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Structural and Functional Diversity of Estrogen Receptor Ligands

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Abstract

Estrogen receptors, comprised of ER α and ER β isoforms in mammals, act as ligand-modulated transcription factors and orchestrate a plethora of cellular functions from sexual development and reproduction to metabolic homeostasis. Herein, I revisit the structural basis of the binding of ER α to DNA and estradiol in light of the recent discoveries and emerging trends in the field of nuclear receptors. A particular emphasis of this review is on the chemical and structural diversity of an ever-increasing repertoire of physiological, environmental and synthetic ligands of estrogen receptors that ultimately modulate their interactions with cognate DNA located within the promoters of estrogen-responsive genes. In particular, modulation of estrogen receptors by small molecule ligands represents an important therapeutic goal toward the treatment of a wide variety of human pathologies including breast cancer, cardiovascular disease, osteoporosis and obesity. Collectively, this article provides an overview of a wide array of small organic and inorganic molecules that can fine-tune the physiological function of estrogen receptors, thereby bearing a direct impact on human health and disease.

Keywords

Estrogen receptors; Endoestrogens; Phytoestrogens; Xenoestrogens; SERMs; Metalloestrogens

1. Introduction

In mammals, estrogen receptor is expressed in two alternative isoforms designated ER α and ER β (1, 2). Together, these mediate a plethora of cellular functions from sexual development and reproduction to metabolic homeostasis. ER α and ER β are members of a family of ligand-modulated transcription factors that have come to be known as nuclear receptors (NRs) (3–6). As their name implies, the binding of ligand is a pre-requisite for the subsequent binding of NRs in a sequence-specific manner to their cognate DNA within the promoters of target genes. This mechanism of action is in sharp contrast to the binding of classical transcription factors to DNA, which are not dependent upon prior activation with a specific ligand.

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Notably, all members of NR family share a core modular architecture comprised of a central DNA-binding (DB) domain flanked between an N-terminal trans-activation (TA) domain and a C-terminal ligand-binding (LB) domain (7–9). A typical scenario for the activation of nuclear receptors, as schematically illustrated for ER α in Figure 1, involves the secretion of lipophilic messengers such as hormones and vitamins by appropriate tissues. Upon their diffusion through the cell membrane, the binding of these ligands to the LB domain culminates in a series of events involving the translocation of nuclear receptors into the nucleus and subsequent modulation of expression of target genes (10–12). While the DB domain recognizes specific promoter elements, the LB domain additionally serves as a platform for the recruitment of a multitude of cellular proteins, such as transcription factors, co-activators and co-repressors, to the site of DNA transcription and thereby allowing nuclear receptors to exert their action at genomic level in a concerted fashion (13, 14). While the trans-activation function of the LB domain is ligand-dependent, the TA domain operates in an autonomous manner and it is believed to be responsive to growth factors acting through the MAPK signaling and may further synergize the action of various co-activators and co-repressors recruited by the LB domain at the site of DNA transcription (15, 16). In this manner, nuclear receptors orchestrate a diverse array of cellular functions from embryonic development to metabolic homeostasis and their malfunction has been widely implicated in disease (7, 17–21).

First discovered more than half a century ago, ER α and ER β mediate the action of estrogens such as estradiol and their hyperactivation leads to the genesis of large fractions of breast cancer (22–28). In addition to breast cancer, estrogen receptors are also implicated in a plethora of other human pathologies including cardiovascular disease (29), osteoporosis (30), and obesity (31). While the physiological action of ER α is highly complex and involves multiple layers at both the genomic and non-genomic level, two major pathways by which ER α participates in the regulation of transcriptional machinery are the classical and the non-classical pathways. In the classical pathway, ER α binds to the promoters of estrogen-responsive genes containing the estrogen response element (ERE) through its DB domain in an estradiol-dependent manner. Remarkably, the ability of ER α to bind to the promoters of target genes in an estradiol-independent manner upon post-translational phosphorylation within the TA domain by kinases such as Cdk2 is also well-documented (32–34). Examples of ER α -responsive genes regulated by ER α through the classical pathway include Myc, Fos, cathepsin D and pS2 (27, 35–39). In the non-classical pathway, ER α regulates gene transcription without directly binding to DNA but in an estradiol-dependent manner. This is made possible by the fact ER α tethers to other transcription factors such as ATF2, Fos, Jun, Elk and SP1 already bound to their cognate elements within the promoters of their target genes and, in so doing, aids in the recruitment of a wide array of transcriptional co-regulators (27, 35, 40, 41). In this manner, ER α modulates expression of estrogen-responsive genes that do not contain an ERE motif within their promoters solely through protein-protein interactions. Examples of estrogen-responsive genes regulated by ER α through the non-classical pathway include Bcl2, ovalbumin, collagenase, cyclin D1 and IGF1 (42–46).

In this article, I revisit the structural basis of the binding of ER α to DNA and estradiol in light of the recent discoveries and emerging trends in the field of nuclear receptors. A

particular emphasis of this review is on the chemical and structural diversity of an ever-increasing repertoire of physiological, environmental and synthetic ligands of estrogen receptors that ultimately modulate their interactions with cognate DNA located within the promoters of estrogen-responsive genes. The role of these ligand-mediated interactions on human health and disease is discussed.

2. Structural basis of ER α -DNA interactions

Upon the binding of estradiol to the LB domain, ER α translocates to the nucleus and binds as a homodimer with a two-fold axis of symmetry to the estrogen response element (ERE), containing the AGGTCA α nnnTGACCT consensus sequence, located within the promoters of target genes (47, 48). DNA-binding is accomplished through a pair of tandem C4-type Zinc fingers located within the DB domain, with each finger containing a Zn²⁺ ion coordinated in a tetrahedral arrangement by four highly conserved cysteine residues to generate the Zn²⁺[Cys]₄ metal-protein complex (49, 50) (Figure 2a). Importantly, while the first Zinc finger (ZF-I) within each monomer of DB domain recognizes the hexanucleotide sequence 5'-AGGTCA-3' within the major groove at each end of the ERE duplex, the second Zinc finger (ZF-II) is responsible for the homodimerization of DB domain. It is noteworthy that environmental metals can exert dramatic effects on ER α -DNA interactions by virtue of their ability to replace the Zn²⁺ divalent ion within the zinc fingers of the DB domain (51–53). In particular, the DB domain of ER α reconstituted with divalent ions of zinc, cadmium, mercury and cobalt binds to DNA with affinities in the nanomolar range, while divalent ions of barium, copper, iron, lead, manganese, nickel and tin are unable to regenerate DB domain with DNA-binding potential though they can compete with zinc for coordinating the cysteine ligands within the zinc fingers.

Interestingly, recent work from our laboratory suggests that the binding of ER α to DNA is coupled to proton uptake by two ionizable residues, H196 and E203, located at the protein-DNA interface (54). Unsurprisingly, alanine substitution of these ionizable residues decouples protonation and hampers the binding of ER α to DNA by nearly an order of magnitude. This pH-dependent effect of ER α -DNA interaction is further illustrated via a thermodynamic cycle (Figure 2b). It is clearly evident from such a cycle that the binding of ER α to DNA may or may not be coupled to proton uptake depending on solution pH. Thus, under low pH values, ER α may become fully protonated prior to binding DNA. On the other hand, under high pH values, ER α may become fully unprotonated and the proton uptake may be decoupled for its binding to DNA, albeit with a much diminished affinity. Although the binding of DB domain of ER α to DNA appears to be coupled to proton uptake, it is not clear how such coupled equilibrium might dictate the physiological role of this important nuclear receptor. It is noteworthy that changes in intracellular pH regulate a multitude of cellular processes such as metabolic homeostasis and apoptosis (55). Furthermore, it is believed that ionizable residues within proteins sense such changes and activate a variety of proton pumps and ion transporters that in turn mediate extracellular transport of protons and anions to regulate intracellular pH (56–58). It is thus conceivable that changes in intracellular pH may also tightly regulate the transcriptional activity of ER α through direct modulation of two ionizable residues, H196 and E203, located at the protein-DNA interface. It should also be noted that H196 and E203 are predominantly conserved across ~50

members of the nuclear receptor family, implying that proton-coupled equilibrium may serve as a key regulatory switch for modulating protein-DNA interactions central to nuclear receptor function and regulation.

3. Structural basis of ER α -estradiol interactions

As noted above, the binding of estradiol to the LB domain represents a key priming step for the subsequent binding of ER α via its DB domain to gene promoters. Importantly, the LB domain of ER α binds to estradiol as a homodimer with a two-fold axis of symmetry, wherein each monomer is largely comprised of a triple-layered anti-parallel α -helical sandwich capped at one end by a small two-stranded anti-parallel β -sheet (20) (Figure 3). Within this α -helical sandwich, the central layer comprised of H5/H6/H9/H10 α -helices is flanked by one layer of H1/H2/H3/H4 α -helices on the left and one layer of H7/H8/H11 α -helices on the right as viewed from the direction of the sole β -sheet (S1/S2) or the C-terminal α -helix H12. Notably, the liganded and unliganded conformations of the LB monomers are very similar except for the spatial orientation and structural ordering of H12 helix relative to the rest of the helical-bundle. In the unliganded conformation (Figure 3a), the H12 helix flips outwards so as to act as a dynamic lid and make way for estradiol to enter into the deep interior hydrophobic pocket. Subsequent to estradiol entry, the repositioning of H12 helix within each monomer seals the pocket accommodating the ligand. Importantly, such ligand-induced trigger allows the entire LB domain to undergo structural re-arrangement to adopt a conformation that best “attracts” a diverse array of transcriptional co-activators (59–61). This model thus exquisitely explains how the binding of estradiol activates ER α for its role in transcriptional regulation.

In the liganded conformation (Figure 3b), estradiol is trapped between the three α -helical layers such that the S1-S2 β -sheet serves as the floor and H12 α -helix as the lid in what is often described as a “mouse trap” model of ligand binding to nuclear receptors (62). The recognition of estradiol is in part achieved through intermolecular hydrogen bonding involving the 3-OH of the A-ring in estradiol with carboxylate moiety of E353 and the guanidinium group of R394 as well as the 17 β -OH of the D-ring with imidazole sidechain of H524. Additionally, van der Waals contacts are established as a result of sandwiching of the A/B-ring interface of estradiol with A350 and L387 on one side and F404 on the other side as well as sandwiching of the D-ring with I424 on one side and L525 on the opposite side. In short, the liganded conformation of LB domain harbors no route for estradiol entry into the deep hydrophobic pocket within the interior of the protein constituted by various α -helices and capped by the sole S1-S2 β -sheet at one end and H12 α -helix at the other. To overcome this physical constraint, the binding of estradiol is coupled to conformational changes in an allosteric manner so as to allow ligand entry and its subsequent entrapment within the deep pocket completely shielded from the external sea of water.

4. Diversity of estrogen receptor ligands

In addition to naturally produced estrogens within the body, a diverse array of small organic and inorganic molecules serve as estrogen receptor (ER) ligands by virtue of their ability to recognize the LB domain in a specific manner. While majority of these ligands display

higher selectivity toward ER α , a number of ER β -selective compounds have also recently been discovered in spite of the rather high similarity between the ligand binding pockets of the two isoforms. It is important to note that ER α and ER β usually act in an antagonistic manner. For example, while the mitogenic action of ER α in breast tissue is responsible for cellular proliferation leading to the development of breast carcinoma, activation of ER β has an anti-proliferative effect in breast (63). In a similar manner, ER β is also believed to exert anti-proliferative effect on other tissues such as prostate, colon, lung, and brain (2, 64–68). Accordingly, the development of small molecule ligands that can selectively activate ER β hold promise for the treatment of indications such as cancers and neurological disorders (69–78). Below, a brief overview of the five major classes of ER ligands is provided.

4.1 Endoestrogens

Endoestrogens are physiological estrogens endogenously produced within the body. Examples include estradiol, estriol and estrone (Figure 4). Endoestrogens are steroidal compounds produced from cholesterol by the sex glands such as ovaries in females and testes in males among other organs such as the liver, brain and adrenal glands. While estradiol is the predominant form in both females and males, estriol is primarily produced during pregnancy and estrone during menopause. In addition to its role in sexual development and reproductive functions in females, estradiol is also involved in a plethora of other physiological functions including lipid metabolism, cardiovascular function and bone maintenance in both males and females (79–83). Endoestrogens are also constituents of oral contraceptives and hormone replacement therapy.

4.2 Phytoestrogens

Phytoestrogens are non-steroidal polyphenolic compounds naturally produced by plants. Three major groups are isoflavones, coumestans and lignans (Figure 5). Because they share chemical and structural similarities with estradiol, phytoestrogens can exert estrogenic or antiestrogenic effects by virtue of their ability to bind to the LB domain of estrogen receptors and block the binding of estradiol in a competitive manner (84). In particular, genistein, coumestrol and liquiritigenin appear to be more selective toward ER β by virtue of their ability to bind to its LB domain with much higher affinity compared to that of ER α (75, 85–87). Dietary sources rich in phytoestrogens include nuts, oilseeds, soy products, cereals, breads, legumes, vegetables and fruits (88). However, phytoestrogens are not considered dietary supplements as their lack in diet does not lead to any deficiency syndrome nor they play any essential biological function. Finally, the role of phytoestrogens in reducing the risk of diseases such as cancer, heart disease and osteoporosis remains controversial (89–94). On the contrary, it is believed that some phytoestrogens may be harmful to health, particularly if their intake is excessive.

4.3 Xenoestrogens

Xenoestrogens are comprised of a wide variety of non-natural synthetic chemical compounds with estrogenic effects. These can be broadly divided into five major categories: medicinal drugs, food additives, body cosmetics, environmental pesticides and industrial chemicals (Figure 6). Although medicinal drugs such as diethylstilbestrol (DES) and ethinyl estradiol were specifically developed to possess estrogenic activity, other xenoestrogens are

also believed to mimic the action of endoestrogens in humans and other animals and, as such, they can also interfere with cellular processes leading to serious implications on health (95–101). In particular, a large body of data suggests that there is a positive correlation between the uptake of xenoestrogens and the development of breast cancer in humans (102–107). There is also growing evidence that xenoestrogens may impact human male reproductive health (108–110). Because of their structural resemblance to endoestrogens, xenoestrogens are believed to exert their estrogenic effects by virtue of their ability to bind to the LB domain of estrogen receptors. It is noteworthy that synthetic non-steroidal compounds with preferential selectivity toward ER β have also been developed (2, 74, 75, 111).

4.4 SERMs

In the so-called ER-positive breast cancer, which accounts for nearly three quarters of all breast cancers, the estrogen receptors are overexpressed and the cancer heavily relies on continuous supplies of endoestrogens to develop. Among the first lines of defense against ER-positive breast cancer and other ER-related syndromes are a group of non-steroidal compounds that have come to be known as the selective estrogen receptor modulators (SERMs) (112). Examples include tamoxifen, raloxifene, clomifene, ormeloxifene and toremifene (Figure 7). What distinguishes SERMs from xenoestrogens is that they harbor functional duality in their ability to act both as agonists and antagonists of ER α in different tissues (113–115). For example, tamoxifen acts as an antagonist in breast but as an agonist in uterus. Thus, while tamoxifen is often the preferred choice of treatment for ER-positive breast cancer, it can also induce endometrial cell growth resulting in uterine cancer. On the other hand, raloxifene acts as an antagonist in both breast and uterus coupled with its agonistic action in bone, making it a preferred choice of therapy in postmenopausal women with osteoporosis. While SERMs are largely selective for ER α , synthetic steroidal analogs that can preferentially modulate ER β have also been reported (2, 74, 75, 116–119).

Importantly, while SERMs exert their antagonistic action by virtue of their ability to compete with estradiol (E2) for binding to a deep interior hydrophobic pocket within the LB domain of ER α , they do so via a distinct mechanism (120, 121) (Figure 8). Binding of E2 agonist induces a conformational change in the LB domain that results in the repositioning of H12 helix so as to enable it to seal the ligand binding pocket (Figure 8a). Additionally, such structural rearrangement ensures that the subsequent binding of transcriptional co-activators, such as TIF2, to the canonical LXXLL motif located within a binding cleft right above H12 helix is not sterically hindered. By sharp contrast, owing to their bulkier groups, the binding of SERMs such as 4-hydroxytamoxifen (OHT)—the active form of tamoxifen prodrug after liver metabolism—to the LB domain results in a slightly differential conformational change compared to that observed upon the binding of E2 (Figure 8b). This in part involves unwinding of H3 and H11 helices by about one turn upon the binding of OHT, thereby relieving the tension on H12 helix and allowing it to occupy the LXXLL binding cleft in lieu of acting as a “lid” on the ligand binding pocket. Accordingly, while the agonist-bound conformation of H12 helix promotes the binding of transcriptional co-activators (Figure 8a), the antagonist-bound conformation hampers the binding of co-activators in a competitive manner (Figure 8b).

How does this explain the antagonistic action of OHT in breast but its agonistic role in uterus? The ability of H12 helix to occupy two distinct positions within the LB domain in response to agonist versus antagonist binding is governed by dynamic equilibrium. In other words, the H12 helix bears the propensity to transiently resonate between these two distinct positions depending on the biological context. In the absence of co-activators, the H12 helix would predominantly occupy the LXXLL site upon OHT binding. As the cellular concentration of transcriptional co-activators rises, the H12 helix would become competitively displaced from the LXXLL site and re-engage at the agonist site, thereby enabling transcriptional activation of LB domain in response to OHT binding. It thus goes without saying that the ability of SERMs to act as agonists or antagonists arises from the differential ratios of transcriptional co-activators to co-repressors in different tissues (27, 122–124).

From a therapeutic perspective, the design of ER agonists should be such that their binding favors the orientation of H12 helix to occupy the agonist site within the LB domain in a manner akin to that induced by the binding of E2. This mode of binding is indeed shared by synthetic agonists such as diethylstilbestrol (DES) (121). On the other hand, the design of ER antagonists should be such that their binding reorients H12 helix to occupy the LXXLL site with high affinity within the LB domain so that it cannot be competitively displaced by rising cellular levels of transcriptional co-activators. As discussed above, this latter phenomenon is indeed widely adopted by SERMs such as tamoxifen and raloxifene (20, 121). Equally importantly, it should also be important to design or screen for new small molecules that could directly bind with high affinity to the LXXLL site in lieu of competing with E2 for binding to the interior hydrophobic pocket within the LB domain. Such novel antagonists may serve as more effective drugs for the treatment of ailments such as breast cancer. Regardless of their precise mechanism of action, a whole new plethora of SERMs is currently under development to modulate the action of ER α in numerous cellular activities. Unsurprisingly, however, clinical trials on many of these promising candidates, such as ERB-041 and ORG37663, have failed to live up to their therapeutic promise (125, 126).

4.5 Metalloestrogens

Unlike all of the ligands of estrogen receptors discussed above, small inorganic compounds in the form of heavy metal ions is rapidly emerging as a new class of ER ligands with estrogenic activity. Several lines of evidence suggest that heavy metal ions adsorbed from various environmental sources mimic the action of endoestrogens and can potentiate the transcriptional activity of estrogen receptors within the body (127–129). High levels of many of these heavy metal ions have indeed been detected in various tissues, including the breast, and there appears to be a direct correlation between their accumulation and the subsequent development and progression of cancer (104, 130, 131). Because of their estrogenic activity, these heavy metal ions have come to be known as metalloestrogens. Examples include a wide variety of cations such as those of aluminum (Al³⁺), antimony (Sb³⁺), barium (Ba²⁺), cadmium (Cd²⁺), chromium (Cr²⁺), cobalt (Co²⁺), copper (Cu²⁺), lead (Pb²⁺), mercury (Hg²⁺) and nickel (Ni²⁺), and anions such as arsenite (AsO₃³⁻), selenite (SeO₃²⁻) and vanadate (VO₄³⁻). Given the environmental contamination of air and water, food and drinks, cigarettes and medicines, and a plethora of cosmetic products with

such heavy metal ions coupled with the knowledge that the hyperactivation of estrogen receptors is linked to the genesis of large fractions of breast cancer (22–27, 104, 130), efforts are being undertaken to decipher the molecular basis of how metalloestrogens activate estrogen receptors. Several studies indicate that the metalloestrogens exert their effects by virtue of their ability to coordinate to specific amino acid residues within the LB domain of estrogen receptors so as to block the binding of estradiol in a non-competitive manner (129, 132–134). It is believed that metalloestrogens may employ various geometries to coordinate with specific residues in the LB domain in a manner akin to their coordination with other proteins (135).

5. Concluding Remarks

In addition to naturally produced estrogens within the body, the library of small organic and inorganic molecules identified as potential ligands of estrogen receptors over the past half century or so runs into hundreds and it is likely that there are thousands of other potential ER ligands that are yet to be discovered from a plethora of environmental and synthetic sources. On the basis of structure activity relationships (136, 137), it has been argued that despite chemical and structural diversity, all organic ligands of ER bind to the LB domain with a remarkably similar mechanism as follows: (1) Hydroxyl groups or similar moieties engage in hydrogen bonding with the LB domain in a manner akin to 3-OH and 17 β -OH moieties of estradiol; (2) The hydroxyl groups, if present, are usually separated by about 12 Å, the distance between 3-OH and 17 β -OH moieties of estradiol; and (3) A rigid hydrophobic planar ring structure mimicking estradiol is usually present. Additionally, a set of hierarchical rules for the identification of novel potential organic ligands of ER have also been developed as summarized here: (1) If a chemical contains no ring structure or a non-aromatic ring structure that does not contain O, S, N, or other heteroatoms for H-bonding, then it is unlikely to be an ER ligand; (2) If a chemical has a non-OH aromatic structure, then it could serve as an ER ligand provided other structural requirements are met; and (3) If a chemical contains a phenolic ring, then it tends to be an ER ligand if it contains any additional structural features similar to estradiol. However, given that a wide spectrum of heavy metals ions can also serve as potential ER ligands, the above set of rules may be of little use in identifying novel ligands of estrogen receptors.

In sum, this review has revisited the structural basis of the binding of ER α to DNA and estradiol in light of the recent discoveries and emerging trends in the field of nuclear receptors. Additionally, I have also provided herein an overview of the chemical diversity of ER ligands and how they employ distinct mechanisms to recognize estrogen receptors. Modulation of estrogen receptors by small molecule ligands represents an important step toward the treatment of a wide variety of human pathologies including breast cancer (22–28), cardiovascular disease (29), osteoporosis (30), and obesity (31). In particular, development of drugs that can selectively activate ER β over ER α would be highly desirable for combating estrogen-related disorders. Toward this goal, clinical trials on MF101 for the treatment of hot flashes in menopausal women show a great deal of promise for the future development of ER β -selective therapy (138, 139). Further research is thus clearly warranted to not only identify highly selective small molecule ligands of ER α and ER β but also to advance our molecular understanding of how they modulate estrogen receptors.

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Abbreviations

DB	DNA-binding
ER	Estrogen receptor
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
ERE	Estrogen response element
LB	Ligand-binding
MAPK	Mitogen-activated protein kinase
NR	Nuclear receptor
SERM	Selective estrogen receptor modulator
TA	Trans-activation
ZF	inc finger

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Figure 1.

A schematic illustrating the activation of estrogen receptor (ER α) by estradiol (E2). ER α is comprised of the core TA-DB-LB modular architecture that is also shared by other members of the nuclear receptor family, where DB is the central DNA-binding (DB) domain flanked between an N-terminal trans-activation (TA) domain and a C-terminal ligand-binding (LB) domain. Notably, ER α exists in dimer-monomer equilibrium in the cytoplasm in association with heat shock proteins (HSPs). Upon its diffusion through the plasma membrane, E2 binds to the LB domain and shifts the monomer-dimer equilibrium in favor of the latter allowing ER α to translocate into the nucleus where it binds to estrogen response element (ERE) element within the promoters of target genes via the DB domain, thereby leading to the recruitment of transcriptional machinery.

Figure 2.

Structural basis of DNA-binding to ERα. (a) Structural model of the DB domain of ERα as a homodimer in complex with ERE duplex containing the AGGTCAcagTGACCT consensus sequence. One monomer of the DB domain is colored green (MonoA) and the other in blue (MonoB). The Zn²⁺ divalent ions are depicted as gray spheres. The DNA backbone is shown in yellow and the bases are colored gray for clarity. The structural model was built using the MODELLER software based on homology modeling (140). The crystal structure of DB domain of ERα in complex with a dsDNA oligo containing the ERE motif but with varying flanking sequences, as determined by Rhodes and co-workers (49), was used as a template (PDBID 1HCQ). The structure was rendered using RIBBONS (141). (b) A thermodynamic cycle for the various equilibria linked to the binding of ERα to DNA: (I) ERα becomes protonated in the free form and the resulting protonated form (P) binds to DNA; (II) ERα binds to DNA in the unprotonated form (U) and the resulting liganded form becomes protonated.

Figure 3.

Structural models of unliganded (a) and liganded (b) LB domain of ER α in complex with estradiol (E2). In each case, only one monomer of LB domain is shown in gray with the C-terminal H12 helix colored yellow for contrast. E2 is depicted as a ball-and-stick model with various atom types and associated bonds shown in green (C) and red (O). In (a), the H12 helix is partially unstructured and displaced resulting in the disorganization of the ligand binding pocket in the unliganded conformation. In (b), subsequent binding of E2 induces the formation of ligand binding pocket, wherein the H12 helix winds up by an extra turn and undergoes $\sim 180^\circ$ rotation about an axis along the H11 helix. Such structural rearrangement allows H12 helix to block the mouth of the pocket accommodating E2 in what has come to be known as the “mouse trap” model. In both (a) and (b), the structural models were built using the MODELLER software based on homology modeling (140). For the unliganded conformation (a), the crystal structure of the LB domain of PPAR γ nuclear receptor, as determined by Milburn and co-workers (142), was used as a template (PDBID 1PRG). It should be noted that the ligand-binding pockets within each monomer of PPAR γ are structurally disordered. For the liganded conformation, the crystal structure of the LB domain of ER α in complex with E2, as determined by Carlquist and co-workers (20), was used as a template (PDBID 1ERE). It is noteworthy that a portion of the H9-H10 loop is missing in the crystal structure of ER α bound to E2. The structures were rendered using RIBBONS (141).

Figure 4.
Chemical structures of endoestrogens estrone (E1), 17β-estradiol (E2) and estriol (E3).

(a) Isoflavones **(b) Coumestans** **(c) Lignans** **Figure 5.**

Chemical structures of three major groups of phytoestrogens. (a) Isoflavones include genistein, daidzein, formononetin and glycitein. (b) Coumestans include coumestrol, repensol and trifoliol. (c) Lignans include lariciresinol, matairesinol, pinoresinol, secoisolariciresinol, podophyllotoxin and steganacin.

(a) Medicinal drugs

(b) Food additives

(c) Body cosmetics

(d) Environmental pesticides

(e) Industrial chemicals

Figure 6.

Chemical structures of five major groups of xenoestrogens. (a) Medicinal drugs include diethylstilbestrol and ethinyl estradiol. (b) Food additives include butylated hydroxyanisole and erythrosine. (c) Body cosmetics include camphors such as 4-methylbenzylidene camphor and parabens such as methylparaben, ethylparaben and propylparaben. (d) Environmental pesticides include atrazine, dichlorodiphenyldichloroethylene, dichlorodiphenyltrichloroethane, methoxychlor, dieldrin, endosulfan, heptachlor and lindane. (e) Industrial chemicals include phenols such as bisphenol A and nonylphenol, biphenyls such as monochlorobiphenyl and dichlorobiphenyl, and phthalates such as di-2-ethylhexyl phthalate, diisodecyl phthalate and diisononyl phthalate.

Figure 7. Chemical structures of SERMs. Examples include tamoxifen, raloxifene, clomifene, ormeloxifene and toremifene.

Figure 8.

Crystal structures of LB domain of ER α in agonist-bound (a) and antagonist-bound (b) conformations. In each case, only one monomer of LB domain is shown in gray with the C-terminal H12 helix colored yellow for contrast. In (a), LB domain is bound to estradiol (E2) and a short helical peptide (colored brown), which is derived from the TIF2 transcriptional co-activator and harbors the canonical LXXLL motif. E2 is depicted as a ball-and-stick model with various atom types and associated bonds shown in green (C) and red (O). In (b), LB domain is bound to 4-hydroxytamoxifen (OHT), depicted as a ball-and-stick model with various atom types and associated bonds shown in green (C), red (O), and blue (N). Note the similarity in the mode of docking of TIF2 peptide to LB domain in response to E2 agonist (a), and that adopted by H12 helix in response to OHT antagonist (b). In (b), the red arrows indicate the unwinding of H3 and H11 helices by about one turn upon the binding of OHT. The structures shown in (a) and (b) were respectively rendered from atomic coordinates provided by PDB codes 1GWR and 3ERT using RIBBONS (141).