Review

A structural perspective on nuclear receptors as targets of environmental compounds

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Nuclear receptors (NRs) are members of a large superfamily of evolutionarily related transcription factors that control a plethora of biological processes. NRs orchestrate complex events such as development, organ homeostasis, metabolism, immune function, and reproduction. Approximately one-half of the 48 human NRs have been shown to act as ligand-regulated transcription factors and respond directly to a large variety of endogenous hormones and metabolites that are generally hydrophobic and small in size (eg, retinoic acid or estradiol). The second half of the NR family comprises the so-called orphan receptors, for which regulatory ligands are still unknown or may not exist despite the presence of a C-terminal ligand-binding domain, which is the hallmark of all NRs. Several chemicals released into the environment (eg, bisphenols, phthalates, parabens, etc) share some physicochemical properties with natural ligands, allowing them to bind to NRs and activate or inhibit their action. Collectively referred to as endocrine disruptors or endocrine-disrupting chemicals (EDCs), these environmental pollutants are highly suspected to cause a wide range of developmental, reproductive, neurological, or metabolic defects in humans and wildlife. Crystallographic studies are revealing unanticipated mechanisms by which chemically diverse EDCs interact with the ligand-binding domain of NRs. These studies thereby provide a rational basis for designing novel chemicals with lower impacts on human and animal health. In this review, we provide a structural and mechanistic view of endocrine disrupting action using estrogen receptors α and β , (ER α/β), peroxisome proliferator activated receptor γ (PPAR γ), and their respective environmental ligands as representative examples.

Keywords: nuclear receptors; endocrine disruptor; environmental pollutant; transcription misregulation; crystal structure

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Introduction

The combination of a large number of molecular, structural, and genetic studies has provided a comprehensive view on the actions of nuclear receptors (NRs) — from the basic molecular events to the effects of receptor ablation on animal development and physiology^[1,2]. NRs are a family of transcription factors that regulate cognate gene networks, resulting in profound physiological changes. Consequently, dysfunctional NR signaling leads to proliferative, reproductive, and metabolic diseases, such as cancer, infertility, obesity, or diabetes. NR-based pharmaceuticals are among the most commonly used drugs. The 48 NRs are modular proteins organized into three major functional domains, namely (i) a variable and intrinsically unfolded N-terminal A/B domain harboring the transcriptional activation function 1 (AF-1), (ii) a

conserved DNA-binding domain (DBD), and (iii) a C-terminal ligand-binding domain (LBD) hosting the activation function 2 (AF-2)^[3]. The crystal structures of several NR LBDs have been determined, revealing a conserved core of 12 α -helices and a short two-stranded antiparallel β -sheet arranged into a three-layered sandwich fold generating a predominantly hydrophobic cavity that may accommodate ligands. Ligand binding relies on the intrinsic dynamics of the small molecule and of the ligand-binding pocket (LBP) that allows a mutual adaptation of both partners^[4–6]. The structures also showed that depending on the nature of the bound ligand, this domain can adopt two predominant conformations, which differ primarily in the position of the C-terminal helix H12^[7] (Figure 1). Upon binding of an agonist (the natural ligand or a synthetic compound mimicking its action), H12 adopts a stable active position, which allows the receptor to interact with transcriptional coactivators. The interaction surface of the receptor is built up from amino acids residing in the helices H3, H4, and H12, which form a predominantly hydrophobic groove

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Figure 1. The position of helix H12 determines receptor activity. Upon binding of an agonist molecule, H12 adopts a stable active position, which allows the receptor to interact with transcriptional coactivators (CoA) (active form, bottom left). Binding of an antagonist ligand redirects H12 to the coactivator binding site, precluding coactivator interaction (inactive form, bottom middle). Inverse agonist compounds stabilize the interaction with corepressors (CoR) (repressive form, bottom right). Molecular representations were generated using the PyMOL software (<http://www.pymol.org/>).

designed to recognize small LxxLL helical motifs present in coactivators. By contrast, binding of an antagonist (generally obtained by chemical synthesis) prevents the positioning of H12 in the active conformation and redirects the helix to the coactivator binding site, where it adopts an inactive conformation and precludes coactivator interaction. Further biophysical analyses revealed that partial agonists/antagonists correspond to a third class of NR ligands that fail to stabilize either of the aforementioned conformations^[8]. In the presence of partial agonists/antagonists, the conformational dynamics of H12 remains high; therefore, the activity profile of these compounds largely depends on the relative abundance of coactivators and corepressors in a cell. They act as cell-selective modulators with agonistic or antagonistic properties according to the cellular context. The fourth class of NR ligands gathers compounds that stabilize the interaction with corepressors and are thus referred to as inverse agonists^[9–11]. Thus, NRs should be viewed as molecular dimmers whose activity can be finely tuned by natural, pharmaceutical, or environmental ligands displaying specific chemical features. It is worth noting that while NR ligands regulate AF-2 activity through direct binding to LBDs and allosteric conformational changes, they also modulate AF-1 activity through domain-domain (Nt-Ct) inter-

actions.

Numerous synthetic substances released into the environment through human activities have been shown to mimic or interfere with the action of endogenous hormones and act as endocrine-disrupting chemicals (EDCs), causing reproductive, developmental, metabolic, or neurological diseases, as well as hormone-related cancers^[12–14]. Several EDCs are man-made compounds, such as bisphenols (BPs), phthalates, parabens, dioxins, pesticides, alkylphenols, organotins, polychlorinated biphenyls, and perfluoroalkyl compounds. Additionally, some natural EDCs can be found in plants and fungi. For more than 20 years, laboratory animals and epidemiological studies have highlighted the pivotal role of NRs in transducing several adverse effects of these chemicals. In addition to the *bona fide* endogenous ligands, the predominantly hydrophobic pocket enclosed in the LBD of NRs can accommodate small lipophilic exogenous compounds with affinities ranging from sub-nanomolar to micromolar values. Certain compounds, such as bisphenol-A (BPA), benzyl-butyl-phthalate (BBP), 4-tert-octylphenol (4-OP), butylparaben (BPB), or chlordecone (CLD), have been reported to activate the estrogen receptors (ER α and β) and act as antagonistic ligands of the androgen receptor (AR). The xenobiotic receptor PXR (pregnane X

receptor) is the target of numerous environmental compounds and natural molecules. However, the affinity of this receptor for chemicals is generally relatively low (micromolar range). The peroxisome proliferator activated receptor γ (PPAR γ) binds to and is activated by molecules such as brominated or chlorinated derivatives of BPA (TBBPA, TCBPA), perfluorinated compounds (PFOS and PFOA), and certain phthalates, such as the mono-ethylhexyl phthalate (MEHP). Several other NRs are responsive to environmental compounds, including the progesterone (PR), glucocorticoid (GR), mineralocorticoid (MR), thyroid hormone (TR α and β), constitutive androstane (CAR), and retinoid X (RXR α , β , and γ) receptors, as well as the peroxisome proliferator activated receptor α (PPAR α) or the estrogen-related receptor γ (ERR γ)^[15]. Considering the conservation of the LBD throughout the NR family and the huge chemical diversity of environmental compounds, all NR family members, including the orphan receptors, represent potential targets of environmental contaminants.

We and other groups have demonstrated the importance of structural studies to reveal the molecular details of the interaction between NRs and compounds structurally and chemically divergent from natural ligands^[15]. They show that environmental pollutants bind to NRs via diverse sets of protein-ligand interactions, thus reflecting their differential activities, binding affinities, and specificities. In this review, a detailed analysis of the various binding/activation mechanisms will be presented using ER α / β and PPAR γ as representative examples. As described below, a large set of environmental ligands has been identified for these NRs.

The estrogen receptors and their environmental ligands

The estrogen receptors in health and disease

Estrogen signaling is primarily mediated by the two estrogen receptors: ER α (also called NR3A1) and ER β (also called NR3A2)^[16, 17]. Similar to most NRs, ERs bind as dimers to DNA response elements in the promoter region of the target genes and respond to the naturally occurring sex hormone 17 β -estradiol (E₂; Table 1) and its metabolites, estrone and estriol, through interaction with coregulator complexes and regulation of target gene expression. The amino acid sequence identity between ER α and ER β is approximately 97% in the DBD and approximately 56% in the LBD, whereas the N-terminus is poorly homologous at 24%. Transcriptional activation by ERs is mediated by the two distinct activation functions AF-1 and AF-2, the relative importance of which depends on cellular and promoter contexts. Both ERs are widely expressed throughout the body, but present different tissue distributions and functions^[18, 19]. ER α is primarily expressed in the uterus, liver, kidney, and heart, whereas ER β is expressed primarily in the ovary, prostate, lung, gastrointestinal tract, bladder, and hematopoietic and central nervous systems. However, ER α and ER β are co-expressed in a number of tissues, including the mammary, thyroid, and adrenal glands, the bone, and some regions of the brain. Although ER α and ER β share similar mechanisms of action, several differences in the transcriptional abilities of each receptor and distinct phenotypes between

gene-null animals have been identified, suggesting that these receptors may regulate distinct cellular pathways. The respective functional roles of ER α and ER β in physiology and disease might result from a complex interplay between the expression levels of each ER, the relative affinity for a specific response element, ligand and cofactor availability, and interaction with other transcription factors. Additionally, ERs can be activated through post-translational modifications and can perform non-genomic signaling^[20].

The primary function of estrogens and their receptors is to regulate female reproduction. They play key roles in the growth and maintenance of the mammary gland and the uterus, promote the formation of female secondary sex characteristics, regulate reproductive cycles, and affect sexual and maternal behavior. In addition, ERs have pleiotropic regulatory roles in a diverse range of tissues, such as the cardiovascular or central nervous systems, bone, prostate, and adipose tissue. They are involved in the regulation of bone density, blood lipid levels, fat deposition, and brain functions^[19]. The importance of ERs in physiology is evidenced in menopausal women, whose endogenous estrogen levels decrease, which then increases the risk of osteoporosis, cardiovascular diseases, and brain disorders (Alzheimer's and Parkinson's diseases). Furthermore, in addition to controlling the normal development and function of the reproductive system and other tissues, estrogens are key regulators of primary breast and prostatic cancer growth^[17]. Approximately 40% of human cancers require steroid hormones for their growth, and the first-line of treatment for hormone-dependent cancers is based on androgen and estrogen antagonists that interact with AR or ERs and shut down the corresponding hormone-responsive pathway. ER β has been shown to antagonize the effects mediated by ER α on cell proliferation in the breast, uterus, ovary, and prostate^[21–23]. In this regard, estrogens with selectivity for either ER subtypes may produce different biological outcomes, particularly on cancer cell proliferation. Considering the widespread role of ERs in human physiology, it is not surprising that environmental compounds that bind to ERs (thereby substituting for the endogenous hormone and deregulating the finely tuned action of E₂) can lead to ER-related disorders. These include breast, endometrial, colorectal, and prostate cancers, as well as neurodegenerative, inflammatory, immune, cardiovascular, and metabolic diseases. In this review, we describe the mechanisms by which compounds that are structurally divergent from natural estrogens and that belong to families representing pollutants bind to ERs and impact their signaling pathways.

Environmental estrogens

A large group of structurally diverse compounds with different binding modes

Cell-based assays and laboratory studies on animals have revealed that the group of molecules acting as ER environmental ligands (xenoestrogens) is highly heterogeneous and includes both natural and synthetic compounds (Table 1). Naturally occurring xenoestrogens, such as phyto-estrogens

Table 1. Structures of ER α and ER β natural and environmental ligands.

PDB	Ligands	PDB	Ligands
ERα		ERα	
1ERE ^[85] / 3UUD ^[29]	Estradiol (E ₂)	1X7R ^[86] / 2QA8 ^[87]	Genistein (GEN)
2QXM ^[87]	PhIP	2QSE ^[87]	4-OH-PhIP
3UU7 ^[29]	Bisphenol-A (BPA)	4MGB ^[24]	Tetrachlorobisphenol-A (TCBPA)
3UUA ^[29]	Bisphenol-AF (BPAF)	4MGD ^[24]	Hydroxyphenyl-trichloroethane (HPTE)
3UUC ^[29]	Bisphenol-C (BPC)	4MGC ^[24]	Benzophenone-2 (BP-2)
4MG8 ^[24]	α -Zearalanol (α -ZA)	4MG5 ^[24]	Chlordecone (CLD)
4TUZ ^[24]	α -Zearalenol (α -ZE)	4MGA ^[24]	4-Tert-octylphenol (4-OP)
4MG7 ^[24]	Ferutinine (FER)	4MG6 ^[24]	Benzyl-butyl-phthalate (BBP)
4MG9 ^[24]	Butylparaben (BPB)	4PP6 ^[27]	Resveratrol (RES)
4TV1	Propylparaben (PPB)	3ERD ^[88]	Diethylstilbestrol (DES)
ERβ		ERβ	
3OLS ^[89]	Estradiol (E ₂)	1X7J ^[86] / 1QKM ^[26]	Genistein

(eg, genistein and ferutinine) and myco-estrogens (eg, zearalenone) are found in plants and fungi, whereas synthetic estrogens comprise both pharmaceuticals, such as ethinylestradiol (EE2; an active component of contraceptive pills) or diethylstilbestrol (DES; used until the 1970s to prevent miscarriage in women with high risk pregnancies) and a variety of industrial chemicals, such as alkylphenols, bisphenols, and their halogenated derivatives, parabens, phthalates, or benzophenones. The pharmaceuticals and some natural estrogens are the ligands that bind to ERs with the highest affinity, with dissociation constant (K_d) values in the (sub)nanomolar range^[24]. For example, DES and α -zearalanol, a metabolite of zearalnone, display an affinity of 0.1–0.4 nM for ERs. Industrial compounds, by contrast, exhibit a significantly lower affinity for ERs, with K_d values ranging from 10 nM to 10 μ M^[24]. Representative examples are the plasticizers bisphenol-C (BPC; 20–40 nM) and bisphenol-A (BPA; 0.5–1.0 μ M), the UV filter benzophenone-2 (BP-2; 0.1–0.5 μ M), and the pesticide chlordecone (CLD; $\sim$ 5.0 μ M). Transactivation and binding assays demonstrated that most xenoestrogens bind to both ER subtypes with similar affinities; however, genistein (GEN) binds slightly more strongly to ER β than ER α ^[25, 26].

Crystallographic studies have revealed that in the ERs, the hormone binding pockets are lined with $\sim$ 18 hydrophobic residues that interact with the steroid scaffold of E₂. Three polar residues located at the two ends of the pockets form hydrogen bonds with the phenolic and hydroxyl groups at the 3- and

17-positions of estrogen (Table 1). These polar residues are His524 (human ER α numbering) in helix H11 on one side and Glu353 (H3) and Arg394 (H5) on the other side (Figure 2A). Thus, the affinity and selectivity of hormone binding derives from both shape of the hydrophobic portion of the pocket and the presence of receptor-specific hydrogen-bond networks. Xenoestrogens exhibit a large structural diversity and vary both in size and chemical features. Several compounds contain two phenolic rings that mimic the A- and D-rings of E₂ (Table 1). Accordingly, these ligands adopt a binding mode reminiscent of that used by E₂, with the two phenol groups hydrogen-bonded to the polar residues of the LBP, as illustrated by BP-2^[24] (Figure 2B) and the phytoestrogen resveratrol^[27] (RES, Figure 2C). The remaining contacts essentially involve van der Waals interactions, the number of which varies from one compound to another and accounts for the variation in binding affinities of the ligands. Another group of estrogenic ligands do not interact with His524 because they either lack a second hydroxyl group, such as ferutinine (FER), the surfactant 4-OP, dichlorodiphenyl-dichloroethylene (DDE), a metabolite of the pesticide DDT, and the preservatives butyl (BPB)- and propyl (PPB)-parabens (Figure 2D), or because they adopt a position that draws this hydroxyl moiety toward Thr347 in H3, such as BPC and *bis*-hydroxyphenyl-trichloroethane (HPTE), a metabolite of the pesticide methoxychlor (Figure 2E). Lastly, some chemicals, such as CLD or benzyl-butyl-phthalate (BBP), have no hydroxyl groups and display weak chemical proximity

Figure 2. Xenoestrogens use diverse binding modes. Close-up views of the ligand-binding pocket of ER α in complex with estradiol (A), benzophenone-2 (B), resveratrol (C), propylparaben (D), HPTE (E), and benzyl-butyl-phthalate (F). Ligands and side chains of residues interacting with them are displayed in stick representation. The three key interacting residues Glu353, Arg394, and His524 are in blue, hydrophobic residues in light orange, and Thr347 in magenta. Oxygen, nitrogen, and chlorine atoms are displayed in red, blue, and green, respectively, and hydrogen bonds are indicated with dashed lines.

with E₂. They are not engaged in any direct interaction with either of the polar residues (Figure 2F).

A wide array of activities associated with ER α and ER β

In contrast to binding affinities, the activity of xenoestrogens depends on the receptor subtype and varies drastically among molecules that range from full agonists to weak agonists/antagonists. When bound to NR LBDs, an agonist (eg, the natural hormone E₂) is involved in a multitude of interactions that stabilize key residue conformations, which are required to hold the activation helix H12 in the active position (Figure 1). Compounds that are engaged in sub-optimal interactions with surrounding residues and fail to stabilize the correct conformers will act as partial agonists or antagonists. This is the case for most ER environmental ligands: they are structurally diverse and often lack several chemical features of E₂. Only a few of them mimic the natural hormone through well-conserved protein-ligand contacts.

BPs form a large family of chemicals that are commonly

used in the manufacture of numerous consumer products. The most widely used BP is BPA (>3 million tons/year), which is found in plastics, food can linings, dentistry sealants, and thermal paper. Several other BPs are used in a variety of industrial applications, eg, BPC (Table 1) is used in the production of fire-resistant polymers. Many studies have revealed large contamination in the general population through food, drinking water, and skin absorption, with a direct correlation between BP exposure and the occurrence of reproductive, developmental, and metabolic diseases (obesity and diabetes) being highly suspected^[28]. Cell-based assays revealed that BPA partially activates luciferase expression in MCF-7 and HeLa cells (inducing 60%–80% of the transactivation seen with E₂) and triggers cell proliferation in a dose-dependent manner, albeit at a concentration higher than that of E₂^[29]. The crystal structure of BPA-bound ER α LBD exhibits the active form of the receptor with H12 capping the LBP. BPA adopts a binding mode reminiscent of that used by E₂ with the two phenol rings hydrogen-bonded to His524, Glu353, and Arg394 (Fig-

Figure 3. Structural determinants of xenoestrogens activity. Interaction network of bisphenol-A (A, in green) and bisphenol-C (B, in pink) with residues of the ER α ligand-binding pocket compared to that of estradiol (in teal), showing the agonist and antagonist position of bisphenols. Structure superposition of E₂-bound ER β LBD (in teal) with ferutinine-bound ER α LBD (C, in orange) or chlordecone-bound ER α LBD (D, in yellow). The presence of I373 in ER β instead of M421 in ER α will induce a shift of bulky ligands towards helix H12, thus lowering the stability of AF-2. Oxygen, nitrogen, sulfur, and chlorine atoms are shown in red, blue, yellow, and green, respectively, and the interactions are indicated with dashed lines.

ure 3A)^[29]. The remaining contacts involve 42 van der Waals interactions. Compared to the E₂-containing structure, the smaller number of interactions accounts for the weaker affinity of BPA. The structure also reveals that key stabilizing interactions are missing in the ER-BPA complex, which possibly renders H12 more dynamic and accounts for the partial agonistic character of BPA. These missing or unfavorable contacts primarily involve H11 residues, including Gly521, His524, and Leu525 (Figure 3A). Because of their bulky chlorine atom substituents, the two bisphenols BPC and the closely related HPTE (Table 1) adopt a different binding mode compared to BPA^[24, 29]. A rotation of 180° around the main axis of phenol A-ring orients ring B toward H12 forming a new hydrogen bond with Thr347 in H3 (Figure 3B). In agonist-bound structures, this residue is involved in a network of van der Waals interactions. Thr347, Leu525, and Leu536 hold together H3, H11, and H12, three main components of the AF-2 function (Figure 3A). The 180° rotation of Thr347 in the BPC and HPTE complexes disrupts the stabilizing hydrophobic cluster. This is in agreement with the AF-2 antagonistic profiles of these compounds and their observed antagonist conformation, with H12 occupying the coactivator binding groove.

FER is a sesquiterpenoid found in the root of plants belonging to the genus *Ferula*, such as *Ferula hermonis*, native to the Middle Eastern region. The roots of *F. hermonis* have long been used in traditional medicine to treat menopausal disturbances in women. FER has an interesting activity profile that illustrates the subtype-dependence of xenoestrogen action. FER is a partial agonist of ER α but an ER β antagonist^[30]. Sequence and 3D-structure alignments reveal that the LBDs of ER α and ER β share a high degree of homology in their primary sequence but exhibit two residue substitutions in their hormone binding pockets. These substitutions correspond to the replacement of Leu384 (ER α) by Met336 (ER β) in H5 and Met421 (ER α) by Ile373 (ER β) in H7. They account for most of the subtype-specific action of ER ligands^[31]. Inspection of the crystal structures of both ER subtypes in complex with E₂ reveals that the variable amino acids reside on each side of the C and D rings of E₂ and create different space constraints in this portion of ER α and ER β LBDs. The structure of FER in complex with ER α reveals that the ligand contains a bulky group that projects toward Met421, which in turn undergoes a large conformational change (Figure 3C). In ER β , the branched and less flexible residue Ile373 is unable to move away from the pocket to make room for the ligand. Instead, a large shift of FER toward H12 possibly destabilizes the active conformation of H12, in line with the antagonistic activity of this compound in ER β (Figure 3C). Interestingly, in contrast with ER α Met421 (H7), ER β Met336 (H5) does not confer adaptability to the LBP of this receptor subtype, because strong constraints imposed by the surrounding residues maintain this residue in a single conformation^[24]. Thus, with two bulky and rigid residues, ER β appears more sensitive than ER α to ligand bulkiness and consequently, more easily antagonized by environmental compounds. Despite a fundamen-

tally different chemical structure (Table 1), the organochlorine pesticide CLD displays a similar activity profile, acting as a weak ER α agonist and an ER β antagonist^[32, 33]. This compound has been used as an agricultural pesticide to control the banana root borer from 1973 to 1993, particularly in the French West Indies. CLD undergoes very slow degradation in the environment, resulting in polluted soils and waterways, and remains a major source of human contamination through the consumption of locally produced foodstuffs. Recent studies have demonstrated that CLD accumulates in the body and that exposure at environmentally relevant levels is associated with a significant increase in the risk of prostate cancer, a decreased length of gestation, and negative effects on the cognitive and motor development of children^[34–36]. Figure 3D shows that CLD occupies a smaller portion of the LBP than E₂. However, it binds between ER α Met421 and Leu384, the LBP region that is sterically constrained in ER β . With its cubic core and additional chlorine atoms, CLD displays a larger volume than the C- and D-rings of E₂. Therefore, the binding mode of CLD in ER β possibly interferes with the positioning of H12 in the active conformation. Because ER β counteracts the action of ER α by exerting anti-proliferative and anti-inflammatory effects, the health impact of compounds acting as ER α agonists and ER β antagonists (eg, FER, CLD, or BBP) is certainly higher than that of compounds with different subtype preferences. These compounds are highly associated with cancer incidence and tumor growth.

The peroxisome proliferator-activated receptor γ and its environmental ligands

The peroxisome proliferator-activated receptor γ in health and disease

The NR subfamily of peroxisome proliferator-activated receptors (PPARs) includes three members, PPAR α (also called NR1C1), PPAR β/δ (also called NR1C2), and PPAR γ (also called NR1C3). These receptors bind to PPAR-responsive DNA regulatory elements in the form of heterodimers with retinoid X receptor (RXR). They control the expression of genes involved in adipogenesis, glucose, lipid, and cholesterol metabolism^[37, 38]. PPARs bind and respond to dietary fatty acids and diverse lipid metabolites, including prostaglandins, eicosanoids^[37, 39], and oxidized phospholipids. These receptors have different tissue distribution and physiological roles^[40]. PPAR α is expressed predominantly in the liver, heart, and brown adipose tissue, whereas PPAR β/δ is ubiquitously expressed. Both play a major role as activators of fatty acid oxidation pathways and therefore in the regulation of energy homeostasis. In addition, PPAR α stimulates heme synthesis, cholesterol catabolism, and participates in the control of amino acid metabolism and urea synthesis. PPAR β/δ has a role in the control of cell proliferation and differentiation and is necessary for placental and gut development. PPAR γ is highly expressed in adipose tissues and plays key roles in regulating adipogenesis^[41], lipid metabolism, and glucose homeostasis through improving insulin sensitivity^[42]. Additionally, PPAR γ is required for the function and survival of mature adipocytes.

Lastly, several studies suggest that all three PPARs have anti-inflammatory activities^[38]. However, the identification of specific endogenous PPAR γ ligands has been difficult. In contrast with classical NRs, such as ERs that essentially bind and respond to a single specific ligand, it appears that natural PPAR γ ligands are diverse and include unsaturated fatty acids, 15-deoxy- Δ 12,14-prostaglandin J2, 9-hydroxy-octadecadienoic acid (Table 2), 15-hydroxy-eicosatetraenoic acid, prostaglandin J2, and nitrated fatty acids^[43, 44].

Consistent with their tissue distribution and their role as sensors of lipids/fatty acids levels, regulating fatty acid catabolism, and lipid storage, all three PPARs are strongly implicated in the metabolic syndrome. Consequently, several PPAR subtype-specific synthetic ligands have been developed as drugs for treating metabolic diseases, such as hyperlipidemia

or diabetes^[45]. As PPAR α agonists, fibrates, such as fenofibrate or clofibrate, are widely used to treat hypertriglyceridemia. By contrast, PPAR γ is the target for antidiabetic agents of the thiazolidinedione (TZD) class, which includes troglitazone, pioglitazone, and rosiglitazone (full agonist reference molecule, Table 2). Because these compounds are potent activators of PPAR γ , they exhibit not only robust insulin-sensitizing activities but also side effects associated with chronic activation, such as fluid retention, higher risk of cardiovascular disease, and weight gain^[42]. However, a recent study has shown that classical agonism is not required for the antidiabetic effect of PPAR γ ligands. Instead, the mechanism involves blockade of the phosphorylation of PPAR γ by cyclin-dependent kinase 5 (Cdk5) at serine 273^[46]. Novel antidiabetic compounds that have a unique mode of binding have been described in vari-

Table 2. Structures of PPAR γ natural and environmental ligands.

PDB	Ligands	PDB	Ligands
PPARγ 2PRG ^[90]	Rosiglitazone	PPARγ 2VSR ^[59]	9-HODE
3OSI ^[51]	Tetrachlorobisphenol-A (TCBPA)	3PO9	Tripropyltin (TPT)
3OSW ^[51]	Tetrabromobisphenol-A (TBBPA)	3QT0 ^[82]	Mifepristone (RU-486)
3PBA ^[72]	Monosulfate TBBPA (Mono TBBPA)	3SZ1 ^[75]	Luteolin
3R5N ^[74]	Magnolol	4JAZ ^[73]	Resveratrol (RES)
	Perfluorooctanoic acid (PFOA)		Mono-ethylhexyl phthalate (MEHP)

ous reports^[47, 48]. They block the Cdk5-mediated phosphorylation without activating the transcriptional function of PPAR γ and are associated with fewer side effects. Considering the physiological role of PPAR γ in adipose tissue development and maintenance, it has been proposed that disruption of the regulatory pathways under the control of PPAR γ may be involved in the onset of diabetes and obesity^[49]. Activation of this receptor by certain xenobiotic compounds has been shown to stimulate adipogenesis *in vitro* and *in vivo* by inducing the differentiation of preadipocytes of the fibroblastic lineage into mature adipocytes^[50–53]. This has led to the “obesogen hypothesis”, which states that in addition to the imbalance between caloric intake and expenditure, the rapidly growing obesity epidemic could implicate environmental risk factors, such as an increased exposure to chemicals that interfere with any aspects of metabolism^[52, 54]. Accordingly, compounds that have the potential to disrupt any metabolic signaling pathways and lead to increased fat accumulation and obesity are referred to as “obesogens”^[55]. Below, we present illustrative examples of the group of environmental obesogens acting through the activation of PPAR γ .

Environmental ligands of PPAR γ

In the early 1990s, the study of a group of xenobiotic compounds termed peroxisome proliferators, which trigger hepatic and renal peroxisome proliferation in rodent cells, had led to the discovery of PPARs as a novel subfamily of NRs^[56]. This set of compounds included phthalates, plasticizers, certain herbicides, and the fibrate class of hypolipidemic drugs. This group of PPAR disruptors now contains additional members, such as the biocides organotins, the perfluorooctanoic (PFOA) and perfluorooctanesulfonic (PFOS) acids, pharmaceuticals (eg, the PR antagonist RU-486), halogenated derivatives of BPA, the imidazole fungicide triflumizole, and some naturally occurring compounds, such as the polyphenols resveratrol, luteolin, and magnolol, which are found in plants. As shown in Table 2, the group of PPAR γ environmental ligands is het-

erogeneous and contains a structurally and chemically disparate ensemble of molecules with very few shared molecular features, suggesting that each of them possibly interacts with the receptor through a specific mechanism.

PPAR γ has a large Y-shaped ligand-binding pocket (LBP) with a volume of approximately 1440 Å³. The diversity of ligands it can accommodate may contribute to the large array of roles assigned to PPAR γ . The LBP extends from the C-terminal helix H12 to the β -sheet S1/S2 and can be divided into two sub-pockets named hereafter the AF-2 and the β -sheet sub-pockets, respectively^[57]. Based on existing PPAR γ complex structures, it has been suggested that full agonists, such as the rosiglitazone (Table 2), occupy both sub-pockets, establishing hydrogen bonds with residues Tyr473 (H12) on one side and Ser342 (S1/S2) on the other side (Figure 4A), whereas partial agonists would bind essentially to the β -sheet sub-pocket^[58]. Interestingly, several structural studies have revealed that the large cavity of PPAR γ can accommodate two ligands occupying both AF-2 and β -sheet sub-pockets^[58, 59] (Figure 4B). In addition, we have provided structural and functional evidence that PPAR γ can bind simultaneously two or three molecules of the phthalate MEHP and the perfluorinated compound PFOA, respectively (Table 2, unpublished data).

Organotins and halogenated BPA as potential environmental obesogens

The first environmental obesogen for which a mechanism of action has been elucidated is the organotin tributyltin (TBT, Table 2)^[60, 61]. Organotins form a diverse group of synthetic tin derivatives ubiquitously found in the environment due to their widespread use since the 1960s in several industrial and agricultural processes^[62, 63]. In the 1980s, these compounds were observed to be responsible for a wide variety of deleterious effects. Despite restrictions on their use, organotins persist in the environment and are absorbed by higher organisms where they accumulate^[64]. Human exposure occurs primarily

Figure 4. PPAR γ bound to pharmaceutical and natural ligands. Close-up views of the ligand-binding pocket of PPAR γ in complex with rosiglitazone (A) and the fatty acid 9-HODE (B), showing the two sub-pockets that can be differentially occupied. Oxygen, carbon and nitrogen atoms are shown in red, yellow, and blue, respectively. The dashed lines depict hydrogen bonds.

through diet, in particular through sea food^[65]; however, some organotins have been documented in house dust, suggesting that exposure from sources other than food may be widespread^[66]. TBT and tripropyltin (TPT) are dual nanomolar affinity agonists of PPAR γ and its heterodimeric partner RXR and were shown to induce adipogenesis in preadipocytes^[60, 61]. A deeper investigation of organotin action indicated that although TBT is a high affinity ligand for both receptors, it acts as a full RXR agonist but activates PPAR γ weakly compared to the reference pharmaceutical compound rosiglitazone. Further structural and biophysical studies revealed that TBT binds covalently to both heterodimer subunits by forming a covalent bond between the tin atom and the sulfur atom of cysteine residues located in the LBP of both receptors^[50] (unpublished data). In RXR, Cys432 is located in helix H11, and although TBT interacts with only a subset of binding pocket residues in the H11–H12 region, it seems to be engaged in enough essential contacts in the AF-2 sub-pocket to stabilize this receptor in its active conformation (Figure 5A). By contrast, the cysteine residue of PPAR γ (Cys285) resides in H3 and anchors the organotin in a region of the β -sheet sub-pocket. As a result, it does not allow the efficient stabilization of the active receptor conformation, which is in line with the weak PPAR γ agonistic activity of the compound (Figure 5B). Thus, the efficient activation of RXR-PPAR γ by TBT results from the combined action of the organotin on the two heterodimer subunits. The discovery of this binding mode suggested that other NRs presenting a cysteine residue in their binding pocket should be considered as potential targets of organotins. The functional outcome of this interaction is dictated by the position of the anchoring cysteine in the LBP. Accordingly, it has been reported that dibutyltin acts as a potent antagonist of the GR, which contains two cysteine residues in its LBP^[67].

Halogenated derivatives of BPA, which feature bromine or chlorine substituents on the phenolic rings, are used as flame retardants. Tetrabromobisphenol-A (TBBPA) is used to produce fireproof epoxy resins utilized in the manufacture of computer motherboards and other electronics. Its production is currently estimated to be approximately 200 000 tons/year, and its presence has been reported in the environment^[68],

in wildlife^[69], and in human samples^[70, 71]. The closely related tetrachlorobisphenol-A (TCBPA) is also used as a flame retardant, but in much lower quantities (<10 000 tons/year). Two recent studies have investigated the capacity of the halogenated BPAs and of their biotransformation products to act as PPAR γ ligands^[51, 72]. Both TBBPA and TCBPA were shown to act as partial agonists of the receptor and promote differentiation of 3T3L1 pre-adipocytes at concentrations within the micromolar range (100-fold less potent than rosiglitazone). These chemicals also activate the corresponding zebrafish and *Xenopus* receptors^[51], indicating that halogenated BPA can disrupt the activity of PPAR γ from different species. In addition, it was shown that the sulfation pathway, usually considered as a detoxification process, leads to the formation of sulfate conjugates, which possess a significant residual PPAR γ binding activity^[51]. The crystal structures of PPAR γ in complex with TBBPA and TCBPA are indistinguishable and reveal the canonical tertiary fold of agonist-bound PPAR LBD. The ligands essentially occupy the β -sheet sub-pocket, with one of the phenol rings nestled between H3 and the β -sheet, and only a small part of the AF-2 sub-pocket with no direct interaction with H12 (Figure 5C). However, the second phenol ring of halogenated BPA is indirectly linked to H12 through a water-mediated hydrogen bond with Tyr473. This mode of binding reflects the partial agonistic profiles of the compounds. The four halogen atoms participate in the interaction by establishing van der Waals contacts with the other residues of the LBP. Cell-based assays showed that the activation of PPAR γ depends on the halogenation degree of BPA derivatives. The bulkier halogenated BPA analogues, the greater their capability to activate PPAR γ . This observation correlates with a higher occupancy of the LBP, resulting in a better stabilization of the active form of the LBD. The crystal structure of the TBBPA metabolite in complex with PPAR γ shows that the TBBPA-sulfate is positioned identically to its parent molecule, with the sulfate group filling a portion of the volume occupied by the solvent in the TBBPA structure. The presence of a conserved water molecule mediates the indirect interaction between TBBPA-sulfate and PPAR γ helix H12. Overall, the crystallographic data validates the notion that halogenated

Figure 5. Binding modes of organotins and halogenated bisphenol A. Close-up views of the ligand-binding pocket of RXR α (A) and PPAR γ (B, C) in complex with the tributyltin (TBT), tripropyltin (TPT) and tetrabromobisphenol A (TBBPA), respectively. Oxygen, carbon, sulfur, and tin atoms are shown in red, magenta, yellow, and gray, respectively. The dashed lines depict hydrogen bonds. Water molecules are displayed as red spheres.

BPA sulfates are partial agonists of PPAR γ .

Other environmental PPAR γ ligands

Recent data have revealed that some natural compounds derived from plants can bind to PPAR γ and modulate its activity^[73–75]. These include RES, luteolin, a flavonoid present in celery, green pepper, perilla leaf and chamomile tea, and magnolol, a lignane, which composes the stem bark of *Magnolia officinalis*. These compounds exhibit anti-cancer, anti-inflammatory, anti-angiogenic, and/or cardioprotective activities, as well as anti-oxidative effects^[76–78]. They bind to PPAR γ with micromolar affinities and display weak agonistic activity; magnolol was reported to induce adipogenesis and glucose uptake in 3T3-L1 cells^[79]. It can additionally bind RXR α with a micromolar affinity and was described as a dual agonist of PPAR γ and RXR α . Because magnolol agonistic activity can be inhibited by RXR or PPAR γ antagonists, magnolol seems to be required on both receptors to positively activate the RXR-PPAR γ heterodimer^[74]. As expected from their activity profiles, the crystal structures show that the compounds bind primarily to the β -sheet sub-pocket with no interaction with H12. Moreover, because of their small size and almost planar conformations, these compounds may be found as either two copies or associated with a fatty acid molecule being trapped during purification of the recombinant protein (Figure 6A, 6B).

The synthetic steroid compound mifepristone (RU-486) is known for its antagonistic properties on the steroid receptors GR and PR and is primarily used for early termination of pregnancy; additionally, it displays an anti-diabetic effect and appears to be a promising drug for the treatment of certain cancers^[80]. RU-486 and its metabolites can be found in the aquatic environment and should be considered as contaminants^[81]. Surprisingly, RU-486 was recently reported to bind to and activate PPAR γ ^[82]. The structure of the PPAR γ LBD in complex with RU-486 shows that PPAR γ adopts an active conformation with the steroid occupying the LBP, similar to rosiglitazone (Figure 6C). The steroid core of RU-486 aligns with rosiglitazone and establishes similar hydrophobic interactions and hydrogen bonds with the residues of the LBP,

except the critical hydrogen bond with Y473 (H12), which is lost. Furthermore, instead of pointing toward helix H12 as observed in the GR^[83] and PR^[84] structures, which account for the antagonistic character of mifepristone, the dimethylamino side chain of this compound projects towards helix H5 in PPAR γ . The difference in the binding modes of RU-486 in steroid receptors and PPAR γ explains the divergence in its activities with the two types of receptors.

Conclusion

EDCs are chemicals of great concern because these compounds, which are ubiquitously present in our daily environment, can alter endocrine functions and cause infertility, malformations, metabolic troubles, or increased incidence of cancers. The deregulation of NR-mediated transcription accounts for the deleterious effects of several EDCs, and the weak structural relationships between EDCs and natural hormones render their interaction with these cellular targets poorly understood and barely predictable. Because of these reasons, it is necessary to characterize the harmful interactions between NRs and environmental compounds, at both structural and functional levels, and develop robust *in vivo*, *in vitro*, and *in silico* screening methods.

Using three major biological targets of environmental contaminants (ER α/β and PPAR γ) as illustrative examples, the present review highlights the diversity of the mechanisms by which distantly related chemicals bind to and activate NRs with graded efficacy and potency. In addition to providing a better understanding of the differential activities, binding affinities, and specificities of environmental NR ligands, such structural knowledge at a near-atomic level provides rational guidelines to design safer chemicals characterized by fewer NR-mediated side effects. This will increase the effectiveness of 3D-structure based computational tools aimed at predicting the NR-disrupting action of environmental pollutants. Moreover, studies aimed at identifying and characterizing EDCs may aid in finding new ligands for NRs, including orphan receptors, and may reveal unforeseen binding modes, thereby providing guidelines for the rational design of novel NR mod-

Figure 6. Differential binding modes of natural and pharmaceutical compounds. Close-up views of the ligand-binding pocket of PPAR γ in complex with magnolol (A), luteolin (B), and RU-486 (C). Oxygen, carbon, and nitrogen atoms are displayed in red, magenta, and blue, respectively. The dashed lines depict hydrogen bonds. Water molecules are displayed as red spheres.

ulators as new therapeutic agents.

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