NUCLEAR RECEPTORS: THE CONTROLLING FORCE IN DRUG METABOLISM OF THE LIVER?

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Keywords: PXR, CAR, PPAR, VDR, Cross talk

Abbreviations: CAR, constitutive androstane receptor; HNF, hepatocyte nuclear factor; LATF, ligand-activated transcription factor; LETF, liver-enriched transcription factor; NR, nuclear receptor; PPAR, peroxisome proliferator-activated receptor; PXR, Pregnane-X receptor; SHP, small heterodimer partner

ABSTRACT

The body is in a constant battle to achieve homeostasis; indeed the robustness to which it can respond to moves away from homeostasis is a vital part in the survival of the organism as a whole (Kitano 2004). There thus exists a need for a network of sensors that are able to capture, interpret and respond to alterations in chemical levels that move the body away from homeostasis and this applies to both endogenous and exogenous chemicals. With respect to external chemicals (xenobiotics), this xenosensing is often carried out through specific interactions with cellular receptors. The phenomenon of "xenosensing" has attracted much interest of late, whereby xenobiotics interact with receptors resulting in the activation of a battery of genes mediating oxidative drug metabolism, conjugation and transport, thereby enhancing the elimination of the xenobiotic by the organism (Gibson et al. 2006). However, this beneficial response is counterbalanced by the increasingly-recognised role of nuclear receptors in mediating drug-drug interactions via enzyme induction (El-Sankary et al. 2001) or the production of toxicity through interaction with endogenous pathways (Dussault et al. 2003; Krasowski et al. 2005; Saini et al. 2005). This review will focus on the role of nuclear receptors in mediating these effects, and how such knowledge will contribute to a mechanism-based risk assessment for xenobiotics.

THE NUCLEAR RECEPTOR SUPERFAMILY

The nuclear receptors form a sub-family of the ligand-activated transcription factors (LATFs), with 48 members being present in humans (Zhang et al. 2004). This number remains relatively constant in higher organisms, with mice and rats having 49 and 47 respectively (Zhang et al. 2004), but outside of the animals this number differs significantly. For example, the telosts (bony fish) have 68 nuclear receptors (Maglich et al. 2003a), a result of a part-genome duplication event that occurred in the actinopterygii lineage approximately 320 million years ago; during this part-genome duplication, approximately 29 % of all *Takifugu* genes underwent duplication (Vandepoele et al. 2004).

Organism-wide tissue profiling suggests that the nuclear receptors play important roles in a number of biological functions, including development, steroidogenesis, energy homeostasis and lipid/drug metabolism (Bookout et al. 2006). In this review we will focus on the role of the major nuclear receptors in the liver and how they control levels of both endogenous chemicals and xenobiotics.

GENERAL STRUCTURE OF NUCLEAR RECEPTORS

As can be seen from Figure 1, the general structure of a nuclear receptor is relatively simple, being comprised of only five sub-regions. The amino terminus is known as the modulatory A/B domain, where the transcriptional activation function (AF-1) is located. This AF-1 domain, along with the AF-2 domain at the carboxyl terminus, is responsible for receptor dimerisation, nuclear localisation and co-activator and co-repressor binding (Leo and Chen 2000; McInerney et al. 1996).

The C-domain, also known as the DNA-binding domain (DBD), is a highly conserved region that has the ability to recognise specific response elements and, hence, initiate transcription of targeted gene sets (Bourguet et al. 2000; Watkins et al. 2003). This DBD consists of two zinc fingers within 60-70 amino acids, plus a carboxyl terminus extension that contains T&A boxes, which are essential for functioning of monomeric nuclear receptors. The first zinc finger contains a highly conserved sequence, the P-box, which is involved in binding between the receptor and the DNA helix. In contrast the D-box region of the second zinc finger is involved in protein-protein interactions and is responsible for binding to response elements within DNA (Aranda and Pascual 2001; Francis et al. 2003). The consensus binding site for the nuclear receptor family members is AGGTCA, with receptors binding to this as either monomers or dimers, the latter being either heterodimers (e.g. PXR/RXR) or homodimers (e.g. GR). Specificity in receptor binding is achieved through both the orientation of the binding sites, which may exist as inverted, everted or direct repeats, and their separation, which varies from 1 to 8 nucleotides. Whereas some nuclear receptors show high stringency towards a single configuration, with the oestrogen receptor binding only to a response element (Metivier et al. 2003), other receptors show more flexibility; the PXR/RXR heterodimer, for example, can bind to DR3, DR4, ER6 or ER8 motifs (Gibson et al. 2006).

Separating the DBD and the ligand binding domain (LBD) is the D-region, which acts as a hinge and allows the receptor to bend and undergo conformational changes in response to ligand binding. Finally, the E region contains the LBD, which is comprised of ten α-helical segments that flex upon ligand binding and cause alterations in co-regulator binding within the AF-1 and AF-2 domains (Ekins and Schuetz 2002; Shao et al. 2004; Watkins et al. 2003).

LIVER-ENRICHED NUCLEAR RECEPTORS

Whereas the majority of tissues within the body express a sub-set of the nuclear receptors, it is in the liver that the majority of nuclear receptors targeted towards xenosensing are located (Bookout et al. 2006). This is a consequence of the unique location of the liver, being the first major organ that orally absorbed compounds encounter, plus the high systemic blood flow through the organ.

These metabolic NRs can be generally categorised into those that mediate transcription of genes whose products that are generally associated with drug metabolism, such as the pregnane X receptor (PXR, NR1I2) and the constitutive androstane receptor (CAR, NR1I3) and those that are important in the metabolic regulation of endogenous compounds. This latter group includes chemicals such as glucocorticoids (Glucocorticoid receptor, GR, NR3C1), lipid oxysteroids (liver X receptor, LXR, with LXRα, NR1H3 being the liver-predominant form), bile acids (farnesoid X receptor, FXR, NR1H4) and lipid metabolism (PPARα, NR1C1). Finally, the hepatocyte nuclear factor nuclear receptors (HNF1, 3 and 4; NR2A21) are fundamental to the functioning of the liver, controlling many basic processes as well as the expression of several other nuclear receptors.

CONSTITUTIVE ANDROSTANE RECEPTOR (CAR, NR1I3)

The constitutive androstane receptor is a LATF that can be termed an orphan receptor, in that no endogenous ligand has been identified to date (Honkakoski and Negishi 2000). However, in contrast to almost every other LATF, CAR is constitutively active unless silenced by the presence of androstane, and is therefore alternatively known as the Constitutive Active Receptor (Sueyoshi et al. 1999). In the absence of activating ligand, CAR appears to be predominantly located in the cytoplasm (Kawamoto et al.

1999), where it is sequestered by the CAR cytoplasmic retention protein, CCRP (Squires et al. 2004). Upon activation, CAR localisation switches to predominantly nuclear and activates target genes as a heterodimer with RXRα (Baes et al. 1994; Kawamoto et al. 1999). CAR is activated by a diverse range of xenobiotics, including 1,4-bis (2-(3,5-dichloropyridoxyloxy)) benzene (TCPOBOP) and phenobarbital, as well as endogenous chemicals such as bilirubin (Saini et al. 2004). Interestingly, whereas TCPOBOP activates CAR via a traditional binding mechanism, both phenobarbital and bilirubin have been shown to not physically bind to CAR, but can still activate the receptors and cause nuclear translocation (Kawamoto et al. 1999; Saini et al. 2004). CAR has also been shown to share some ligands with PXR. Both phenobarbital and clotrimazole are ligands for both PXR and CAR (Moore et al. 2000), although as noted previously there is some specificity with TCPOBOP being murine CAR-specific (Wei et al. 2000), whereas CITCO is human CAR-specific (Maglich et al. 2003b).

CAR binding to regulatory regions of target genes appears to occur at a number of different response elements: Activation of CYP2B genes occurs through binding to an imperfect DR-4 site within the phenobarbital response element (PBRE) (Honkakoski et al. 1998). However, CAR has also been shown to be capable of binding to the ER6 normally bound by PXR within the proximal promoter of CYP3A4 (Sueyoshi et al. 1999; Xie et al. 2000). This is presumably due t the similarity of DNA binding domains, with CAR sharing 66% identity with the DBD of the PXR(Blumberg et al. 1998).

FARNESOID X RECEPTOR (NR1H4)

The farnesoid X receptor (FXR) is a nuclear receptor known to be a bile acid sensor (Makishima et al. 1999). It is most highly expressed in the liver, intestine and kidney

(Bookout et al. 2006) and forms a heterodimer with the RXRα upon activation by bile acids (Makishima et al. 1999). This heterodimer binds to an inverted repeat with one separating nucleotide (IR1) within the regulatory regions of target genes (Pineda Torra et al. 2003).

As stated above, FXR is involved in the regulation of the bile salt homeostasis, and in particular the regulation of their biosynthesis. FXR is able to down regulate the expression of the central enzyme involved in the bile salt biosynthesis, namely cholesterol 7α hydroxylase (CYP7A1), although this action appears to be indirect; FXR induces the expression of the small heterodimer partner (SHP), an unusual nuclear receptor that lacks a DBD. SHP forms a heterodimer with the orphan nuclear receptor the liver receptor homolog 1 (LRH-1), which then inhibits the transcriptional activation of LRH-1 towards CYP7A1 and CYP8B1 (Bavner et al. 2005; Eloranta et al. 2005). In addition to directly impacting on bile salt biosynthesis, FXR also regulates genes whose products affect bile salt disposition, generally acting to concentrate bile salts within the liver, ready for excretion. These genes include OATP8, an SLCO uptake transporter with specificity towards organic anions, xenobiotics, and peptides(Jung et al. 2002) and the hepatic canalicular bile salt export pump (BSEP) (Plass et al. 2002). FXR also induces the human MDR-3 mediating the phospholipids secretion into bile (Huang et al. 2003) and ileal bile acid binding protein, a protein for the transport of the bile salt in the intestine back to the liver (Grober et al. 1999). Finally, activation of FXR not only impacts on bile salt biosynthesis, but also lowers triglyceride levels in both the liver and serum, again through activation of SHP (Watanabe et al. 2004).

It should be noted that FXR does not only regulate genes whose products are involved in the biosynthesis of bile salts, but also genes metabolic genes such as UGT2B4 (Barbier et

al. 2003a) and SULT2A1 (Song et al. 2001) as well as the drug transport protein ABCC2 (Kast et al. 2002).

Overall, the FXR plays an important role in maintaining bile acid homeostasis in the body, by repressing their biosynthesis, inducing their conjugation, and their elimination.

GLUCOCORTICOID RECEPTOR (GR, NR3C1)

The glucocorticoid receptor (GR) is one of the most characterised nuclear receptors, having been studied for over fifty years. In a similar fashion to CAR, when not activated by ligand the majority of GR protein is sequestered in the cytoplasm in a complex with heat-shock protein 90 (Tago et al. 2004). Upon binding of a ligand, heat-shock protein dissociates from the receptor and GR translocates to the nucleus (Freedman and Yamamoto 2004). However, in contrast to CAR, GR functions as a monomer, with no equivalent of RXRα binding to the activated receptor (Freedman and Luisi 1993).

Interest in GR with respect to target genes involved in drug metabolism has existed because of a seeming paradox: No consensus binding site for GR was identified in the CYP3A4 regulatory regions, despite the fact that treatment of hepatic cells with dexamethasone induces the expression of CYP3A4 in a GR-dependent manner (El-Sankary et al. 2000). In addition, several lines of evidence showed that the CYP3A enzymes were induced in vivo by glucocorticoids, including the assessment of the ratio of urinary 6-β-hydroxycortisol to cortisol and the increase in metabolic ratio observed in the presence of rifampicin (Pascussi et al. 2003b). In addition, it was observed that the classical anti-glucocorticoid PCN was also able to activate CYP3A gene transcription (Plant 2007), and that the activation of CYP3A transcription by glucocorticoids was maintained in GR null mice (Schuetz et al. 2000). These two latter pieces of evidence suggested that there existed a non-GR mediated activation of CYP3A genes by

glucocorticoids, and this was confirmed through the identification of PXR as a low affinity glucocorticoid sensor (El-Sankary et al. 2001; Pascussi et al. 2000a; Pascussi et al. 2003a). A role for GR in the transcriptional activation of CYP3A genes still exists however, although through an indirect mechanism. Glucocorticoids have been demonstrated to activate the expression of a number of genes encoding LATFs, including PXR, RXRα and CAR, through the sub-micromolar activation of GR (Pascussi et al. 2000a; Pascussi et al. 2000b; Pascussi et al. 2003a); the net effect of this is a feed-forward loop that ultimately increases the expression of target genes for these receptors, including CYP3A4, CYP2B6 and CYP2C8/9. Hence, at low concentrations of glucocorticoids, body responses are mediated through GR interactions with other nuclear receptors, whereas higher concentrations of glucocorticoids activate these nuclear receptors directly. Such a two-tier response system provides the most efficient response to stimuli.

In summary, the GR acts as a central hub for nuclear receptors, not only controlling the expression of a number of genes whose protein products are central to metabolism, but also regulating the expression of a number of other NRs, and, hence, indirectly the expression of their target genes.

Hepatocyte Nuclear Factor 1 (HNF1)

The hepatocyte nuclear factor 1 family (HNF1) are expressed mainly in the liver, kidney, intestine and pancreas (Cereghini 1996), with HNF1α being the predominant isoforms. HNF1α has been shown to be a positive regulator for a number of cytochrome P450 enzymes, such as CYP1A2 and CYP2E1 (Akiyama and Gonzalez 2003), and a negative regulator of CYP4A, CYP7A1 and CYP27 hydroxylase enzymes (Cheung et al. 2003). This regulation of cytochrome P450s involved in fatty acid metabolism could, in part, explain

the phenotype of patients with MODY 3 diabetes, which results from a mutation in the HNF1α gene (Elbein et al. 1998). These patients suffer from high levels of fatty acids and increased resistance to anti-diabetic drugs (Elbein et al. 1998).

Taken together, it can be seen that HNF1α plays an important role in the regulation of CYPs involved in drug metabolism and toxicity, as well as bile acid and fatty acid metabolism.

HEPATOCYTE NUCLEAR FACTOR 3 (HNF3)

HNF3 exists in three isoforms, HNF3α, HNF3β and HNF3γ, which have 90% identity in their DNA binding domains, and indeed bind to the same response element in target genes (Cereghini 1996). In HNF3β null mouse embryonic cells, the expression of HNF4α and HNF1α are both reduced, and HNF3α expression was undetectable, suggesting that HNF3β is an important regulator of the expression of other liver-enriched transcription factors, including HNF1α, HNF3α and HNF4α (Duncan et al. 1998).

HNF3 has been linked to the regulation of a number of cytochrome P450 enzymes, including CYP2C (Bort et al. 2004) and CYP3A4. Interestingly, in the latter case there are two response sites that appear to function differently; disruption of one HNF3/CEBPα binding site within the CYP3A4 proximal promoter reduced the xenobiotic-mediated expression of CYP3A4 by glucocorticoids but not the macrolide antibiotic rifampicin, (El-Sankary et al. 2002), whereas disruption of a second binding site for HNF3 within the CYP3A4 proximal promoter led to a diminished activation of CYP3A4 expression in response to phenobarbital and clotrimazole, and an increase in response to metyrapone (Bombail et al. 2004).

Taken collectively, these data suggest that HNF3s play an important role in regulating/refining the activation of cytochrome P450 gene expression in both basal and in response to different xenobiotic exposure.

HEPATOCYTE NUCLEAR FACTOR 4 ALPHA (HNF4, NR2A2)

HNF4 is a liver-enriched transcription factor that is classified as an orphan nuclear receptor due to its lack of known endogenous ligand (Cereghini 1996); however, the elucidation of the HNF4 crystal structure complexed with a fatty acid suggests that this class of chemicals may act as endogenous ligands, consistent with the known role of HNF4 in fatty acid sensing (Dhe-Paganon et al. 2002). The LBD of HNF4α contains two binding sites, an acyl binding site and a site conferring thioesterase activity, with both of these sites able to modulate the transcriptional activity of the HNF4α (Hertz et al. 2005). In addition to a potential role in fatty acid homeostasis, HNF4 has also been reported to be involved in cholesterol and glucose metabolism (Gold et al. 1999; Spath and Weiss 1997), foetal development (Kamiya et al. 2003) and liver maturation (Watt et al. 2003).

With respect to drug metabolism, HNF4α has been associated with the expression of a number of cytochrome P450 expression, including CYP3A4, CYP3A5, CYP2A6 and to a lesser extent CYP2B6, CYP2C9 and CYP2D6 (Jover et al. 2001). Control of cytochrome P450 expression may occur at two levels: First, direct activation of cytochrome P450 promoters, such as occurs within the CYP3A4 promoter (Ogino et al. 1999). Second, HNF4α can activate other nuclear receptors, which in turn regulate cytochrome P450 target genes; examples of this latter scenario include activation of the nuclear receptors CAR and PXR (Tirona et al. 2003). With respect to activation of CYP3A4 transcription, this occurs through an HNF4α response element within the CYP3A4 distal promoter, where HNF4α enhanced the PXR-mediated transactivation of CYP3A4 in both basal

conditions and in presence of the PXR ligand, rifampicin (Tirona et al. 2003). In a similar fashion, HNF4α can impact upon expression of PXR and CAR, in foetal and adult liver mouse respectively. The molecular mechanism for this induction by HNF4α is via an interaction with response elements within the proximal promoters of both PXR and CAR (Ding et al. 2006; Kamiya et al. 2003).

Taken together, these data show that the HNF4α is involved in the regulation of the transcription of several cytochrome P450 genes by direct binding to its promoter, but also through regulating expression of nuclear receptors; these, in turn, regulate their target gene sets, which include many drug metabolising enzymes and cytochrome P450s.

PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR ALPHA (NR1C1)

The peroxisome proliferator-activated receptors (PPARs) are involved in regulating the expression of genes involved in lipid metabolism (Francis et al. 2003). Peroxisomes are small cytoplasmic organelles that perform both β-oxidation of fatty acids and cholesterol metabolism. There are three PPAR isoforms: PPAR α, PPARβ or δ and PPARγ, which have been shown to activate distinct, but overlapping, target gene sets (Lee et al. 2003). PPARα is the major family member in the liver, but plays a role in fatty acid metabolism in several other organs, including the kidney and intestine, where it is also highly expressed (Bookout et al. 2006). PPARs forms a heterodimer with RXRα when activated, with the resultant complex binding to an imperfect direct repeat of AGGTCA separated by one nucleotide (DR1), and this was the first demonstration that the 5' flanking region of the binding site also influenced nuclear receptor binding (Juge-Aubry et al. 1997).

PPARα is activated by endogenous ligands, fatty acids, both saturated and unsaturated fatty acids, with a preference for higher acyl chain length species. Xenobiotic ligands for PPARα include hypolipidemic drugs such as the fibrates, as well as some herbicides, plasticizers and food flavourings (Francis et al. 2003). Classic target genes for PPARα include CYP4A and acyl CoA oxidase (Bell et al. 1991). However, in addition to stimulation of β-oxidation, PPARα has also been implicated in the regulation of the human SULT2A1 gene (Fang et al. 2005), human UGT2B4, murine UGT2B (Barbier et al. 2003b), and cholesterol 7α-hydroxylase (CYP7A1) and sterol 27-hydroxylase (CYP27) (Post et al. 2001).

PPARγ shows a much more restricted expression profile, being expressed predominantly in adipose tissues, intestine and spleen (Bookout et al. 2006). Its major physiological role is in regulating adipocyte differentiation, with polyunsaturated fatty acids as its major endogenous ligands (Lee et al. 2003).

The final member of the PPAR sub-family is PPARβ/δ, which is expressed nearly ubiquitously (Bookout et al. 2006). The exact role of PPARβ/δ is still a matter of debate, although a role in differentiation has been postulated (Werling et al. 2001).

PPARα has been of considerable interest due to a striking species difference that occurs upon activation of the receptor. In rodents exposed to peroxisome proliferators in long term studies, hepatocarcinogensis is evident, primarily due to an increase in cell division and decrease in apoptosis, leading to hepatocyte hyperplasia and fixation of spontaneous mutations (Plant et al. 1998a; Plant et al. 1998b). However, peroxisome proliferating chemicals are still used today, and in fact human exposure is relatively frequent: How can this be reconciled with the known carcinogenic effects observed in rodents? The use of PPARα null mice demonstrated that the hyperplasia was PPARα-

dependent (Lee et al. 1995), although there is some evident to suggest that PPARα-independent mechanisms may also exist (Crunkhorn et al. 2004), and hence the higher levels of PPARα in rodents compared to humans may be responsible for species differences in peroxisome proliferation and cancer (Peters et al. 2005), with humans expressing much lower levels (Palmer et al. 1998). There has also been shown to be species difference in the effect of PPARα-activation, with increased fatty acid oxidation but not peroxisome proliferation occurring in humans (Choudhury et al. 2000). Recently, several potent PPARα/γ agonists have been developed that are capable of eliciting peroxisome proliferation in higher primates (Cariello et al. 2005), and this has again opened the debate on the correct risk assessment for this class of chemicals.

Taken together, it is clear that PPARα regulates several endogenous processes, including bile and cholesterol metabolism, and in doing so its functions overlap with those of the pregnane X receptor.

PREGNANE X RECEPTOR PXR (NR1I2)

The Pregnane X receptor (PXR) also termed the pregnane activated receptor (PAR) and steroid X receptor (SXR), is a LATF activated by naturally occurring pregnanes and was initially identified in mouse liver (Kliewer et al. 1998). PXR is initially expressed at very low levels in the mid-foetal liver, and this expression increases postnatal and throughout adult life (Masuyama et al. 2001). In addition to the liver, PXR is also highly expressed in the intestine as well, mirroring the expression of its target genes, such as the CYP3A family members (Bookout et al. 2006).

PXR has been shown to interact with CCRP, and HSP90, which tend toward cytoplasmic retention of the protein in HepG2 cells (Squires et al. 2004). Localization of PXR is, as with CAR, equivocal with differing results seen in mouse liver, mouse primary

hepatocytes and cell lines (Squires et al. 2004). What is clear however the requirement for PXR to interact with the nuclear import machinery via a nuclear localisation signal, as is seen for CAR (Kawana et al. 2003).

As detailed previously, both GR and HNF4α have been demonstrated to regulate the expression of a number of different nuclear receptors, including PXR, and hence may be seen as master regulators. In comparison, analysis of the PXR regulatory regions suggests that it is able to be regulated by a wide range of nuclear receptors itself, rather than impact on the expression of other NRs (Aouabdi et al. 2006; Gibson et al. 2006). In particular, PPARα has been shown to be able to activate PXR gene expression (Aouabdi et al. 2006), as well as GR (Pascussi et al. 2000a) and HNF3(Gibson et al. 2006). Further analysis of the PXR regulatory regions also suggests regulation by CAR, VDR and ERα (Gibson et al. 2006). Taken together, this would suggest that PXR is able to integrate signals from a great many nuclear receptors, potentially placing it at an important node in the biological response network to xenobiotics.

PXR is a central mediator of CYP3A gene expression, being activated by many of the xenobiotics that induce CYP3A expression, and which are substrates for CYP3A (El-Sankary et al. 2002; El-Sankary et al. 2000). This role as a central mediator of CYP3A activity is further confirmed by studies in the PXR knock out mouse, which is non-responsive to the CYP3A inducers dexamethasone and PCN (Staudinger et al. 2001). In addition to the activation of CYP3A gene expression, PXR also activates gene expression of a range of other drub metabolising enzymes and transporters, including CYP2B6 (Wang et al. 2003), UGT1A1 (Hartley et al. 2004), ABCB1 (Geick et al. 2001), ABCC2 (Kast et al. 2002) and OATP2 (Hartley et al. 2004): Indeed, there is a large overlap in the target gene set between PXR and CAR, reflecting their roles in acting as a 'metabolic

safety net' for the removal of potentially toxic levels of xenobiotics (Plant 2004; Xie et al. 2000).

Interaction Between Nuclear Receptors

As detailed above, the nuclear receptor superfamily is a complex set of interacting proteins that allow the body to co-ordinate responses to fluctuations in chemical levels. As such, it is vital that the nuclear receptors undergo 'cross-talk'; this has the twin advantages of ensuring the most efficient response to a given stimuli, and in providing a safety net to ensure that there is always an active capture system for a stimulus, even should the cognate receptor be deficient for some reason. It is becoming increasingly clear that all nuclear receptors interact together, and one of the great challenges is to ascertain how this interaction network fully functions and to be able to predict what the biological response will be for any given stimuli. Such information will become particularly relevant in the case of multiple stimuli within the system, for example during polypharmacy, when the ability to identify potential drug-drug interactions would be highly beneficial

Interactions between nuclear receptors may occur at the level of sharing ligands, sharing co-regulator, sharing heterodimer partners or sharing DNA binding elements. Perhaps the best studied of these interactions is at the level of the target gene sets activated by nuclear receptors: For example, CAR and PXR co-ordinately regulate a battery of genes involved in all aspects of drug metabolism including oxidative metabolism, conjugation and transport, as previously described. Recent studies have identified approximately 69 genes that are under CAR regulation (Ueda et al. 2002) and 40 genes under PXR regulation (Maglich et al. 2002), with many of these genes being co-regulated by both PXR and CAR. In reality, this number of genes is likely to be

considerably higher, with different ligands potentially activating specific subsets of the target genes. It should also be noted that this overlap does not only extend to those gene targets traditionally associated with the 'core function' of a particular nuclear receptors. For example, whereas it is perhaps intuitive that both CAR and PXR are able to influence the expression of an overlapping set of drug metabolising enzymes and drug transporters (Maglich et al. 2002), as this is a common feature of their biology, it has also been shown that many nuclear receptors, including CAR and PXR may have shared effects on other biological processes, such as nuclear import via the karyopherin family of transport proteins (Plant et al. 2006). An important question now is to decipher the biological impact of such a co-ordination and how it may impact body responses to chemical stimuli

CONCLUSIONS

Research over the past decade has demonstrated that the nuclear receptor superfamily is intrinsic to the body's response to fluctuations in the levels of many chemicals within the body. These chemical levels may relate to endogenous processes, xenobiotics, or the interaction of these two chemical spheres, and it is perhaps at this interaction that our greatest challenge lies. To produce safer, more effective drugs and prescribe them in a manner that means they have the best chance of reaching efficacy it is imperative that we understand the pharmacokinetics and pharmacodynamics of these drugs. Understanding nuclear receptor biology, and how the network of nuclear receptors can act to respond to xenobiotics, will help us to better predict biological response to a single chemical. Perhaps more importantly, it should provide important insights into how the body responds to the multiple stimuli of polypharmacy, and how such stimuli can impact on endogenous processes, potentially leading to adverse events.

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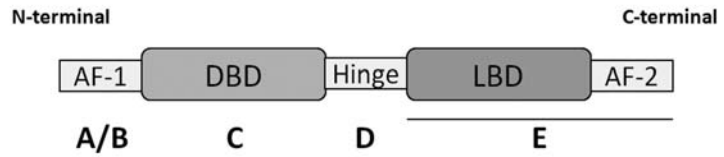

Figure 1: Generic Structure of a Nuclear Receptor