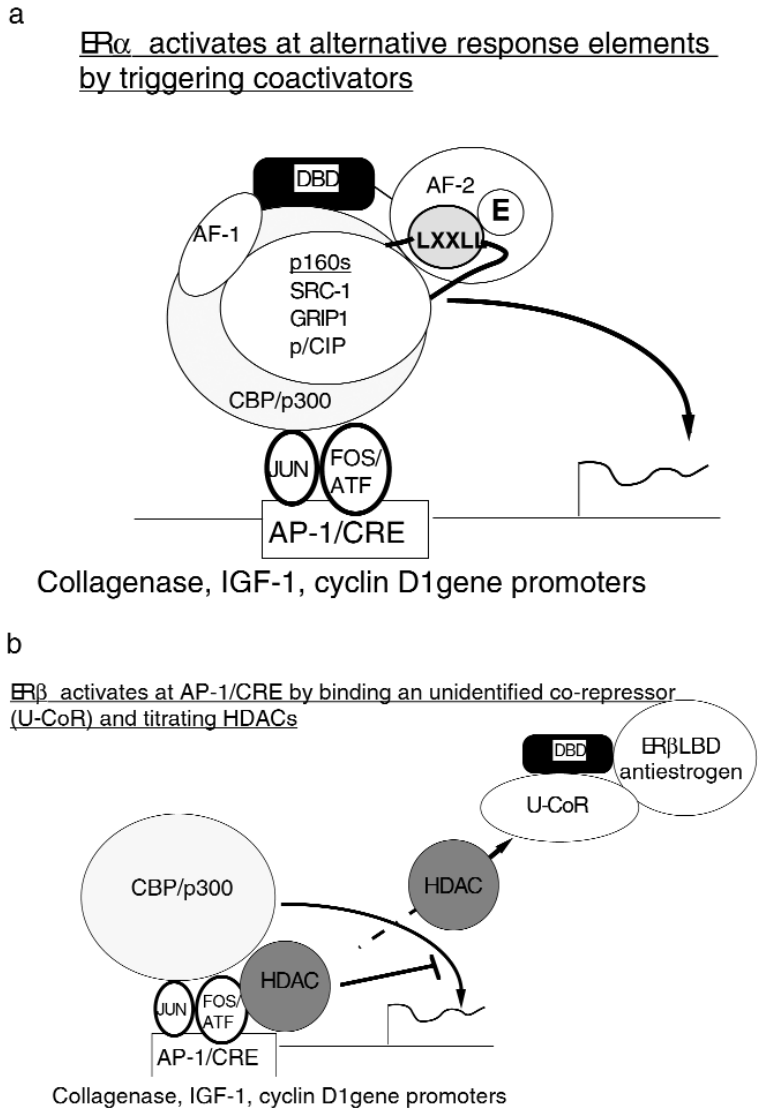

Fig. 4 *How ERs activate at AP-1/CRE. (a) ERα.* At AP-1/CRE sites, ERα uses its AF functions, to nucleate formation of a complex of CBP/p300 with p160s. The complex is tethered at the promoter by contacts with Jun/Fos (or in the case of the CRE with Jun/ATF-2). In the absence of ERα only CBP/p300 is recruited. *(b) ERβ.* ERβ activates at AP-1/CRE independently of AF functions by tethering to a sequestration site, binding N-CoR or some other unidentified co-repressor (U-CoR), and then titrating HDACs away from the promoter and thereby allowing full action of CBP/p300.

dependent on the integrity of the NTD, which harbors AF-1. The above studies suggest that ERα works at AP-1 via AF-1 and AF-2 contacts with p160s. Indeed, Myles Brown and colleagues have recently demonstrated with chromatin immunoprecipitation assays that both ERα and p160 coactivators are present at the IGF-1 (an AP-1-regulated gene ref), c-MYC (a non-ERE-regulated gene with Sp1 sites), and cyclin D1 promoters within a few minutes of estrogen addition [45,53]. Furthermore, in uterine but not in mammary cells, the p160 coactivator SRC3 is present at these non-ERE promoters after tamoxifen exposure [53].

ERβ activation with antiestrogens at AP-1/CRE and Sp1 targets, by contrast, is completely independent of AF function. ERβ does not have a conventional AF-1, and antiestrogens do not allow ERβ AF-2. Neither does mutation of the AF-2 surface of ERβ impede its robust ability to activate at AP-1 with antiestrogens [72]. The amino terminal domain of ERβ does contribute to activation with antiestrogens [67,80], but the nature of this contribution is unknown. Certainly, it is not a conventional AF-1 function.

As noted earlier, the primary structural difference between ERα and ERβ appears to be the presence of an AF-1 function in the amino terminal domain of ERα, and the absence of an AF-1 in ERβ. The presence or absence of an AF-1 function appears to be a key feature in controlling the ligand preference of ERs at AP-1. Thus, replacing the amino terminal domain of ERβ, with that of ERα completely prevents ERβ from activating with antiestrogens and allows it to activate with estrogens [67]. Similarly, the progressive loss of AF-1 function by deletion of the N-terminal domain of ERα progressively allows ERα to activate AP-1 with raloxifene and ICI [72]. Complete deletion of the N-terminal domain of ERα converts it into a pheno-copy of ERβ; ERα deltaAF-1 activates at AP-1/CRE sites only with raloxifene and ICI. These studies thus indicate that the presence of an efficient AF-1 prevents ERs from activating at AP-1 with antiestrogens such as raloxifene and ICI.

We propose that there are two different pathways whereby ERs act at AP-1/CRE sites [26,69,72]. The ERα pathway requires estrogens and AF functions and is illustrated in Fig. 2 below. We propose that the AP-1 element is bound by Jun/Fos and that ERα is not bound at the AP-1 DNA. Jun/Fos recruit their coactivator CBP/p300, which may also bring in a p160 such as GRIP1 (illustrated). ERα could join this complex through the AF-1 and AF-2 surfaces that contact GRIP1, or by direct contacts with Jun [44,62]. In doing so, ERα could either increase the amount of the CBP-p160 complex, or modulate its activity, thereby enhancing transcription. ERα, in brief, is a coactivator for Jun/Fos family proteins at AP-1/CRE sites.

Our model for ERβ action at AP-1 is completely different than that for ERα. We presume, although our evidence at the moment is indirect, that ERβ acts, not at the complex of proteins at the promoter, but at a sequestration site away from the promoter, for example, on bulk DNA. ERβ at the sequestration site and in the presence of antiestrogens such as raloxifene associates with an unidentified corepressor, possibly N-CoR or SMRT that we here call "U-CoR". U-CoR in turn binds histone deactylases (HDACs) which are titrated away from the AP-1 regulated promoter. Since HDACs in general counter the HAT activity of coactivators and repress transcription, their removal from the promoter leads to activation of transcription. Tests of this model are in progress.

ERα AND ERβ ACTION AT Sp1 SITES RESEMBLES, BUT IS SUBTLY DIFFERENT FROM ACTION AT AP-1/CRE ELEMENTS

Binding sites for the Sp1 transcription factor (GC-rich elements) are present in many estrogen response gene promoter regions especially those involved in regulation of proliferation: cyclin D1, E2F1, DNA polymerase, IGFBP4, telomerase, and possibly c-myc [51]. These elements cooperate with EREs, ERE half-sites, and binding sites for AP-1/CRE factors in promoting estrogen response. Moreover, simplified reporter genes with tandem Sp1 binding sites respond to estrogen. A particular interesting example of Sp1 contribution is in the cyclin D1 promoter where a tandem repeat of Sp1 elements cooperates with the CRE element to yield a full estrogen response [8]. ERα activates at Sp1 elements, but unlike activation at AP-1/CRE elements the activation is strongest in mammary cells and weak or nonexistent in HeLa and other uterine cells [52]. Furthermore, both estrogens and antiestrogens tend to activate. ERb, in contrast, inhibits expression at Sp1 elements with estrogen, and activates with antiestrogens [52,80].

The functions of ERα involved in activation of Sp1 elements are similar to those involved in activation at AP-1/CRE elements: the AF-1 domain plays a central role, whereas the ERE recognition function of the DNA-binding domain does not [20,52,69,73]. ERs and Sp1 bind to each other, and the

presence of ER can promote recruitment of Sp1 to GC-rich elements [8]. This promotion of binding to the GC-rich elements may underlie ERα activation. However, the complexities of ligand spectrum have suggested that ERs may also behave as coactivators and corepressors at the complex of proteins on the GC-rich elements [52].

ERα AND ERβ DOWNREGULATION OF TARGET GENES THAT HAVE BEEN ACTIVATED BY CYTOKINES

ERs also downregulate many genes as have become very evident with RNA expression micro-array studies [59]. A well studied instance is ERα and ERβ inhibition of TNFa action at so-called TNF response elements that are found in the promoters of many cytokine genes including TNF itself [3,4]. The NFκ-B, AP-1, and Ets1 transcription factors bind to these elements, but ERs do not. Instead, ERs appear to inhibit the ability of these factors to stimulate transcription via protein–protein interactions. Interestingly, inhibition requires the ability of ERs to interact with the p160 coactivators, which appear to play a key role in mediating inhibition, by a mechanism that is presently unknown. ERβ is especially efficient at the inhibition of the TNFa response, which is also produced by phytoestrogens. This may reflect a potential function of ERβ in suppression of inflammation. SERMs reverse the estrogen-dependent inhibition and, in fact, further activate transcription from this type of response element, much as they do at AP-1 sites.

SPECULATIONS ON THE PHYSIOLOGICAL RELEVANCE OF ANTIESTROGEN (SERM) INDUCTION OF TARGET GENES

As described above, SERMs can activate AP-1, SP-1, and EphRE-responsive genes in conditions in which estrogens either elicit a weaker response, or actively repress transcription. Why would a gene respond to an artificial compound that is not found in nature, yet not respond to the cognate receptor ligand? We envision at least two possible explanations. The ERs can be activated both by ligands and, in the absence of ligands, by second messenger inputs (see Topic 1.8). Perhaps SERMs create an ER conformation that resembles that of unliganded ERs and somehow mimics these ligand independent modes of activation. Second, it is possible that there are natural ligands that resemble SERMs and that SERM responses mimic a natural response to these unspecified ligands. One possibility is that the SERMs mimic natural steroid metabolites [79]. Alternatively, there may be unspecified sources of SERM-like compounds in the environment and SERM-dependent gene induction might represent a response to exposure to these environmental SERMs. In any of these cases, one of the implications of the findings presented above is that environmental ER interacting compounds could show estrogen-like or SERM-like activities. It is therefore necessary to monitor each of these activities.

IMPLICATION FOR DEVELOPING SYSTEMS TO MONITOR AND EVALUATE ENVIRONMENTAL ESTROGENS

It would be highly desirable to have a simple and uniform system to monitor environmental estrogens in a laboratory setting, and that would be some indication, even if imperfect, as to how the environmental estrogens would affect human health. Systems using reporter gene response in cultures of human cells expressing ERs are very attractive. The above considerations put a limit on how simple these systems can be. First, both ERα and ERβ must be included, because they have both different binding affinities for different ligands, and more importantly, different preferences for activation of transcription at some response elements. Thus, and secondly, both reporter genes with EREs, and reporter genes with alternative response elements will have to be included. The AP-1/CRE elements are among the most important alternative response elements, but for some purposes Sp-1 and NFkappaB reporter genes may have to be included as well. Of course, the development of RNA expression arrays with every gene rep-

resented is ideal in one way. But even complete microarrays do not substitute for the use of idealized reporter genes with simplified response elements, which gives information of its own sort.

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