A Molecular Guidance System Based Upon Target Genes, Nuclear Receptors and Ligands Applied to Drug Discovery and Prediction of Toxicity

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Abstract: A molecular guidance system useful in drug design is described in which nuclear receptors position ligands in intercalation sites in responsive genes. Evidence is based upon positions of agonists in receptors and the transcriptional activity of a designed estrogen that is 3 times more potent than the steroid hormone estradiol.

Key Words: Nuclear receptors, intercalators, DNA unwinding, steroid hormones, hormone response elements, computer modeling, transcription, drug design.

INTRODUCTION

The discovery and development of many future pharmaceuticals will be inextricably linked to how genes function. Such function includes normal physiological processes as well as aberrant processes associated with various diseases. As we gain a detailed knowledge of the structure of genes and the factors that regulate them, the ability to develop new safe and effective pharmaceutical candidates will improve and expand dramatically. Drug candidates will include small molecular weight compounds (ligands) that affect gene function though nuclear proteins including enzymes, e.g. topoisomerases [1] and nuclear receptors [2-4]. This review focuses on the latter and the hypothesis that ligands are being inserted into DNA by nuclear receptors leading to gene regulation [5-7]. New evidence is presented based upon modeling of published X-ray structures of the ligand binding domains (LBDs) of nuclear receptors and the transcriptional activity of a new potent estrogenic drug that was designed using this model. These findings extend the ligand insertion hypothesis by proffering that nuclear receptors provide a remarkably precise molecular guidance system that delivers drugs and other ligands to specific sites in the DNA of responsive genes. Given the explosion of literature on drugs that act *via* nuclear receptors, we have chosen to limit this initial review to the study of naturally occurring, mammalian steroid hormones.

NUCLEAR RECEPTOR LIGANDS AND DRUG TARGETS

Nuclear receptors are transcription factors that bind both small molecule ligands and certain DNA sequences leading to activation or suppression of gene function [2-4]. Common natural ligands include the mammalian steroid hormones estradiol, progesterone, testosterone, aldosterone and cortisol as well as triiodothyronine (T_3), trans-retinoic acid, 9-cis-retinoic acid, the secosteroid 1α, 25-$(OH)_2$-vitamin D_3 and the insect steroid hormone ecdysone (Fig. (**1**)) [8]. The list of natural ligands is expanding and includes prostaglandins, eicosanoids, bile acids, cholesterol analogs as well as a long list of synthetic drugs that can act as agonists or antagonists of transcription (cf. www.nursa.org). Both the normal physiological and pathological targets that involve nuclear receptors are vast and include contraception, male sterility, hormone replacement therapy, breast cancer, prostate cancer, anti-fertility agents, anti-inflammatory agents, thyroid disease, bone disease, diabetes, obesity and acne. Relatively new targets are cardiovascular disease [9], lipid disorders [10], asthma [11] and diseases of the central nervous system including Parkinson's disease [12], Alzheimer's disease [13] and depression [14]. Nuclear receptors are also known to affect drug metabolism, drug transport and the interindividual variability in response to drugs [15]. It is not surprising that in addition to governmental agencies and academic institutions worldwide, virtually all major pharmaceutical companies as well many biotechs have ongoing programs devoted to the study of nuclear receptors.

Nuclear receptors are also known to bind to certain co-regulators i.e. proteins that can either stimulate or repress transcription [16]. These proteins number in the hundreds, are known to bind to nuclear receptors and function as critical components of the transcriptional machinery. Impairment of co-regulator function is associated with a very wide range of human diseases. Also of great interest is a large group of proteins that have tertiary structures in common with nuclear receptors but whose ligands are unknown. These "orphan receptors" play a critical role in the regulation of certain genes [17]. In some cases, studies of orphan receptors have resulted in the identification of ligands and thus novel drug candidates. It is also known that some receptors do not require ligand to activate or repress transcription [18]. This process is known as ligand independent transcription.

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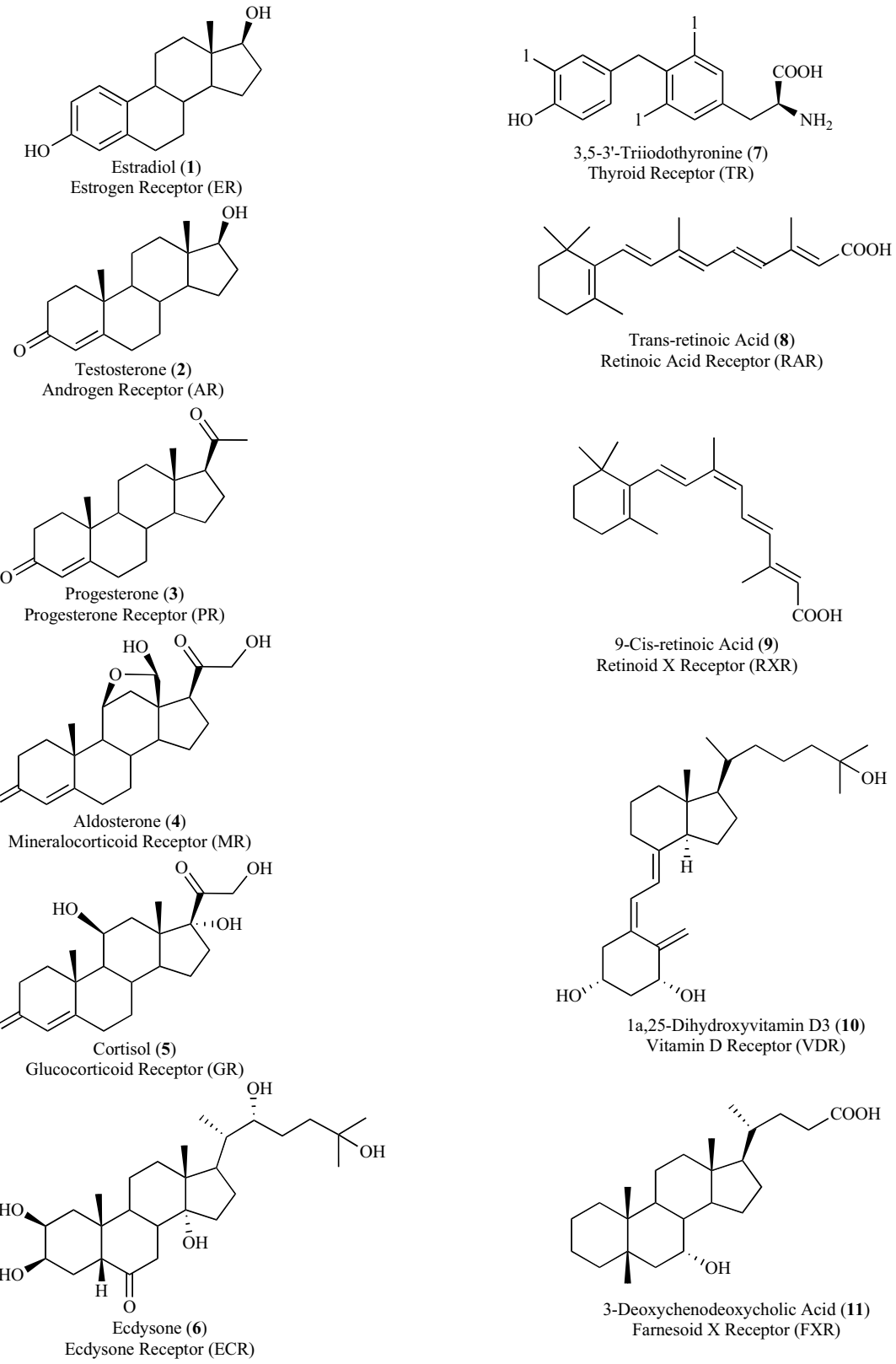

Fig. (1). Structures of natural ligands of nuclear receptors: the mammalian steroid hormones estradiol, testosterone, progesterone, aldosterone and cortisol, the insect hormone ecdysone, the thyroid hormone 3,5,3'-triiodotyronine (T_3), trans- and 9-cis-retinoic acid, 1α,25-dihydroxyvitamin D_3, the bile acid metabolite 3-deoxychenodeoxycholic acid and the names of their associated nuclear receptors. The orientation of the steroid hormones in the X-ray crystal structures of the ligand binding domains of their receptors is provided in Figs. (**3-4**). The synthetic glucocorticoid analog dexamethasone is depicted in the crystal structures in lieu of cortisol.

Nuclear receptors are phosphoproteins and phosphorylation is also known to have pronounced effects on receptor action [19]. Despite an extensive literature, the complete picture of how nuclear receptors act remains to be elucidated. This review will focus primarily on the role of steroid hormone ligands and will be limited in scope to selected receptor isoforms (e.g. estrogen receptor α). The reader is directed to several reviews on nuclear receptors [2-4], the RCSB Protein Databank (www.rcsb.org) and the Nuclear Receptor Signaling Atlas (www.nursa.org) for additional information.

NUCLEAR RECEPTOR STRUCTURE AND FUNCTION

Nuclear receptors are a family of proteins that despite widely varying amino acid sequences have conserved secondary and tertiary structures. The major features are a highly variable amino terminal domain, a centrally located DNA binding domain (DBD), a hinge region and a ligand binding domain (LBD) at the carboxy terminal end of the protein [2-4]. Although the three dimensional structures of full length nuclear receptors have not yet been characterized, NMR and X-ray crystallographic data of DBDs bound to DNA and LBDs bound to various ligands have been reported. The DBDs are short sequences of approximately 70 amino acids which contain zinc fingers. The DNA sequences that bind and are activated by nuclear receptors in transcriptional assays have been identified and termed hormone response elements (HREs). The sequences of the HREs are highly conserved i.e. 5'-AGGTCA-3' and 5'-AGAACA-3' [3]. These sequences are termed half sites as many receptors bind as dimers to two half sites with a wide range of motifs e.g. palindromes, direct repeats and everted repeats. Nuclear receptors that are activated by mammalian steroid hormones have palindromic or pseudopalindromic HREs with three base spacers between the half sites and bind predominantly as homodimers i.e. one monomer to each half site. As an example, the consensus estrogen receptor response element (ERE) is 5'-AGGTCAnnnTGACCT-3' where n stands for any base. Other steroid hormone receptors, e.g. those that are activated by the androgen testosterone, the progestin progesterone, the mineralocorticoid aldosterone and the glucocorticoid cortisol, bind as homodimers to the consensus HRE 5'-AGAACAnnnTGTTCT-3'. The natural ligands thyroid hormone, trans-retinoic acid, 9-cis-retinoic acid, 1α, 25-$(OH)_2$-vitamin D_3, PGE-2α and ecdysone activate receptors that bind as heterodimers in a variety of HRE motifs e.g. direct repeats. Certain orphan receptors also form heterodimers with liganded receptors and some receptors can bind and activate transcription at a single response element half site. The primary structures of the LBDs of nuclear receptors are poorly conserved yet all have a common tertiary structure including 12 alpha helices that form a compact shape. The X-ray crystal structures of LBDs bound to ligand show that all ligands are similarly positioned near helices 11 and 12 at the carboxy terminal end of the receptor.

While the exact details of how receptors and their ligands regulate genes are not fully understood, a general picture has emerged. Namely, upon binding ligand, the receptor undergoes a conformational change that involves helix 12 of the LBD. Many investigators have shown that different conformations of the receptor LBD give rise to agonist or antagonist effects [20]. Thus, conformational changes in the receptor affect transcription by specifically binding to the HREs. It is also understood that full activation of many genes is dependent upon the stereochemistry of the ligand, its full length receptor and the associated response elements. Co-factors [16] also help modulate this process as mentioned above.

DRUG DISCOVERY USING NUCLEAR RECEPTORS: UNANSWERED QUESTIONS

In order to exploit nuclear receptors in drug discovery, one approach is to screen candidate ligands for their relative strength of binding. Another approach is to employ NMR or X-ray crystal structures of the LBDs and map the cavities known to bind ligands. New compounds can be screened and/or designed by fit into the ligand binding sites. Both of these approaches combined with other methods, e.g. transcriptional assays and animal testing, have been used. A complicating factor has been that relative binding of ligands to receptors does not always correlate well with biological activity [21, and references therein]. In the case of estrogens, some compounds bind strongly to the estrogen receptor yet have poor activity in animals relative to the natural hormone estradiol. Conversely, certain compounds bind weakly to the estrogen receptor yet are more potent than estradiol *in vivo*. Albeit unlikely, such discrepancies could conceivably be due to the metabolism of the compounds in animal tests. Very recent studies demonstrated that certain compounds which have equivalent binding to estradiol are more potent than estradiol in animal assays [22]. The investigators employed molecular modeling to show that the potent compounds stabilize the interaction of certain co-activators with the LBD thereby effecting receptor dynamics including subsequent events i.e. transcription and associated biological responses. Another puzzling observation is that some receptors apparently lack specificity in binding certain natural hormones. For example, the mineralocorticoid receptor which is activated by aldosterone binds the hormones progesterone and cortisol with equivalently high affinity yet the latter lack mineralocorticoid activity [23,24]. Moreover, the strength of binding of ligand/receptor complexes to their hormone response elements does not necessarily correlate with transcriptional activity leading investigators to search for mechanistic explanations for this contradiction [25].

The universally accepted notion of how receptors act is that binding of the ligand to the LBD results in a specific conformational change in the receptor that imparts transcriptional activity. Thus, the conformational change in the ligand/LBD complex must be communicated to the HRE/DBD complex to effect transcription. The DBD and LBD in the intact receptor are tethered together by the hinge region domain and in most theoretical models are shown distal to one another. This raises the question about exactly how the signal imparted by the ligand is transmitted to the genes.

While the discussion here has been limited to nuclear receptors, it should be noted that similar questions have arisen in studies with other receptor systems e.g. G protein coupled receptors [26]. For example, certain analogs of thyrotropin-releasing hormone act as super-agonists yet have

low receptor affinity. In fact, the authors demonstrate an inverse correlation between activity and receptor binding. Although beyond the scope of this review, explanations for such observations include the presence of different receptor active states induced by agonist binding [26,27] which may have analogies with nuclear receptors.

THE LIGAND INSERTION HYPOTHESIS: NEW EVIDENCE THAT NUCLEAR RECEPTORS GUIDE LIGANDS TO HORMONE RESPONSE ELEMENTS

We have proposed that ligand binding causes a conformational change in the receptor resulting in folding in a manner that orients the ligand/LBD complex in close proximity with the HRE [6,7,28,29]. To our knowledge, such an orientation does not contradict published experimental data and provides a direct way that ligand can effect transcription. Namely, concomitant with the conformational change in the receptor, we envision unwinding of the HRE and transfer of ligand to cavities formed between base pairs. Strong experimental evidence that the LBD must be in very close contact with DNA is provided by the observations of independent investigators that estrogens radioactively labeled with short acting Auger electron emitting isotopes, i.e. 17 alpha ^{125}I estrogens, cause DNA damage in cells containing the estrogen receptor and not in cells without the receptor [30]. Auger electrons deposit their energy near the radioactive atom i.e. within a small sphere of 2 nanometers (20 Å). For comparison, the length of the steroid nucleus is approximately 15 Å. In order for the steroid to damage DNA, the receptor and ligand must be very close to the DNA surface. While the authors did not mention intercalation, they concluded that the ^{125}I of the steroid must be directed to the nuclear estrogen response element (ERE) by the estrogen receptor. As described in further detail below, our laboratories reported the sequence that best fit ligands and predicted its occurrence in hormone responsive genes before HREs were characterized [5,6,31]. This sequence is not only universally present in HREs but alterations of the sequence diminish transcriptional activity elicited by ligand. That HREs can unwind and bend upon binding to nuclear receptors has also been shown by both molecular dynamics [32] and *in vitro* experiments [33-37]. Folding of receptors has been also demonstrated in which the distal amino and carboxy terminal ends interact with each other [38-40]. The path that ligands can take upon unbinding to receptors has also been studied leading investigators to suggest that such information will be useful in drug design [41, 42].

Synopsis of Observations Leading to the Ligand Insertion Hypothesis

A chronological review of the theoretical framework and evidence leading to the ligand insertion hypothesis has been reported elsewhere [28]. Briefly, studies with crude space filling models first reported in 1977 showed that certain biologically active compounds fit between base pairs in double stranded DNA [43]. Most notable was the fit in DNA (intercalation) of the mammalian steroid hormone estradiol. Because estradiol was thicker than classical intercalating drugs known to bind strongly to DNA, it was likely that other factors such as the estrogen receptor might aid in the intercalation process. Physicochemical experiments with the relatively flat phytoestrogen coumestrol showed it to be a weak DNA binder and as expected ruled out classical intercalation [44]. A consistent finding was that estradiol, other steroid hormones and thyroid hormone T_3 fit particularly well into a cavity formed in a single unwound double stranded dinucleotide sequence, i.e. 5'-CA-3'·5'-TG-3', and in a very specific orientation [5-7,31,45]. Each hormone formed specific and unique hydrogen bonding linkages to DNA. The hypothesis was advanced that this sequence would be present in critical locations in genes regulated by these hormones. Computer modeling confirmed these early observations and demonstrated that the degree of fit into the site in DNA within a given set of ligands correlated with degree of hormonal activity. When the structures of HREs activated by nuclear receptors and their ligands were eventually characterized, the site 5'-CA-3'·5'-TG-3' was located at the end of the consensus half sites, i.e. 5'-AGGT**CA**-3' and 5'-AGAA**CA**-3' (bold letters), and found to be requisite for transcriptional activity. This finding combined with other experimental evidence [21] strengthened the hypothesis that ligands were being actively inserted into genes by nuclear receptors. Various models of the HREs with the DNA unwound at this locus were created and ligands (***L***) were inserted as previously reported [5-7,29,31] i.e. 5'-AGGT**C*L*A**-3' and 5'-AGAA**C*L*A**-3'.

Structures of Ligand/DBD/unwound HRE Complexes

When X-ray crystal structures of HREs bound to DBDs became available, it was determined that the putative insertion site 5'-CA-3'·5'-TG-3' was exposed and could be unwound in the presence of the DBDs [7,21,29]. It was also possible to construct models of DBDs bound to unwound HREs with ligand inserted. Based upon this evidence, the LBD of receptors was predicted to play a key role i.e. assisting in the unwinding and bending of DNA in a manner required for ligand insertion, folding to allow the LBD to contact DNA, orienting the ligand to the unwound insertion site and guiding ligand into the DNA [29]. It followed that fit of the ligands into the unwound DNA by the LBDs would be directional and highly specific, e.g. in the case of all mammalian steroid hormones and the insect hormone ecdysone, the D ring of the steroids would be inserted between the bases T and G with the beta side facing T. The DBDs restricted the approach of ligand to the 5'-CA-3' strand of the unwound HRE.

In the case of HREs containing two half sites, it was also possible to construct models in which two ligands were inserted i.e. two molecules estradiol could be intercalated into the unwound estrogen response element (ERE) 5'-AGGT**C*L*A**nnn**T*L*G**ACCT-3' (Fig. (**2**)) [28,29]. Models based upon X-ray structures of the estrogen receptor DBD dimer/ERE complex (pdb entry 1hcq) [46] revealed that DNA could indeed be unwound at both loci to form two cavities approximately 25 Å apart and ligands could be inserted into both cavities in the same orientation as in a single unwound site. Moreover, the palindromic structure of the ERE placed the two estradiol molecules in the DNA virtually parallel to one another with the beta sides facing. The dimer interface of the two DBDs was separated upon unwinding the ERE but the interaction of the DBDs with DNA remained largely undisturbed. This model containing two half

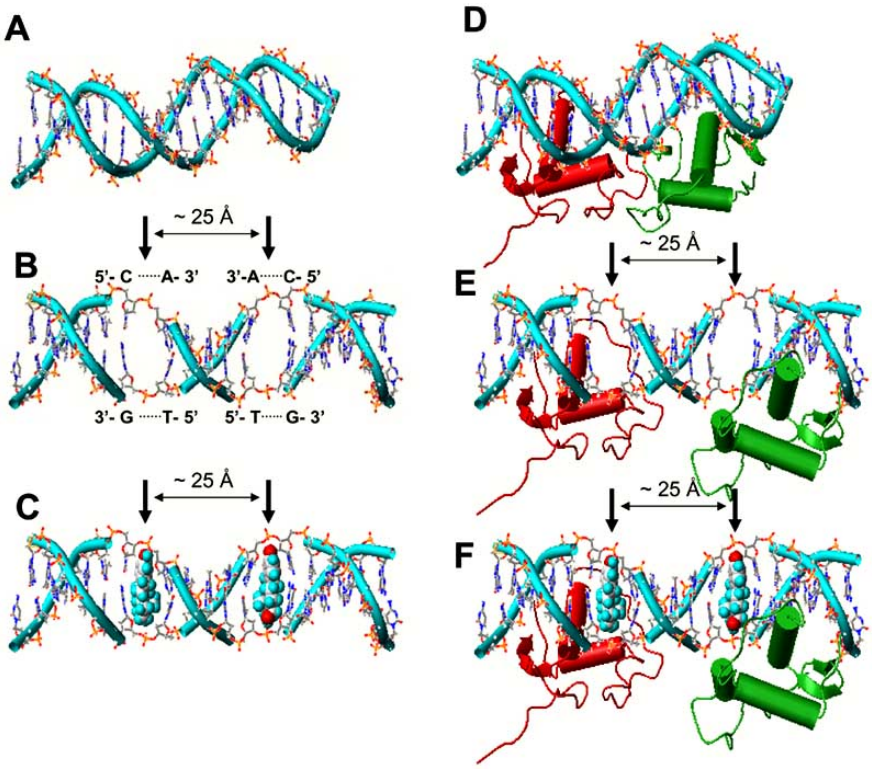

Fig. (2). Computer modeling of sites in the DNA of a pre-gene sequence HRE that can be unwound while bound to the DNA binding domains of nuclear receptors and accommodate ligands inserted in the cavities formed between the base pairs. In this example, the HRE sequence is the known palindrome that binds to a homodimer of the estrogen receptor and contains two half sites, each of which binds receptor i.e. 5'-AGGT**CA**nnn**TG**ACCT-3' 3'-TCCA**GT**nnn**AC**TGGA-5'. The sites of local unwinding are two 5'-**CA**-3'5'-**TG**-3' double stranded dinucleotides previously shown by modeling to stereospecifically fit agonist ligands. **A**) the consensus ERE 5'-AGGT**CA**nnn**TG**ACCT-3' from the crystal structure (pdb entry 1hcq) [46]); **B**) unwinding of the response element DNA in panel A at the two sites 5'-**CA**-3'5'-**TG**-3'; **C**) fit of two molecules of the natural mammalian steroid hormone estradiol (***E***) into the unwound ERE i.e. 5'-AGGT**C*E*A**nnn**T*E*G**ACCT-3'; the orientation of both steroids is the same in both cavities with the D ring end between the **T** and **G** bases and the A ring end between the **C** and **A** bases as previously described [5-7, 29,45]; the structure of the palindrome necessitates that the two steroid molecules are essentially parallel to one another approximately 25 Å apart with the beta sides facing each other in the orientation shown; **D**) the estrogen response element in panel A with the DBD of the estrogen receptor which binds as a homodimer (red and green); for clarity, only the tertiary features of the receptor protein are shown with the cylinders designating helices; **E**) the estrogen response element unwound as in panel B in the presence of the DBD; the dimer interface of the DNA binding domain is separated while the interaction with the DNA at each half site is maintained; **F**) fit of two molecules of estradiol into the unwound estrogen response element as in panel C in the presence of the DNA binding domain of the receptor. Note that the DBD partially restricts access to the 5'-TG-3' strand of the unwound site necessitating that estradiol enter the DNA cavity from the direction shown by the arrows. Given the common tertiary structural features found to be conserved in the receptor DBDs throughout the nuclear receptor superfamily, the conservation of base sequences of the HREs and the presence of 5'-**CA**-3'5'-**TG**-3' at the same location in the half sites, the direction of entry of a single ligand is proposed to be the same for other receptors including homo and heterodimers.

sites was particularly intriguing since it made further predictions possible about the role of the LBDs in ligand insertion. In the case of homodimers, because the spatial location of the insertion sites in the unwound ERE was fixed in space relative to each other, the resulting orientation of the ligands was also fixed in space relative to each other. Thus, one would expect that the position of ligands in homodimeric LBDs should be consistent with their location in the unwound ERE. More specifically, the two estradiol molecules in the LBD: 1) should be approximately parallel to one another; 2) have their beta sides facing each other; 3) should be separated a distance of approximately 25 Å; 4) should be oriented as in the DNA i.e. the D rings of both steroids facing the DNA.

Structures of Ligand/LBD/Unwound HRE Complexes

When X-ray crystallographic structures of the LBDs of nuclear receptors bound to ligands were first reported, new light was shed on the ligand insertion hypothesis. Particularly striking was the orientation of the estradiol molecules in the estrogen receptor LBD homodimer which matched that predicted by fit into DNA (pdb entry 1ere) [47] (Fig. (**3**)). Namely, the two estradiol molecules were parallel to each other, the beta sides were facing, and they were separated by

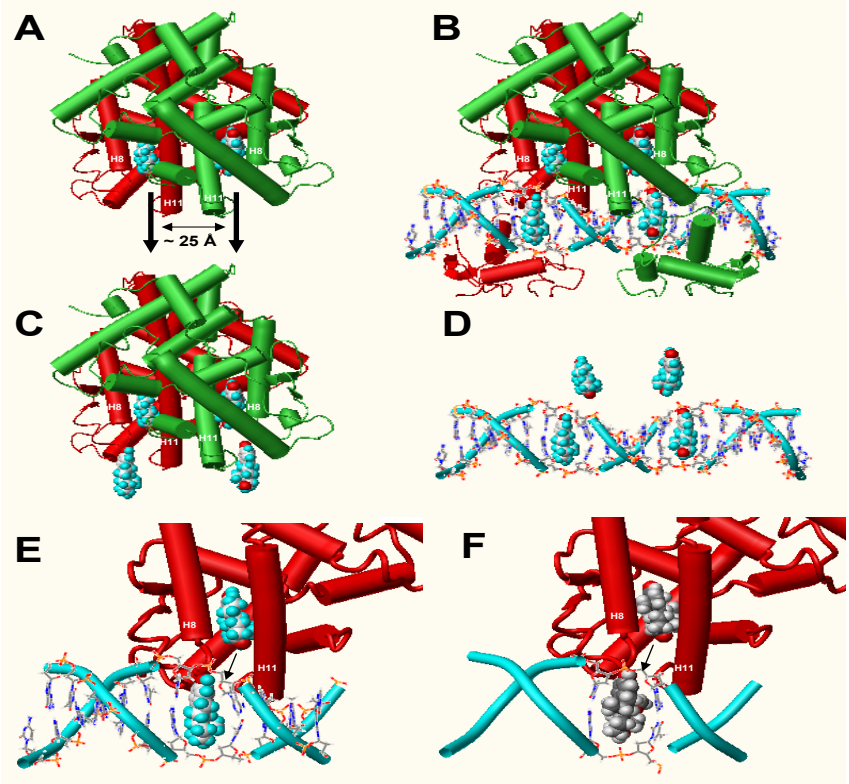

Fig. (3). Proposed interaction of the LBDs of nuclear receptors that are bound and activated by agonists with their cognate HREs. To be consistent with the example shown in Fig. (**2**), the estrogen receptor interaction with the estrogen response elements was chosen for analysis. For simplicity, only the tertiary structure is shown with the cylinders depicting helices in the protein; the LBD is shown as a dimer (red and green). **A**) the LBD of the estrogen receptor bound with two molecules of estradiol obtained from the crystal structure (pdb entry 1ere) [45]; the proposed direction of exit of the estradiol molecules unbinding from the receptor is depicted by arrows; **B**) model depicting the complementarity of the ligand binding domain of the receptor with that of the unwound ERE shown in Fig. (**2**); note that the orientation of the two estradiol molecules in the receptor is virtually the same as the orientation of the ligands in the model of the unwound ERE i.e. they are approximately the same distance apart (25 Å), pointing in the same direction with the beta sides of the steroids facing each other; **C**) further clarification of the orientation of the ligands based upon the receptor with the orientation of the ligands in the unwound DNA below (unwound DNA removed); **D**) clarification of the orientation of ligands in the unwound DNA with the orientation of the ligands in the LBD (protein removed); **E**) the model as in B with the only one monomer of the LBD shown; note that the molecule of estradiol is situated in the LBD between helices 8 (labeled H8) and helix 11 (labeled H11) of the protein which is predicted to help guide estradiol into the unwound ERE (arrow); the view is with the minor groove side of the DNA proximal with the receptor guiding ligand from the major groove; because the LBDs of nuclear receptor proteins bound to agonists have the same basic tertiary structure of helices including the position of helices 8 and 11 surrounding the ligand binding pocket in the protein, this view is shown for other ligand/receptor/HRE complexes in Fig. (**4**); **F**) the synthetic drug PDC-7 which was designed by improving the fit of estradiol in the dinucleotide site 5'-**CA**-3'5'-**TG**-3'; PDC-7 is shown docked into the estrogen receptor LBD with the proposed entry into DNA depicted by an arrow. PDC-7 is a relatively weak binder to the estrogen receptor, 2-3 times more potent than estradiol *in vivo* and 3 times more potent in transcriptional assays than estradiol as first reported herein (cf. Fig. (**5**) and Table **1**). Arrows depict the direction of transfer of ligands from the receptor to the DNA cavity.

approximately 25 Angstroms. When attempts were made to place the estradiol molecules in the LBDs in proximity of the EREs using molecular modeling, the surface of the LBD was shaped such that it was complementary to the shape of the unwound ERE. Docking of the LBD along the unwound HRE revealed that the two estradiol molecules were in exact register to be transferred to the DNA with the D rings of both steroids facing the DNA as hypothesized. While this finding was consistent with earlier predictions, the complementarity of the surface of the LBD and the DNA coupled with the remarkably close match of the relative location and orientation of the ligands in the DNA and LBD was unexpected and frankly astonishing. It was concluded that nuclear receptors are not only transferring ligands to their cognate responsive genes but that nuclear receptors are providing a highly fine tuned molecular guidance system. Evidence that such a relationship is not unique to estrogen receptors was provided by the crystal structures of other LDBs in the nuclear receptor superfamily which have common tertiary structures. Below, the putative molecular guidance system is further explored by examining the relationships of the steroid ligands in the unwound DNA and in the LBDs.

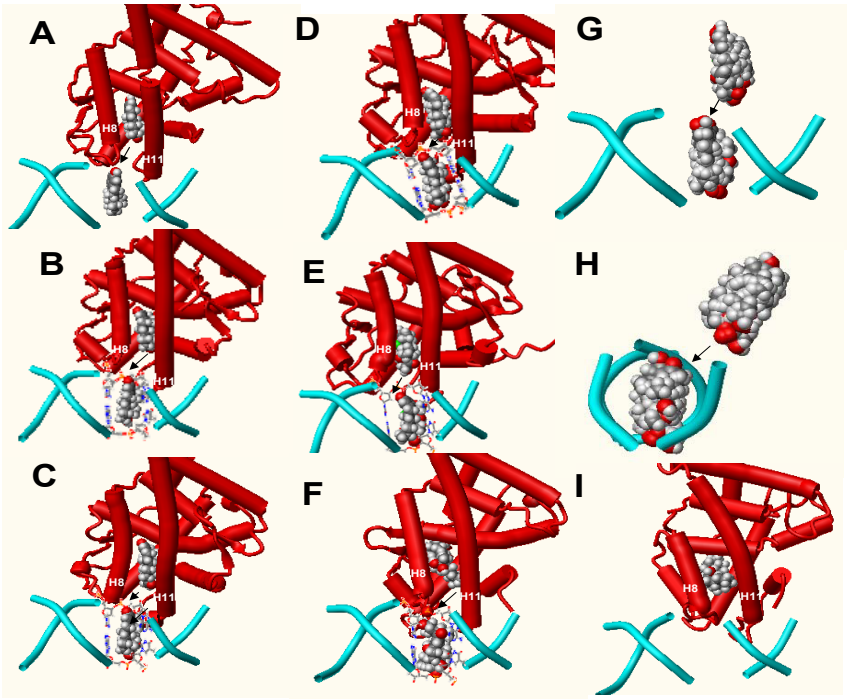

Fig. (4). Comparison of the fit of agonists in partially unwound DNA in the site 5'-**CA**-3''5'-**TG**-3' which is conserved in the sequences all HRE half sites and positioned as in Fig. (**2**) along with the fit of ligands into the LBDs of nuclear receptors as in Fig. (**3**). For clarity, a single monomer is shown. The fits of the ligands in the DNA are as previously reported [5-7,29,45]. The LBDs were oriented relative to one another using the conserved helices i.e. helices 8 and 11. **A**) estradiol in the ER LBD and DNA (cf. Fig. (**3E**); **B**) testosterone in the androgen receptor LBD (pdb entry 2am9) [48] and DNA; **C**) progesterone in the progesterone receptor LBD (pdb entry 1a28) [49] and DNA; **D**) aldosterone in the mineralocorticoid receptor LBD (pdb entry 2aa2) [50] and DNA; **E**) dexamethasone in the glucocorticoid receptor LBD (pdb entry 1m2z) [51] and DNA; **F**) ponasterone A in the ecdysone receptor LBD (pdb entry 1z5x) [52] and DNA; **G**) composite made by overlapping the mammalian steroids A-E based upon fit into the LBDs relative to DNA; the composite shows that the ligands are oriented in the same manner relative to one another in both the LBDs and DNA e.g. with the D and A rings and the same sides of the steroid skeleton overlapping; **H**) composite in G viewed down the helix axis of the DNA; **I**) bile acid 3-deoxychenodeoxycholic acid (3-DCDA) in the farnesoid X receptor LBD (pdb entry 1ot7) [53]; 3-DCDA is a relatively poor fit in DNA due to the cis A/B ring junction (Fig. (**1**)) and thus not shown in DNA. The orientation of this steroid in the receptor is opposite that of the mammalian steroids and ponasterone A; moreover, the location of the ligand in the receptor LBD is shifted relative to the other agonists which may explain the weak transcriptional activity elicited by this bile acid catabolite compared to the steroid hormones. The arrows depict the direction of entry of ligands from the receptor to the DNA cavity.

Relative Alignment of Agonist Ligands in Unwound DNA and LBDs Match

As described for the estrogen receptor, the surfaces of the homodimeric LBD conform to the surface of the ERE half sites unwound at the locus that fits the ligand (5'-CA-3''5'-TG-3') (Fig. (**4**)). The interaction of both LBDs with the ERE half sites in the model is the same with their orientation and location constrained by the DBD. The guanine in the DNA strand containing 5'-TG-3' is proximal to the DBD whereas the strand containing 5'-CA-3' is readily accessible. This phenomenon has been observed in the available X-ray crystal structures of DBD/HRE complexes of other receptors including both homo and heterodimers. Thus, the relationship between the tertiary structure of the LBDs and the DBD/unwound HRE complexes of these receptors is very similar. For this reason and because viewing and comparing models of the entire LBD/ligand/unwound HRE complexes is inherently difficult, only those parts of the LBD of the receptor, ligand and unwound DNA models involved in the putative transfer of ligand will be shown. Moreover, in order to simplify the comparisons and for brevity, only complexes involving X-ray structures of the natural steroid agonists with their LBDs [47-53] will be shown.

A predominant feature of the putative interaction surface of the LBDs with unwound HREs involves amino acids at the amino terminal end of helix 8 and carboxy terminal end of helix 11 of the LBD. Thus, the LBDs were aligned with one another by overlapping these features (Fig. (**4**)). Please note that some investigators have different helix numbering systems notably for helix 7 and helix 8. For consistency, the numbering of the helices employed was that recently reported by other investigators [41]. The estrogen receptor LBD in the monomeric form was used as an index structure (Fig. (**3E**)). The fits of the ligands in the DNA were as previously described by docking into the unwound dinucleotide 5'-CA-3''5'-TG-3' [5-7, 29,44]. To simplify comparisons of these complexes, only those features of LBDs proximal to the putative transfer of ligand are shown. However, the com-

plete structures of the LBDs are similar in tertiary structure to that of the estrogen receptor LBD shown in Fig. (**3**). It should also be emphasized that in order for ligand to be transferred to the DNA, local changes will need to be made in the conformations of the amino acid side chains in each of the LBDs to permit ligands to exit into the DNA. Such changes would most likely be similar to those occurring naturally when ligand is reversibly bound to the receptor [41,42]. These amino acids vary from receptor to receptor yet the secondary and tertiary structures remain constant.

The putative LBD/unwound HRE complexes for the steroids estradiol, testosterone, progesterone, aldosterone and dexamethasone (cortisol analog) demonstrate a striking similarity in the orientation of the ligands in the LBDs. Namely, each has the D ring of the steroid oriented in the same manner pointing into the unwound HRE. Moreover, the orientation of the beta face of the steroid is the same in all cases and this orientation matches that of the ligand in the DNA; the relative orientation of the steroids relative to one another (as shown by the overlay in Fig. (**4**)) is the same in the LBD as it is in the unwound DNA. The insect hormone ponasterone A (ecdysone analog) is larger than the mammalian steroid hormones but exhibits the same properties in the unwound DNA and LBD. Namely. the orientation of ponasterone A in the ecdysone receptor LBD is the same as that of the mammalian steroids and the fit in the DNA is the same i.e. with the D ring facing the unwound site and the beta face in the same orientation as in the DNA. The puffing in the polytene chromosomes of drosophila caused by ecdysones [54] may be related to the fit of this relatively bulky steroid in the DNA.

Although not shown, the X-ray complexes of LBDs and unwound HRE for T_3, 1α, 25-$(OH)_2$-vitamin D_3, 9-cis-retinoic acid and trans-retinoic acid [55-58] were analyzed using the same procedure. The orientation of the ligands are as they were published in the unwound DNA [5-7,29,31,45]. Unlike the steroid hormones which are rigid molecules, these ligands have conformational flexibility allowing for the ligands to adapt to the DNA cavities. Allowing for conformational flexibility, the orientation of the ligands relative to one another is again the same in the LBD as it is in the unwound DNA. Details of these findings will be reported elsewhere.

The X-ray crystal structure of the LBD of the farnesol X receptor (FXR) has been published in complex with the bile acid ligand 3-deoxychenodeoxycholic acid (pdb entry 1ot7) [53]. Of interest is that certain end products of metabolism including the bile acids are poor fits into unwound DNA [6, 28 and references therein]. While bile acids are steroids, the stereochemistry of the A/B ring junction is cis making them fit poorly in DNA compared to the steroid hormones. As shown in Fig. (**4**), the orientation of 3-deoxychenodeoxycholic acid in the LBD is also opposite of that of the steroid hormones; the A ring and not the D ring is facing the unwound DNA. The weak activity of the bile acids relative to the steroid hormones may be explained by these observations. Since bile acids are also known to be toxic, it raises the question of whether bile acids are successfully transferred to DNA and whether their poor fit may be related to toxicity. The orientation of the ligand within the FXR LBD may also reflect a protective mechanism in which certain nuclear receptors prevent certain toxic ligands from damaging genes. If so, there could be an even greater role for nuclear receptors and their co-activators in modulating gene activation and repression.

Design of New Drugs Based Upon Receptor/Ligand/DNA Interactions

Structure-activity correlations have been derived from the relative fit of ligands into DNA and biological testing in laboratory animals. In the case of estrogens, results of early molecular modeling showed that the capacity of compounds to fill the space between base pairs and form hydrogen bonding linkages to two phosphate groups when intercalated into DNA were highly active in uterotropic assays [59]. Compounds lacking these properties had little or no biological activity. Later studies with computer modeling and energy calculations confirmed these results [21]. These finding led to the development of a compound PDC-7 (11β-methoxy-7α-methyl-estradiol) which was designed as an estrogen by improving the fit of the natural hormone estradiol in DNA. The fit of PDC-7 in the unwound ERE is shown in Fig. (**3**). PDC-7 was synthesized and found to have estrogenic activity (uterotropic activity) in laboratory animals 2 to 3 times greater than estradiol (Table **1**) [21]. PDC-7 binds to the estrogen receptor but the strength of binding is only 15-18 % of estradiol. Such a discrepancy could be due to *in vivo* conversion of PDC-7 to a more active metabolite that had enhanced binding to the receptor. To further explore this possibility, the activity of PDC-7 was examined in *in vitro* assays. Namely, the ability of PDC-7 versus estradiol to transactivate a "classical" ERE such as that found in the vitellogenin gene promoter was evaluated [60]. In these experiments, induction of luciferase production by PDC-7 and estradiol was compared in ER-positive MCF-7 breast cancer cells that were transiently transfected with ERE(v)-tk-Luc, a reporter

Table 1. Comparison of the Chemical and Biological Properties of the Natural Hormone Estradiol with those of PDC-7 (11β-methoxy-7α-methylestradiol); PDC-7 was Designed by Fit into DNA at the Unwound, Double Stranded Dinucleotide 5'-CA-3'.5'-TG-3'. This Dinucleotide Occurs at the end of Consensus Estrogen Response Element Half Sites in Estrogen Responsive Genes e.g. the Palindrome 5'-AGGTCA nnnTGACCT-3' that is Activated by Estrogen Receptor Homodimers Bound with Ligand (cf. Figs. (2) and (3)). The Data in the Table [21] and the Transcriptional Activity Reported here for the First Time (cf. Fig. (5)) were Normalized to that of Estradiol set at 100

Compound	Estradiol	PDC-7
Relative Fit in DNA Measured by Energy Calculations	100	110
Relative Transcriptional Activity on Estrogen Responsive Genes	100	300
Relative Estrogenic Activity in Animals (Uterine Weight Gain)	100	240
Relative Binding Affinity to the Estrogen Receptor	100	20

construct containing 2 vitellogenin EREs directly upstream of thymidine kinase-luciferase [61]. As shown in Fig. (**5**), PDC-7 was three times more active than estradiol (ED_{50} = 3.3 pmol PDC-7; ED_{50} = 10 pmol estradiol). This experiment was repeated several times with the same result i.e. PDC-7 was approximately 3 times more active than estradiol. The superior activity of PDC-7 over estradiol in these transcription assays is consistent with the results of animal testing.

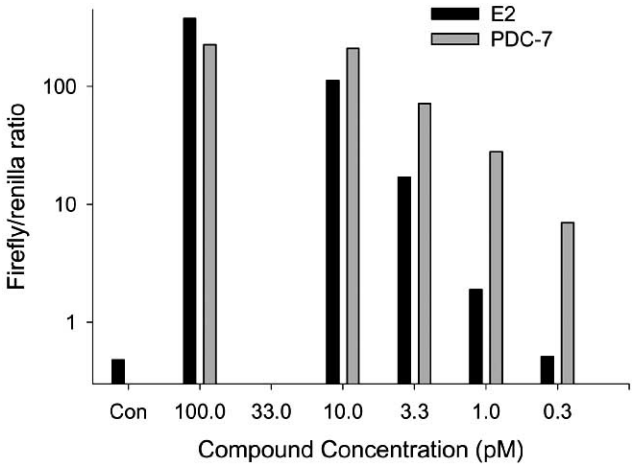

Fig. (5). Comparison of the ability of 17β-estradiol (E2) and PDC-7 to activate a canonical estrogen response element (ERE) as assessed by reporter gene activity. In these experiments, MCF-7 human breast cancer cells were cultured in phenol red-free medium containing 2% charcoal-stripped serum, then co-transfected with ERE-tk-Luc (firefly) along with tk-Luc (renilla) as control (CON) vector for standardization purposes [60]. After 24 hr, the cultures were treated with the indicated concentration of E2, PDC-7, or solvent control, followed 24 hrs later by the Dual-Luc assay as described [61]. Results are expressed as the ratio of firefly to renilla activity as an indication of ERE transactivation. Data shown are representative of 4 independent experiments.

When docked into the estrogen receptor LBD, PDC-7 fits but not quite as deeply in the pocket as estradiol [21]. This finding may explain the relatively weak binding of PDC-7 to the estrogen receptor. Our modeling studies of other very potent synthetic estrogens i.e. 11β-acetoxyestradiol and moxestrol (11β-methoxy-17α-ethinylestradiol) that have weak receptor binding show similar properties [21]. The relative fit in the LBD measured by energy calculations for these compounds was also lower than estradiol. Each of these compounds was also a better energetic fit in DNA than estradiol. Metabolites of these compounds, e.g. to 11-hydroxy analogs, are poorly active *in vivo* [59].

Because both the *in vitro* transcriptional data and the *in vivo* estrogenic activity in laboratory animals show PDC-7 to be more active than estradiol and PDC-7 was designed as a better fit in DNA, it is very likely that estrogens are being inserted into DNA during their mode of action. Moreover, the relatively weak binding of PDC-7 and certain other potent estrogens to the estrogen receptor which is consistent with results of computer modeling of their docking into the estrogen receptor LBD has important consequences. Namely, while binding to the receptor is necessary to get the ligand to the DNA, it follows that the unbinding from the receptor and transfer of the ligand to the DNA ultimately results in full transcriptional activity. Such a scenario explains the apparent paradox throughout the nuclear receptor superfamily described earlier i.e. that strength of receptor binding does not correlate with biological activity. Further support for this notion is provided by independent studies of certain indenestrols which are strong binders to the estrogen receptor but weak estrogens *in vivo*. Very strong binders such as the indenestrols [62, 63] may be transferred poorly to the DNA. Compounds that are strong receptor binders and poor fits in DNA as is the case with indenestrols would also have poor activity.

The lack of specificity of ligand binding among certain members of the nuclear receptor superfamily described above may also be explained by transfer of ligand to DNA e.g. the mineralocortcoid receptor which binds progesterone and cortisol with equal affinity [23, 24]. Given the apparent precision of the putative transfer of ligand coupled with the remarkably conserved tertiary structure of the LBDs, it is likely that specific amino acids unique to individual receptors will be found to be critical for guiding certain ligands to the DNA thereby giving rise to specificity of action. By helping maintain the structural integrity of the LBD of receptors and thus transfer of ligand, co-activators will also be critical components that likely govern both the specificity and potency of candidate ligands.

IMPLICATIONS OF THE NEWLY DISCOVERED ROLE OF NUCLEAR RECEPTORS: A FINELY TUNED MOLECULAR GUIDANCE SYSTEM FOR REGULATING GENES

Intercalation in DNA and Topoisomerases

Classical intercalators are generally flat aromatic molecules that fit easily between base pairs in DNA. Although some intercalators may be transferred to DNA by nuclear receptors, most have not been shown experimentally to insert between base pairs by themselves. We have previously used the term nonclassical intercalators to describe compounds that have a greater molecular thickness but are still capable of fitting between base pairs [28,44]. The agonist ligands in the nuclear receptor superfamily shown here fall into that category and would not be expected to facilely intercalate on their own. We envision that the nuclear receptors, coactivators and possibly other factors provide the capacity to unwind the DNA and guide nonclassical intercalation. Precedence that the action of nuclear proteins can guide molecules into DNA is provided by certain topoisomerases that can form complexes with DNA aiding the intercalation of some anticancer drug ligands such as the camthothecin topotecan [64].

The role of intercalation in the genomic action of drugs and natural ligands has been recently reviewed [28,65]. The reader is also directed to other recent and more general reviews of intercalating drugs which are taking on a rapidly expanding role in pharmaceutical development [66-71].

Revised Mechanism of Receptor Mediated Ligand Insertion into DNA

Based upon the weight of experimental evidence reported here and in previous publications, we extend ligand insertion

hypothesis in the following manner. The conformational change in the receptor elicits a conformational change in the DNA that is proposed to involve: 1) folding of the protein such that the LBD faces the DNA; 2) unwinding of the DNA in the cognate response element to create a ligand intercalation site; 3) intercalation of ligand in the site in DNA. The intercalation site results from local unwinding at the locus 5'-CA-3'·5'-TG-3' at the end of the half sites of the HRE. Transcription is the result of the proper physicochemical arrangement of the nuclear receptor, DNA and intercalated ligand. The receptor orients the ligand such that it is inserted into DNA from the major groove approaching from the 5'-CA-3' strand.

The conformational change in the receptor may allow the protein to wrap around the DNA such that the N-terminal domain of the receptor participates in the stabilization of the transcription complex [38-40]. Such a model could allow the N-terminal and C-terminal portions of the receptor protein to interact with each other while wrapped around the DNA. It is expected that the receptor protein will also provide added specificity to the intercalation of various ligands i.e. by modulating hydrogen bonding relationships as previously postulated [5-7].

Antagonists, Ligand Independent Transcription, Orphan Receptors and Co-regulators

The focus of this review has been purposefully limited in scope to nuclear receptor agonists. The critical role of antagonists in the current proposed mechanism is mentioned only briefly here but will be the subject of subsequent papers. In short, the putative molecular guidance system provides many ways one can design an antagonist. For example, we have previously proffered that many antagonists, e.g. the antiestrogen and breast cancer drug tamoxifen, can intercalate into the same site as agonists but with extensions out of the unwound DNA cavity and different hydrogen bonding patterns to DNA than the agonists [72]. These observations have been employed in the development of antagonists [73]. If ligand insertion is occurring with agonists as described above, antagonists would be agents that attenuate the formation of the proper transcriptional machinery. Studies by other investigators demonstrate that the tertiary structure of the LBDs of nuclear receptors bound to antagonists are different than those bound to agonists. In particular, there is a well described difference in the orientation of helix 12 [74] and this observation has been employed in the development of antagonists. In our model, helix 12 is facing the DNA surface at the intercalation site and would be involved in the exit of ligand from the receptor and interaction with the transcriptional complex.

Another way to create an antagonist is to design a compound that binds directly to hormone response elements at the intercalation site of agonists i.e. 5'-CA-3'·5'-TG-3' (Fig. (**2**)) [72]. Such a compound would block agonists from intercalating and forming a functioning transcriptional complex. The compound need not bind to the receptor to have such an effect [72]. In fact, a new drug candidate developed using this strategy has been shown to intercalate into the two sites in the ERE at (5'-CA-3'·5'-TG-3') and inhibit transcription by preventing the estrogen receptor complex from binding to DNA [75]. This bis-intercalator, XR5944, has been tested in Phase I clinical studies of cancer subjects but, to our knowledge, has not been given to patients with estrogen-driven tumors such as breast cancer [76]. In our model, such compounds should prevent the receptor mediated transfer and insertion of ligand by competing with the estrogen receptor for binding to DNA at the half sites. Ligand insertion would be impaired as the ligand biding domain would be incapable of folding properly around the DNA.

It is well established that genes can be activated by nuclear receptors without ligands (either inhibited or enhanced) [18]. Even in ligand dependent systems, there is a certain degree of basal transcriptional activity but introduction of ligand usually induces activity multifold. Our current thinking is that ligand should not be necessary to elicit some activity if the proper transcriptional complex has been established. That could be achieved by folding of the receptor without ligand particularly if the tertiary structure of the ligand binding domain is the same with and without ligand. Further light may be shed when the X-ray structures of apo receptors (those without bound ligand) become generally available. Our examination of the LBD of the apo retinoid x receptor (RXR) [77] reveals a distortion relative to the LBD bound to the ligand 9-cis-retinoic acid. Namely, the putative channel in the receptor is partially collapsed and the LBD does not form a complementary surface with the unwound DNA. Such a conformation without ligand would probably attenuate transcription. Initial examination of the LBDs of certain orphan receptors show common tertiary structural features with the ligand activated receptor but not the apo receptor RXR. In some cases, the area of the LBD which would normally bind ligand is occupied by amino acids. While it may not be possible for ligand to be transferred to the DNA i.e. there is no obvious ligand binding pocket, a conformational change in the receptor is still possible orienting the LBD facing the DNA thereby resulting in constitutive transcriptional activity. Other orphan receptors may not be able to form a ligand binding pocket in DNA due to an inability to unwind DNA. We have already commented on the possibility that certain ligands bind to nuclear receptors yet may not be transferred (or transfer is impaired) i.e. as may be the case with the bile acid/FXR receptor complexes. We also envision that there will be receptors that do not have classical binding pockets but can be activated by ligands that bind at other locations in the LBDs. Such receptors might undergo conformational changes that interfere with natural gene regulation as may be the case with certain pesticides known to interact with nuclear receptors [78].

If the ligand insertion mechanism is taking place, co-regulatory proteins may play a critical role. Our initial examination of the structures of the LBDs of several nuclear receptors bound to co-activators shows that the locations of the co-activators are not near the putative LBD/DNA interface. Conversely, certain co-repressors are located where they could interfere with transfer of ligand to DNA e.g. near helices 11 and 12. If such observations can be generalized, it would help explain how these proteins function.

Genotoxicity and Alterations of the Putative Molecular Guidance System

One of the obvious concerns during drug discovery is avoiding the development of compounds that exhibit unexpected toxicity. In the case of certain genotoxins, their adverse effects can be attributed to covalent binding to DNA thereby damaging gene function. Examples include certain carcinogens e.g. aflatoxins [79] and benzo[a]pyrene oxides [80] that intercalate and form covalent complexes with DNA. Some drug discovery programs attempt to design compounds that are incapable of intercalation. Our laboratories have developed methods to screen for such compounds using computer modeling of intercalation complexes [81,82]. A good correlation was observed between the capacity to intercalate and various *in vitro* tests for intercalation. These correlations have been discussed in several recent reviews [26,65 and references therein]. In short, while it is important to consider whether a drug candidate intercalates, ruling out such compounds in drug discovery should be dependent upon the exact nature of the interaction with DNA. Covalent interactions of a drug with DNA are likely to damage gene function but may be desirable e.g. in attempts to destroy certain aberrant genes. Compounds that reversibly interact with DNA and do not cause damage to the DNA, which would include agonist ligands in the nuclear receptor superfamily, may be highly desired. If the mode of action of agonist ligands is dependent upon proper guidance by nuclear receptors to intercalation sites, designing out intercalation would result in compounds that are inactive or poorly active at best. Moreover, compounds that bind only to the receptor and are not capable of intercalation could prevent normal gene function.

Drug Design and Computational Technology for Drug Discovery

Our laboratories have developed an integrated drug design technology based upon the capacity of drugs to interact with DNA in concert with nuclear receptor proteins. Reviews of these drug discovery tools have been recently published [28,81]. Briefly, pharmacophores and search engines have been created based upon intercalation of agonists, antagonists and various toxins in DNA. These tools can be employed to simultaneously screen large numbers of compounds for various activities and potential untoward effects e.g. genotoxicity. While such technology has proven useful in the design of novel drug candidates as described above, an equally valuable application is the prediction and detection of unanticipated side effects of such compounds.

Caveats

While the ligand insertion hypothesis is clearly strengthened by the discovery of the alignment of the LBDs and associated ligands with the cognate unwound response elements in DNA, much is unknown about this putative process. Most importantly, because the three dimensional structures of full length receptors are not yet available, one cannot predict with certainty the structure of the transcription initiation complexes. Clearly, each complex will be different and dependent not only on the structure of the receptor but also the composition and arrangement of response elements, the structure of intercalated ligands, whether a given receptor forms homodimers and heterodimers (including orphan receptor partners), the proper binding of co-activators and co-repressors, etc. It follows that alteration of any component of a given transcription complex, especially those that effect unwinding of DNA and ligand transfer, will have important consequences and may be associated ultimately with various diseases. These could include mutations in parts of the receptors forming contacts with DNA, changes in base composition of the HREs, and changes in those amino acids guiding transfer of ligand in the LBD. Specific examples would include changes in amino acids at key locations in LBDs including helix 8 and helix 11 which are proximal to the DNA. Although apparently distal to the site of ligand exit from the LBD, ongoing studies indicate that mutations in co-activators could have profound effects on this process. Certain amino acids in parts of the LBD not directly involved in contacting ligand are also likely to be important e.g. those involved in folding the receptor and unwinding the DNA.

We wish to emphasize that while we have previously discussed in detail evidence for the ligand insertion mechanism [21], the ability to orient the LBDs of receptors to their cognate response elements in the manner described is new and surprising. Clearly, the almost exact match of the orientation of ligands in the receptor with the orientation we had reported in unwound DNA of HREs are not chance observations. In the case of estrogens (Fig. (**3**)), we have not been able to arrive at any orientation of the LBD with the ERE that would permit close contact of the two estradiol molecules other than that shown. The distance that estradiol, and for that matter other ligands, need to travel to contact the DNA in this orientation is very short i.e. approximately the length of the ligand itself (15 Å). This distance approximates the minimum distance that is required for radioactively labeled estrogens to damage DNA [30]. Other orientations of the estrogen receptor LBD result in one or both of the ligands as much as 40 Å away from the surface of the DNA and in some cases orthogonal to the orientation in the DNA. Moreover, the preferred orientation of ligands to be transferred to the DNA is also the best orientation for the surface of the LBD to form a complementary complex with the unwound ERE. These results are strong support for the premise that the LBD of the full length receptor is folded back on the DNA with the ligand proximal to the DNA surface. A major unanswered question remains, however, i.e. what is the driving force for ligand insertion. Our studies using energy calculations indicate that the electrostatic fit of estradiol in DNA is better than in the receptor suggesting that there may be a delicate balance between the fit in the receptor versus DNA. However, conclusions from such calculations are limited by the static nature of the models. Clearly, ligand binding is known to cause a reordering of helices in the LBD of the receptor accompanied by very specific conformational changes in the receptor/DNA complex. Such ordering may give rise to a lowering of entropy in the transcriptional complex. Clues to this process may be gained from molecular dynamic studies that have demonstrated the exit of ligand from the receptor can take several paths including a novel one that involves helices 8 and 11 consistent with our model [41]. Given the structural plasticity of ligand binding pockets [83], it is expected that the HREs will have

important allosteric effects on the receptors as proposed by others [84].

The theoretical framework provided here raises many more questions than it answers. Is the process reversible, does the DNA structure remain intact, what eventually happens to the ligands and their receptors, and how did such a system evolve? As such, we must caution against over interpretation of the current findings. Although models have inherent value in drug discovery and are essential to advancing the field, they are snapshots of a complex dynamic process and thus only approximations of what may be occurring *in vivo*. Space considerations do not permit discussion of all of our current findings and relevant literature including studies of other important receptors e.g. PPAR (peroxisome proliferator-activated receptor) isoforms. As new evidence becomes available, modification, refinement and reinterpretation of existing data are expected. Current publications are intended to provide a testable hypothesis for future experimental studies.

SUMMARY AND CONCLUDING REMARKS

Thirty years ago, the study of compounds that interact directly with DNA and those that act indirectly with genes through nuclear receptors diverged. The former led to the discovery of numerous drugs designed to bind to DNA as well as intercalate between base pairs during their mode of action. The latter resulted in drugs whose effects are known to be transmitted to DNA through specific conformational changes in receptors bound to DNA. Such changes are brought about by binding of ligands to the receptor.

Based upon multiple lines of evidence, we have advanced the hypothesis that nuclear receptors mediate the transfer of ligands to sites in DNA. New compelling evidence is presented here based upon available X-ray crystal structures of LBDs. Namely, the orientations of ligands in the receptors and their correspondence to one another matches those previously published based upon intercalation into sites in unwound DNA. Moreover, when oriented toward unwound HREs, the ligand binding domains align the ligands with the unwound sites. Experimental evidence that ligand is being transferred is provided by the drug PDC-7 which binds weakly to the estrogen receptor but has potent transcriptional activity. The transcriptional activity of PDC-7 matches *in vivo* potency and is approximately 3 times greater than the natural hormone estradiol. PDC-7 was designed by computer modeling fit into the unwound HRE. Taken as a whole, these findings force a convergence in thinking about how drugs regulate genes and extend the ligand insertion hypothesis. Namely, nuclear receptors in concert with other co-regulatory proteins provide a finely tuned molecular guidance system in which ligands are placed precisely in intercalation sites in DNA. Alterations of this guidance system interfere with normal physiological activity and are proposed to be associated with altered gene function including the presentation of various diseases. Exploiting this mechanism will be key to the future development of many gene regulatory drugs.

ABBREVIATIONS

HRE	=	Hormone response element
ERE	=	Estrogen response element
LBD	=	Ligand binding domain
DNA	=	Deoxyribonucleic acid
DBD	=	DNA binding domain
PDB	=	RCSB Protein Data Bank
L	=	Ligand
3-CDCA	=	3-deoxychenodeoxycholic acid
PDC-7	=	11β-methoxy-7α-methylestradiol
moxestrol	=	11β-methoxy-17α-ethinylestradiol
PPAR	=	Peroxisome proliferator-activated receptor
E2	=	Estradiol
T_3	=	3,5,3'-triiodothyronine
FXR	=	Farnesoid X receptor

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Received: 02 May, 2008 Revised: 29 August, 2008 Accepted: 09 September, 2008