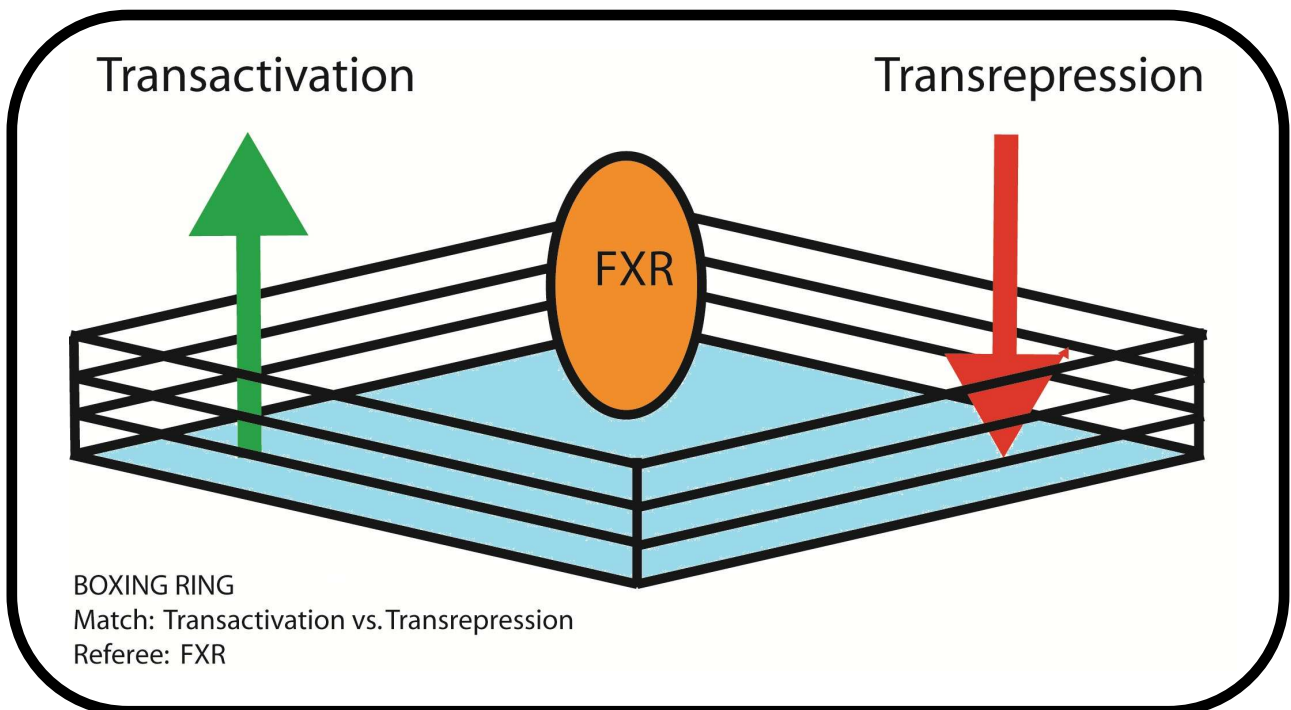# Transactivation versus transrepression in FXR:

lessons learned from other nuclear receptors

Master Thesis
Cancer Genomics and Developmental Biology
May-August 2011
Supervisor: Saskia W.C. van Mil

Danielle A.A. Hollman
3052443

Abstract

Farnesoid X Receptor (FXR) is an important player in the upregulation of genes (transactivation) in bile acid homeostasis and fat and glucose metabolism. Recently, it has become clear that an additional important role for FXR consists of downregulating genes (transrepression) in inflammation. Because of this dual role of FXR, full agonists will likely have serious side effects, which would be similar to what is known for other members of the Nuclear Receptor (NR) family. Therefore selective modulators of FXR should be developed. However, the molecular mechanisms deciding between transactivation and transrepression in FXR are currently unknown. For the NR family, cases of SUMOylation and phosphorylation have been reported to be distinctive between transactivation and transrepression. SUMOylation can either diminish transactivation selectively or increase transrepression. Phosphorylation was observed to prime for SUMOylation, thereby also selectively augmenting transrepression. Here, we review the state of current knowledge about FXR transactivation and transrepression and compare this to what is known in other members of the NR family. Ultimately, increased knowledge on the differential mechanisms of transactivation and transrepression will lead to the development of more specific drugs with less serious side effects.

1. Nuclear Receptors

One of the largest groups of transcription factors (TFs), the Nuclear Receptor (NR) superfamily regulates genes required for virtually all aspects of development, reproduction and metabolism (1). The most distinguishing feature of this superfamily of 48 transcription factors is that their activity is regulated by small lipophilic, diffusible ligands, making them ideal drug targets (2-5). Indeed, 13% of all FDA-approved drugs target a NR (6), treating a wide range of diseases, such as metabolic syndrome, inflammation and cancer (7-10).

Originally discovered as receptors for steroid hormones (GR; glucocorticoid receptor, ER; estrogen receptor, PR; progesterone receptor, AR; androgen receptor), the NR family also encompasses metabolic receptors (such as Peroxisome Proliferator Activated Receptor (PPAR), Liver X Receptor (LXR) and Farnesoid X Receptor (FXR). While many new NR ligands have been discovered in the past couple of decades, approximately half of the 48 human receptors are still orphans (1).

Molecularly, nearly all NRs share a common architecture (figure 1), with a N-terminal AF1 (Activation Function) domain, highly conserved DNA binding domain (DBD) and ligand binding domain (LBD) (11, 12). The DBD is linked to the ligand binding domain (LBD) by a flexible hinge. The LBD is relatively conserved as well and changes conformation upon ligand binding (13, 14). The main function of NRs is to positively or negatively regulate gene expression at the level of transcription. NR target genes are generally inactive when corepressors are bound to their promoters. Positive regulation, transactivation, is generally achieved by the binding of NR homodimers (steroid receptors) or heterodimers (metabolic receptors heterodimerize with RXR) to nuclear receptor response elements (NR-REs) in the vicinity of target genes and a recruitment of coactivators to the promoter (1). In addition, many NRs are capable of negative regulation of gene expression in a ligand-dependent manner by antagonizing the activities of other TFs, termed transrepression. Examples of this are members of the nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) families. Transrepression typically involves indirect association (tethering) of the NR with target genes rather than direct DNA binding (15-18).

Upon activation by their respective ligands, the steroid receptors form homodimers on the DNA, whereas the metabolic receptors all form heterodimers with Retinoid X Receptor (RXR)(1). The regulation of the transcriptional effect of NRs contains many layers of complexity and is not understood to a full extent yet. However, the use of full NR agonists in a clinical setting leads to serious side effects, which currently limit their utility and safety. To understand this, knowledge of the molecular pathways regulating the differential transcriptional activities of NRs is required. So far, differential cofactor recruitment as well as diffential post-translational modifications (PTMs) have been implicated in distinguishing between transactivation and transrepression. Also, it has become clear that also different ligands can cause differential NR responses (19, 20). The separation of transactivation and transrepression separately offers great therapeutic possibilities.

This review highlights the current knowledge on the molecular mechanisms of NR signaling in transactivation and transrepression in general, and for FXR in particular. We consider recent findings that certain PTMs in NRs indeed appear to differentiate between molecular mechanisms of transactivation and transrepression. We believe that selective ligands targeting such PTMs will boost generation of selective NR targeting drugs.
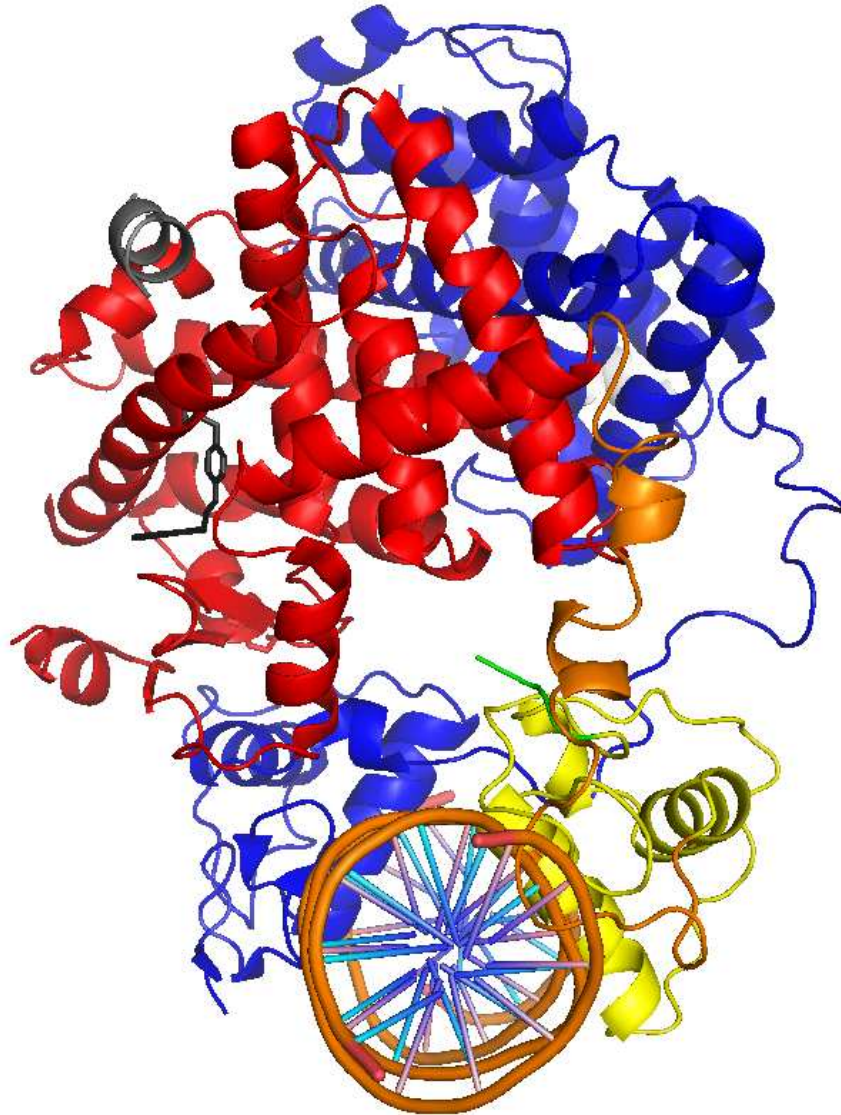
A)

B)

Figure 1: Overview of the structure of a NR. A) Schematic overview of a NR showing the AF1, DBD, hinge and LBD domain present in almost every member of the NR family. B) Structure of PPARγ/RXRα heterodimer on the DNA. RXR is shown in blue, PPARγ is shown in several colors; AF1: green (tiny section only), DBD: yellow, hinge: orange, LBD: red. In gray interactions of PPAR with a cofactor are shown and in black the PPARγ agonist Rosiglitazone. Structure as crystallized by Chandra et al, 2008, visualized using PyMOL.

2. Farnesoid X Receptor

In this review, we focus on Farnesoid X Receptor (FXR, NR1H4), which belongs to the subclass of metabolic receptors within the NR-family (21). The endogenous ligands for FXR are bile acids (BA), the most effective one being chenodeoxycholic acid (CDCA) (22-24). Next to CDCA, other BAs such as lithocholic and deoxycholic acid activate FXR (25-27). BAs circulate between the liver and the intestine and are essential for uptake of vitamins and dietary fats from the intestine. Upon ligand binding, FXR can induce gene transcription of its target genes by dimerization with Retinoid X Receptor (RXR) to an FXR responsive element (FXRE) (21).

The primary role of FXR is to serve as a BA-sensor. Since BAs are toxic in high concentrations, an important function of FXR is to regulate its synthesis and accelerate its biotransformation and excretion into the bile. Therefore, many target genes of FXR are involved in these processes. In the liver, active FXR inhibits synthesis of bile acids by CYP7A1 and CYP8B1 by activation of SHP (Small Heterodimer Partner) (28). Furthermore, FXR regulates BA uptake from the sinus via NTCP (Sodium Taurocholate Cotransporter Protein) (29, 30) and canalicular secretion via BSEP (Bile Salt Export Pump) (31). In the enterocyte, FXR controls the absorption of BA via ASBT (Apical Sodium-dependent Bile Acid Transporter) (32), transport from the apical to the basolateral membrane via IBABP(Intestinal Bile Acid Binding Protein) (33) and finally secretion of BA from the enterocyte into the portal blood by OSTα and β (34). Because BAs are toxic, they are made less biologically active and more easily secretable by UGT2B4 (Uridin 5'-diphosphoate-glucuronosyltransferase 2B4), which glucuronidates the BA in the liver and is also a FXR target (35-37).

Mutations as well as polymorphisms in FXR have been shown to lead to cholestasis (38), an impairment in bile flow which causes accumulation of toxic bile salts and other compounds in the liver and is a common and devastating liver disease (39). In mouse models with ligated bile ducts and α-naphthylisothiocyanate treatment, which are acute models for cholestasis, the synthetic FXR agonist GW4064 markedly reduced liver injury (40). Also in more chronic models of cholestasis, induced by 17α-ethinylestradiol, the FXR agonist 6-ethyl chenodeoxycholic acid (6-ECDCA) protected from cholestasis by, among others, increasing SHP and decreasing CYP7A1, CYP8B and NTCP, thus proving its important role *in vivo* (41).

The role of FXR was also extended to fat and glucose metabolism (42, 43), as well as liver and intestine inflammation (44, 45), liver growth and regeneration (46), and liver carcinogenesis (47), even though the exact mechanisms remain elusive. Fxr-knock out mice display increased susceptibility to tumorigenisis in liver and colon, suggesting that FXR plays a protective role (47-49).

Recently, we discovered that FXR is an important player in the counter-regulation of intestinal inflammation, possibly by transrepressing NF-κB signalling. Stimulation with the potent semi-synthetic FXR agonist INT-747 improved clinical symptoms and histology in DASS and TNBS murine models of colitis. This beneficial effect was not observed in Fxr-knock out mice. Moreover, Fxr activation inhibited mRNA expression of pro-inflammatory genes such as TNFα and cytokines (45).

This multi-level protection against inflammation makes FXR an interesting novel therapeutic target. Currently, drugs targeting FXR in cholestasis, Diabetes Mellitus type II and metabolic syndrome tested in phase I and II clinical trials. However, caution should be taken for the use of full FXR agonist in this respect now FXR also appears to

have transrepressive actions. For many other NRs, full agonists have been reported to cause serious side effects, generally accepted to be due to concurrent activation of all transcriptional actions of a NR. Therefore, there is a therapeutic need to develop selective FXR synthetic ligands which modulate specific sets of genes. Much is known about *in vivo* mechanisms of FXR, however in order to be able to develop such ligands, it is vital to dissect the molecular pathways by which FXR regulates gene transcription. Differentially recruited proteins to FXR and different PTMs are likely to be decisive in determining specific FXR signaling in one or the other direction. The mechanisms already known for FXR as well as other NRs will be discussed below.

3. FXR transactivation mechanisms

Simple transactivation
The main function of NRs is to regulate gene transcription, either positively via transactivation or negatively via transrepression. Positive regulation is achieved by the binding of NR homo- or heterodimers to hormone response elements (HREs). Classical mechanisms of transactivation have been known for many years, however recently additional mechanisms of binding to HREs have come to light.
The most straight-forward way of transactivation is simple transactivation (figure 2A). In this process, the NR dimerizes (either as a homodimer or RXR heterodimer) and binds directly to the respective responsive element (RE) (50, 51). This mechanism of DNA binding is very common among NRs and responsible for induction of many target genes. For FXR, heterodimerization with RXR and binding to an FXRE is the most frequently described mechanism of transactivation (23).
The highest affinity FXREs contains an inverted repeat with 1 nucleotide spacing (IR-1) motif to which the FXR/RXR heterodimer binds (31, 33, 52) e.g. for SHP transcription (28), but FXR has also been observed to bind to IR-0 (53), as well as direct repeat (DR-1) sequences (54). Since FXR binds to these various REs with different affinity, the expression of these target genes can be differently regulated (21).

Composite transactivation
In a composite situation, the NR binds to a NRE and synergizes with other DNA binding TFs that are present (figure 2B) (55). This mechanism has been known to exist for a long time for other NRs, such as GR, in which synergy between NF-κB and GR has been observed to occur; the endogenous ligand for GR, glucocorticoids, were observed to positively regulate NF-κB-dependent responses (56-58)f.
Recently, composite transactivation has also been described for FXR. Using ChIP-seq (Chromatin Immuno Precipitation-sequencing), Chong et al. recently showed in murine hepatic chromatin that many FXREs contain another half NRE-site (Nuclear receptor Response Element) in close proximity. This could indicate that other NRs can modulate FXR function. Additional NR half-sites were present proximal to 71% of the IR-1 FXREs. As an example, addition of Lrh-1 in a luciferase assay showed increased Fxr activity for the *Shp*, *Rdh9*, *Pcx* and *Pemt* promoters. Also, Lrh-1 binding to Fxr was demonstrated using a co-IP (Immuno Precipitation). Many of the Lrh-1 half-sites were located less than 50 bp from the FXREs in the promoter, which gives a strong indication that Lrh-1 indeed bound as a monomer proximally to Fxr/Rxr heterodimers, thereby enhancing its gene transcription (59). It will be interesting to investigate which other NRs could potentially influence FXR-mediated gene transcription in the liver and other tissues as well as whether this mechanism can also be found in human chromatin extracts.

Monomeric transactivation

Transactivational activity of FXR does not always require RXR. FXR has been shown to possess RXR-independent transcriptional activity, most likely through monomeric binding (figure 2C). This was the case for UGT2B4 (Uridin 5'-diphosphoate-glucuronosyltransferase 2B4), where FXR bound to a NR half site regardless of RXR presence and induced BA glucuronidation via UGT2B4. RXR presence actually inhibited FXR-mediated UGT2B4 induction, but this was due to the formation of inactive FXR/RXR complexes, which was shown by EMSAs (Electrophoretic Mobility Shift Assay) (36) using a L433R FXR mutant, which is incapable of dimerizing with RXR (60)

Also GLUT4 (Glucose Transporter type 4), the main insulin-responsive glucose transporter, has been reported to be activated by monomeric FXR. CDCA-liganded FXR induced GLUT4 transcription in HepG2 liver cells and 3T3-L1 adipocytes. Mutational analysis of the GLUT4 promotor and EMSAs suggested that FXR was able bind the GLUT4 promoter as a monomer on a half-site, independently of RXR. Addition of liganded RXR did not further induce FXR-mediated GLUT4 transcription, nor did RXR siRNA decrease GLUT4 induction. In C57BL/6J mice CDCA injection increased the GLUT4 protein amount, confirming the role of CDCA-mediated induction of GLUT4 *in vivo* (61).

Tethering transactivation

Next to directly contacting the DNA via NREs, TFs may also induce gene transcription without direct DBD binding. In a tethering situation, a NR does not contact the DNA directly, but exerts its effect via another, DNA binding, TF (figure 2D) (55). This transactivating mechanism has not yet been reported for FXR, but has been shown in GR for multi-protein complexes called enhanceosomes. In these complexes, GR was tethered to the DNA, and combinatorial positive responses were observed. (62, 63). As an example, for the α2-Macruglobulin (α2-M) promoter, presence of GR was not essential for transcription, but did increase transcriptional activity on the promoter, via tethering of GR probably to cJun or STAT3 (signal transducer and activator of transcription) (63).
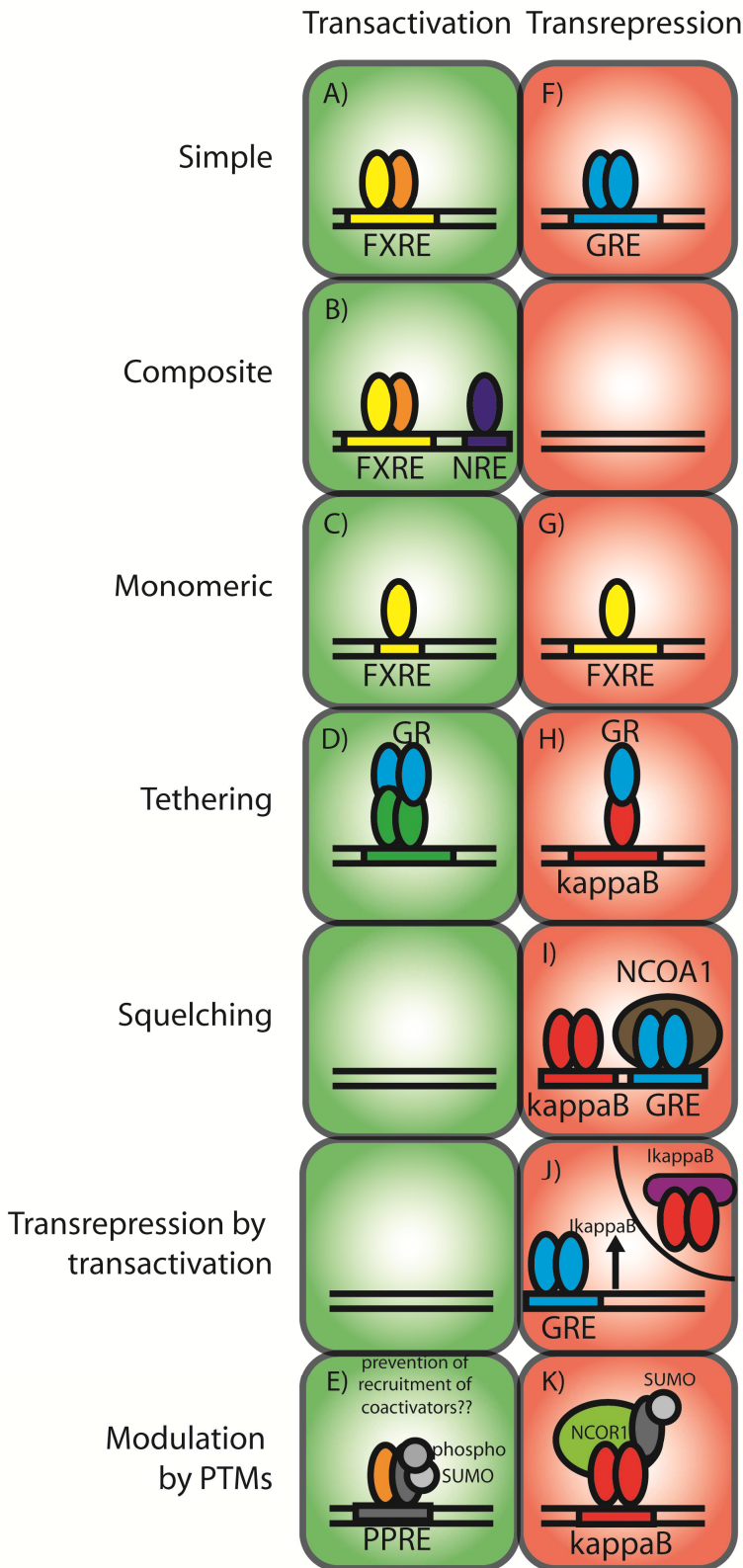
Figure 2: Schematic model of differences between DNA binding mechanisms between transactivation and transrepression.
A) Simple transactivation of a FXR/RXR heterodimer on a FXRE.
B) Composite transactivation of a FXR/RXR heterodimer together with another NR on a NR half-site.
C) Monomeric transactivation of FXR on a FXRE half site.
D) Thethering transactivation of a GR homodimer binding to another transcription factor (e.g.) cJun.
E) Inhibition of transactivation by a priming phosphorylation and following SUMOylation of PPARγ/RXR heterodimer on a PPRE.
F) Simple transrepression by an GR homodimer on a GRE.
G) Monomeric transrepression by FXR on a FXRE.
H) Tethering of GR to NF-κB on a κB RE inhibits NF-κB activity.
I) Binding of GR to a GRE invites NCOA1 binding, inhibiting NF-κB signally by scavenging the available coactivators needed for transcribing genes.
J) GR homodimerization on a GRE increases I-κB transcription. I-κB translocates to the cytosol and inhibits NF-κB by retaining it in the cytoplasm.
K) SUMOylation of PPARγ prevents release of NCOR1, thereby keeping NF-κB inactive on the κB RE.

4. FXR transrepression mechanisms

The mechanisms of FXR transrepression are currently under study and yet unknown for the greatest part. However from other NRs it is known that inhibition of the NF-κB pathway and transrepression of other genes can occur in a multitude of ways. This is discussed below, sorted by the mechanism of DNA-binding by the NRs modulating the response.

Tethering transrepression
Unlike transactivation, examples of simple and composite models for transrepression are rare. The best studied nuclear receptor for mechanisms of transrepression is GR, therefore this NR will be used as an illustration for mechanisms undiscovered in FXR.
Until recently, for GR only a few cases of direct transrepressive binding to a promoter were observed, and no consensus sequence could be assigned to these so-called negative GREs (nGRE) (64). Instead, the standard model of transrepression became tethering of a NR to the DNA by other TFs (figure 2H). The direct binding of a NR to its target TF sterically inhibited the binding of coactivators to this TF, thereby inhibiting transcription (65). This model has been well known for its function in inflammation. It is mediated via other TFs which bind to the DNA. Upon NR binding to the TF, RNA polymerase is inhibited in transcribing the target genes of the TF. Well studied examples of this are NF-kB and AP-1. These TFs bind to κB and AP-1 REs respectively (15-17) and are involved in transcribing a plethora of immunomodulating factors (18). In fact, many signaling mediators that are produced during chronic inflammation are under command of these two responsive elements (66). Many of the anti-inflammatory capacities of NRs (e.g. GR, PPAR) have attributed to direct binding to NF-κB and AP-1 and therefore inhibiting transcription of their target genes.
For NF-κB tethering interactions with post-translationally modified NRs have been described recently. The molecular mechanism of this transrepression manner will be discussed extensively in the next two chapters. In short, PTMs on a NR block the clearance of corepressors from the NF-κB complex, thereby refraining it from inducing its promoter (figure 2K) (65).

Simple transrepression
Recently, GR was observed to be able to transrepress many genes by direct binding to their promoters (figure 2F). Negative GREs (nGREs) were discovered to be present on more than 1000 human/mouse genes which are repressed by glucocorticoids *in vivo*. Stimulation with an agonist induced the binding of *cis*-acting GRs to an inverted nGRE. In turn, this downregulated genes by interacting with NCOR1 and 2 (Nuclear Corepressor). IR-0, IR-1 and IR-2 containing nGREs could be efficiently transrepressed by agonist-liganded GRs (67). So far, this has only been observed in GR, so whether this holds true for other NRs remains to be investigated.

Monomeric transrepression
Only two examples of FXR transrepression have been known to date, both use the monomeric transrepression mechanism. First, FXR was observed to inhibit the *ApoA-I* gene. *ApoA-I* is involved in cholesterol and phospho-lipid efflux from tissues (68). Bile-acid liganded FXR has been shown to negatively regulate ApoA-I expression by binding independently of RXR to a negative FXRE, presumably as a monomer (figure 2G). The RXR/FXR heterodimer only showed very slight binding compared to FXR alone on an EMSA and the L443R mutant did not show decreased repression of ApoA-I, despite being unable to dimerize with RXR. Thus, both findings indicate that binding is RXR

independent. Furthermore, *in vivo* negative regulation of ApoA-was shown by feeding hApoA-I transgenic mice with the FXR agonist taurocholic acid. This decreased serum concentrations and mRNA levels of ApoA-I, arguing for a relevant biological mechanism (60).

Second, *APOA* was also inhibited by monomeric FXR. APOA is known to form lipoprotein(a) together with apoB100, which is an important plasma lipoprotein (69). High levels of lipoprotein(a) are correlated with development of thrombo-artherogenic diseases (70, 71). FXR was observed to inhibit *APOA* expression in a SHP-independent manner, by binding as a monomer to the second half site of a DR-1 FXRE. Also *in vivo* the relevance of the mechanism was shown by transgenic *hAPOA* (tg*APOA*) mice, which showed low expression of APOA upon bile duct ligation. Comparison of *tgAPOA* mice with tgA*POA*/*Fxr*$^{-/-}$ mice, showed that upon administration of BA, plasma concentrations and hepatic expression of *APOA* were reduced in FXR containing mice, but not in FXR knock out mice. This established that *APOA* inhibition by FXR also occurs *in vivo*. Interestingly, FXR was found to compete with the activatory nuclear receptor hepatocyte nuclear factor 4α (HNF4α) for binding to the DR-1 motif (72). This provides a mechanism how NRs can compete for a binding site and how the balance between activity of these NRs can decide the transcriptional effect on a target gene.

Transrepression by squelching
The amount of cofactors present in a cell can be rate-limiting for transcription. Since NRs and pro-inflammatory TFs compete for the same pool of cofactors, this can lead to a process called squelching (figure 2I). In this way, the pool of NRs binding to the DNA compete away the available co-factors from NF-κB, thereby inhibiting transcription of its target genes (73). This was first observed for GR, RAR (Retinoic Acid Receptor) and RXR on AP-1 targets; heightened presence of the coactivator CBP (CREB Binding Protein) diminished transrepression of AP-1 target genes (74). Moreover, GR-mediated inhibition of NF-κB could be overcome by supplementary NCOA1 (Nuclear coactivator) and CBP (75). Even though squelching would be an attractive mechanism to explain the often observed antagonism between inflammatory and NR signalling, the physiological relevance remains to be established still (65).

Transrepression by transactivation
A final mechanism for NF-κB inhibition is the upregulation of the cytosolic Inhibitor of κB (IκB), which binds to NF-κB. In this way, it tightly controls NF-κB localization in the cytoplasm, thereby preventing it from activating genes (76). Initially, GR was observed to upregulate IκB expression. Even though no classical GRE was present in this promoter, homodimerization was required for binding (77). Later, this was also observed for PPARα, which has been shown to inhibit the induction of the inflammatory cyclooxygenase-2 (COX2) gene at the transcriptional level via repression of NF-κB signalling (65, 78), as well as for AR (Androgen Receptor) (65). However, conflicting results in which GR and AR-mediated induction of IκB did not uniformly alter NF-κB DNA binding have been reported (79). This suggested that the multiple ways of NF-κB mediated transrepression occur in a cell type or tissue specific manner (65).

FXR in inflammation
The role of FXR in inflammation has only been discovered recently. It was observed that FXR activation decreased the severity of inflammation of the intestine *in vivo* (80). Furthermore, pro-inflammatory cytokines that activate NF-κB signaling repressed FXR activation in the intestine (45) as well as the liver (44). Currently, it is unclear yet how exactly FXR interacts with NF-κB to repress genes (81). Limited experiments

suggested that NF-κB shows reduced affinity to its RE (44) but there is also evidence that FXR inhibits co-repressor clearance from the NF-κB promoter (82).

Comparison with other NRs can provide us with other mechanisms which could potentially be involved in FXR-mediated NF-κB inhibition. Since other NRs, such as GR, are able to attenuate the NF-κB pathway in a multitude of ways, it is a very likely possibility that FXR is similar in this respect. Indications are that FXR and NF-κB are able to regulate each other through reciprocal inhibiting interactions, both *in vitro* as well as *in vivo*. (44, 81, 83). This mechanism has already been positively established in GR and PXR (Pregnane X Receptor) (84, 85). Using FXR DBD mutants it would be interesting to investigate whether DNA binding is required for NF-κB binding or whether the tethering mechanism is dominant.

Which modes of action a NR will ultimately use to exert its transcriptional effect is most likely to be dependent on multiple factors, differing between cell types and promoters, thereby allowing target genes to be regulated specifically. Differential cofactor interactions have been reported to be selective between transactivation and transrepression, and these interactions varied in efficiency between different ligands (20). However, next to the ligand-dependent part, the properties of the NR are also known to change by post-translational modifications. Recently, there has been a growing focus on how these PTMs influence the balance between transactivation and transrepression. We will discuss what is known about the effect of PTMs in distinguishing between transactivation and -repression for FXR, as well as for other NRs.

5. FXR PTMs in transactivation and transrepression

FXR phosphorylation in transactivation and transrepression
Three phosphorylations have been discovered on FXR to date, all of which augment its transactivation activity (86, 87). S135 and S154 were observed in the DBD of endogenous FXR in HepG2 cells and were shown to be phosphorylated by PKC using pharmacological inhibitors. Despite the fact that phosphorylations of these sites did not increase DNA binding nor change its subcellular localization, they did promote recruitment of the coactivator PGC1α (PPARγ Coactivator). In this way, transactivation was achieved. Mutations of either or both phosphorylation sites severely diminished PKCα induced activation and ligand induced FXR activation, indicating a role for the LBD in changing the conformation of FXR so that these sites become available to PKCα. Because S135 and 154 both caused SHP and UGT2B4 to increase and ApoA-I to decrease (86), these two phosphorylations did not distinguish between transactivation and transrepression.

The third phosphorylation, Y442, was observed to play a role in nuclear localization as well as transactivation in overexpression and mutational studies. PKCζ was the kinase responsible for phosphorylating Y442. In turn, PKCζ was activated by phosphorylation by FIC1 (87). FIC1 is crucial for normal bile acid transport (88). Unfortunately, the effect of this phosphorylation was only studied on the SHP promoter (87), therefore the effects of Y442 phosphorylation on transrepression are unknown. Importantly, all phosphorylation data were generated *in vitro*, and the functional relevance of these sites *in vivo* is currently unknown.

FXR SUMOylation

FXR SUMOylation was discovered to mediate anti-inflammatory transrepression in the intestine, and to be protective in a mouse colitis model. Two potential SUMO sites were present in FXR, K277 and K460, which both complied to the wKXE/D motif, where w represents a hydrophobic and X any amino acid. Vavassori et al. showed by IPs and luciferase assays that *in vitro* recruitment of FXR to the iNOS (inducible Nitric Oxide Syntase) promoter required SUMOylation, and addition of NCOR1 siRNA caused THP-1 cells (human acute monocytic leukemia cells) to display an enhanced response to LPS by increased mRNA transcription of the iNOS and IL-1ß (InterLeukin) inflammatory factors. Since pre-treatment with INT-747 prevented clearance of NCOR1 from these promoters, this pointed to a role for FXR in retaining NCOR1 at the promoters, which is similar to the mechanisms observed in other NRs as discussed in the next chapter. However, conformation of the latter data *in vivo* is necessary in order to establish whether this is a biologically relevant mechanism. Also, focus should be on elucidating the exact binding mechanism of FXR-SUMO to the iNOS promoter, whether it is mediated by NF-κB or not and whether DNA binding of FXR is required. Even so, it is clear that SUMOylation of liganded FXR is able to differentially inhibit inflammatory genes such as IL-1ß, iNOS and TNFα, but not SHP (89), pointing to a distinctive mechanism to separate between transactivation and transrepression.

FXR acetylation

FXR has been reported to be acetylated at residues K157 and K217. K217 was observed to be acetylated by p300 and deacetylated by SIRT1. Acetylation on K217 inhibited dimerization with RXR and diminished binding to the DNA. Furthermore, luciferase assays using the SHP and (FXRE)3-tk promoter showed that K217R mutants display increased transactivation ability (90).

Decreased transactivation by acetylation was also shown in functional studies and this was especially the case for K217 acetylation. Presumably, FXR dissociated from the promoter (91). However, the function of acetylation has not been studied in a transrepressive context, therefore its effect on inflammation is yet unknown. Since evidence points to FXR losing its DNA binding and heterodimerization capacities, acetylation might only interfere with repressive mechanisms that require either or both of this mechanisms. This would still allow tethering interactions of monomeric FXR to NF-κB to happen (figure 2). Therefore, we speculate that acetylation can be a potential distinctive factor between transactivation and transrepression.

FXR ubiquitilation

Ubiquitilation of FXR is an understudied area. FXR has been reported to be ubiquitilated *in vitro*, as well as in cells. Inhibition of the proteasomal degradation using MG132 resulted in a noticeable reservoir of ubiquitilated FXR. Also, K157R or K217R mutants displayed three times shorter half-lives than wild type FXR, indicating that acetylation might increase FXR stability and thus decrease ubiquitin-mediated proteasomal degradation. However, little is known about the *in vivo* occurrence of FXR ubiquitination, and the site of ubiquitilation remains to be determined (90). If, for example, an acetylation site would be discovered that distinguishes between transactivation and transrepression, this observed difference in FXR half-life would be of some significance, as it could mean that acetylated FXRs would be less prone to ubiquitin-mediated degradation and thus selectively target either transactivating or transrepressing FXRs for degradation. However, so far such as effect has not been observed. Even though it is known that in general for TFs activation is correlated with degradation, so this might be an attractive hypothesis (92).

6. The role of PTMs in transactivation versus transrepression for other NRs

For other NRs, more research has been performed for the function of PTMs in distinguishing transactivation from transrepression, especially for GR. So far, SUMOylation, in interplay with other PTMs, is the major player involved in making this distinction.

NR SUMOylation
Shared between many NRs (including GR, ER, RAR and FXR) are the inflammatory genes. Each NR represses an overlapping, but distinct pattern of ligand-dependent inflammatory gene expression (44, 93-96). At first, it was unclear how this was regulated at a molecular level, however, SUMOylation brought an explanation. SUMO (Small Ubiquitin-like Modifier) is an 11 kDa protein, that can be covalently attached to proteins by a system of 3 ligases.
The first SUMO-sites observed in PPARγ were at K107 in the AF1 and K367 in the LBD (97, 98). SUMOylation of K107 inhibited ligand-independent transactivation, but left transrepressive functions in luciferase assays intact. Moreover, mutation to R107 increased transactivational functions (97), but did not interfere with recruitment to the iNOS promoter and transrepressive capacities, as shown by ChIP and luciferase assays. Thus, SUMOylation of this site selectively inhibited transactivational functions of PPARγ (figure 2E and 3), at least for the PEX11, Perilipin and AokTK promoters (97, 98). In contrast, the K367R mutant was not recruited to the iNOS promoter in the presence of the PPARγ agonist rosiglitazone, and K367R showed a similar transactivational activity on the AoxTK luciferase promoter as WT PPARγ. Both indicate that K367 SUMOylation is important in distinguishing between transrepression and transactivation and that SUMOylated PPARγ is important in transrepression. Moreover, it was suggested that NCOR1 was required for ligand-dependent recruitment to the iNOS promoter (figures 2K and 3) (98).
Next to PPARγ, an increasing amount of SUMO-sites were discovered in a wide range of NRs. Distinct transrepressive SUMO-sites have also been observed in LXR, GR and FXR (see chapter 5 for FXR SUMOylation). For LXRβ, SUMOylation sites have been discovered at K410 and K448, mutation of these to arginine lead decreased transrepressive activity in a iNOS luciferase assay. Moreover, mutation of both abolished transrepression completely, whereas transactivational activity on the ABCA1 promoter was not affected (99).
A further step of regulation is formed by different SUMO mechanisms, which are responsible for differentially placed SUMOylations on LXR as opposed to PPARγ. In this way, these NRs are able to regulate overlapping but distinct subsets of proinflammatory genes (99).
SUMOylation has also been reported to increase transactivation. For RORα (RAR-related Orphan Receptor) it was discovered by Hwang et al. that SUMOylation on K240 in the hinge region lead to an increased transcriptional activity, as shown using a 5x RORE and p21 promoter. Mutation to arginine lead to decreased luciferase activity. Unfortunately, the effect of K240-SUMO on transrepression was not investigated (100). An increased transactivation has also been reported for SUMOylation in the hinge region of ERα (Estrogen Receptor) (101). Unfortunately, the effects on transrepression of these SUMOylations remain unknown to date.

Crosstalk between SUMO and other PTMs
It has been clear that many effects of NRs are caused by intensive cross-talk. This is the case for PTMs in general as well as for PTMs distinguishing between transactivation and transrepression. For PPARγ, phosphorylation of S112 primed it for SUMOylation on K107 (97, 102, 103). SUMOylation of K107 without prior S112 phosphorylation caused less potent repression of transactivational functions (97, 103). Thus, inhibition of transactivation was stronger upon S112 phosphorylation (figure 3).

Crosstalk between phosphorylation and SUMOylation has also been observed for GR, where phosphorylation of S246 by JNK facilitated subsequent SUMOylation of K297 and 313 and repressed genes in a promoter specific manner (104).

An additional layer of regulation by PTMs has come from indications that SUMOylation might prevent heterodimerization with RXR, thereby inhibiting gene transcription. This was observed for LXR using ChIP; RXR was not recruited to promoters containing SUMOylated LXR, unlike unSUMOylated LXR which did dimerize with RXR (105). A putative SUMO-acceptor site (K410) in LXRß was discovered close to the RXR-heterodimerization site, offering a potential mechanism. However proof is still required to adopt this hypothesis (106). Interestingly, if this were true, this would mean that SUMOylated LXR would not transrepress genes as a RXR heterodimer, but rather as a monomer or perhaps homodimer. This provides a distinct regulatory mechanism for a cell, as many methods of transrepression require dimerization with RXR (see figure 2). For TR (Thyroid hormone Receptor) something similar has been shown; transcriptional repression was mediated via NCOR2 interaction with TR homodimers, not TR/RXR heterodimers (107).

An other interesting aspect about covalently attaching a SUMO protein to a NR, is that it creates new interaction surfaces. These interaction surfaces have been reported to recruit corepressors, interacting through so-called SUMO-interaction motifs (SIMs) (106, 108). Recently, GPS2 (G-protein Pathway Suppressor) was observed to serve as a bridge between agonist-bound, SUMOylated LXR and the NCOR1 complex (109), indicating that the interaction between a SUMO-NR and NCOR1 causing transrepression is not a direct one, but rather regulated by another (set of) protein, offering additional regulatory possibilities.

In conclusion, PTMs offer a wide range of regulatory possibilities to fine-tune the target genes affected by a NR. This ranges from recruitment of coregulators to inhibiting RXR heterodimerization and facilitating interactions with other proteins. Thus, PTMs form an important aspect to study when trying to understand the mechanisms separating transactivation from transrepression.

To the best of our knowledge, no reports about acetylation and ubiquitilation have been made to be distinctive in separation between transactivation and transrepression.

GRdim mutants and mice
In GR, a mutant is known that was able to transrepress, but lost its transactivation ability, which offers possibilities to study these processes separately. The mutation, A458T, is situated in the DBD of GR and is known to prevent homodimerization and DNA-binding-dependent transactivation (110). A knock-in of this mutation into mice was viable, unlike GR knockout mice, which died soon after birth (111). In the A458T mice (also called GR^{dim} mice) GR showed impaired DNA binding and dimerization and therefore reduced transactivation. In contrast, tethering interactions were not compromised, so transrepression function was largely unaffected (112). GR^{dim} mice were observed to retain their ability to inhibit local inflammation of the skin as well as the repress of systemic inflammation upon glucocorticoid-stimulation. This means that these responses were not dependent on DNA binding capacity of GR. Since GRE-mediated genes were not induced by glucocorticoid administration, this lead to less

off-target effects (112, 113).The GR^{dim} mice open up possibilities to study transactivation and transrepression separately and feed the belief that it is possible to develop specific transrepression inhibiting drugs

Importantly, since Surjit et al. recently observed that GR-mediated transrepression not only occurred in tethering interactions but also via direct binding to nGREs, this means that a GR^{dim} mutation does affect nGRE-mediated transrepression as GR can no longer bind to the DNA. Therefore, potential drugs targeting tethering transrepression activities only and not DNA-binding mediated transactivation, will probably also not interfere with nGRE-mediated targets. Since many of the nGRE mediated targets were observed to have important physiological functions such as metabolism, immune and stress systems and the circadian clock, it is highly desirable to not target this mechanism (67). For FXR, a RXR dimerization defective mutant is also known, the L433R mutant (60), however this mutation is in the LBD, not the DBD, so monomeric DNA binding can still occur. Nevertheless, it would be very interesting to do similar experiments for FXR and investigate which target genes require FXR heterodimerization capacity *in vivo* and which genes can no longer be inhibited.
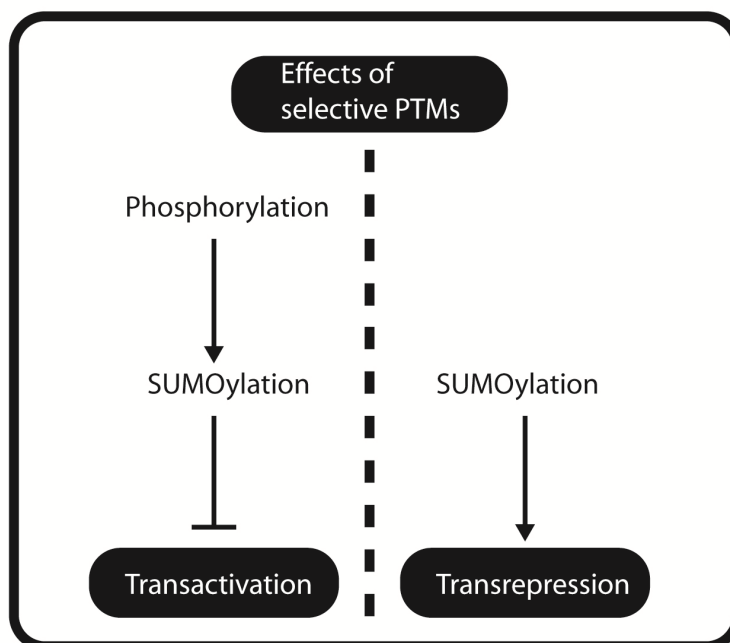

Figure 3: Model of effects of PTMs distinguishing between transactivation and transrepression. Selective regulation of transactivation via PTMs has been found for phosphorylation and SUMOylation. In PPARγ, phosphorylation was found to increase SUMOylation, and SUMOylation decreased transactivation specifically. For transrepression, SUMOylation was found in increase the transrepressive activity of PPARγ, as well as LXRβ and FXR.

7. Discussion

FXR is the master regulator of bile homeostasis. Aberrant regulation of this nuclear receptor is at the base of several serious diseases, such as cholestasis and inflammation of the liver and intestine. No FDA-approved FXR-agonists have been marketed yet, therefore, it is important to develop a therapeutic agent targeting this NR. Currently, clinical trials for FXR ligands targeting cholestasis, diabetes mellitus type II and metabolic syndrome are being conducted. However, since full agonists targeting other NRs show serious side effects, this is expected for FXR as well. Therefore, there is a great need for selective FXR modulators. FXR modulation of target genes can be dissected into transactivation and transrepression. More and more

evidence from other NRs is emerging that indeed transactivation and transrepression can be separately targeted by selective ligands. Recently, many selective ligands have been discovered for RXR (reviewed in (114)). FXR/RXR form a permissive heterodimer, meaning that RXR ligands alone are also able to activate the dimer. Since FXR/RXR heterodimers are likely to participate in transactivation as well as transrepression, targeting RXR to specifically transrepress but not transactivate might attenuate FXR-mediated transactivational side effects.

In a similar way, for GR selective ligands have been developed. Ronacher et al. showed that the efficacy of a ligand for transactivation and transrepression is irrespective of its relative affinity for the NR. The affinity of different ligands did correlate with GRE-mediated transactivation, but not with NF-κB/AP-1-mediated transrepression. Also ligand-selective differences between promoters for some ligands in reporter assays were shown (20). This is an important concept for future selective drug development. Studies such as Ronacher et al. show that the effect of selective ligands can already be visualized in a very artificial setting containing artificial promoters and no chromatin. However, drug development requires a more *in vivo* approach, so that effects of ligands will be determined in an *in vivo* setting.

Modelling the different mechanisms for DNA interactions of FXR and comparing this to other NRs can greatly facilitate knowledge about the different mechanisms that drive the separation between transactivation and transrepression. At first glance, the DNA binding mechanisms seem quite similar. However, what has not been covered in this review, is that the recruitment of cofactors and other transcriptional regulators between these different mechanisms can be quite different, thus exerting very different effects on gene transcription. For example, FXR monomers were observed to both induce genes (e.g. GLUT4 (61)) and inhibit others (e.g. ApoA-I (60)). This selective effect on gene transcription by FXR recruitment might be dependent on promoter context and surrounding TFs as well as on PTMs.

So how do we know what precise mechanism of NF-κB inhibition FXR will use to exert its anti-inflammatory functions? For GR, the answer contains multiple variables. Glucocorticoids probably use different mechanisms to establish their anti-inflammatory functions, depending on environmental cues such as the particular promoter sequence and cell-type dependent differences like the availability of co-factors and differential histone modifications (115, 116). Therefore, it is important to realize that NF-κB is not a single pathway, but rather a context-dependent, multitargetable effector (117). Also for FXR, it is likely that NF-κB inhibition is mediated via several DNA binding or tethering mechanisms.

With the discovery of PTMs as being causative for the distinction between transactivation and transrepression, comes the notice that if SUMOylation can be selectively induced or mimicked by certain ligands, anti-inflammatory functions of FXR can be induced, while bile homeostasis is not perturbed. Similarly, in diseases that require increased transactivational but not transrepressive FXR activity such as cholestasis, transactivation should be selectively induced.

Many previous studies undertaken on the effect of PTMs with regard to gene expression cannot be used, since they either focus on genes that are activated or genes that are repressed upon PTM acquirement. Currently, PTMs leading to distinctive transcription are more extensively studied, and we expect more differential PTMs soon to be uncovered, also in FXR. This is of great importance for FXR selective modulation. Development of selective ligands for FXR will lead to selective therapeutic agents, therefore greatly improving both research and patient care in a bench to bedside setting.

References

1. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: Opening the X-files. Science. 2001;294(5548):1866.

2. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, et al. The nuclear receptor superfamily: The second decade. Cell. 1995;83(6):835-9.

3. Lonard DM, O'Malley BW. Nuclear receptor coregulators: Judges, juries, and executioners of cellular regulation. Mol Cell. 2007;27(5):691-700.

4. Nagy L, Schwabe JWR. Mechanism of the nuclear receptor molecular switch. Trends Biochem Sci. 2004;29(6):317-24.

5. Bourguet W, Vivat V, Wurtz JM, Chambon P, Gronemeyer H, Moras D. Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. Mol Cell. 2000;5(2):289-98.

6. Overington JP, Al-Lazikani B, Hopkins AL. How many drug targets are there? Nature reviews Drug discovery. 2006;5(12):993-6.

7. Altucci L, Leibowitz MD, Ogilvie KM, de Lera AR, Gronemeyer H. RAR and RXR modulation in cancer and metabolic disease. Nature Reviews Drug Discovery. 2007;6(10):793-810.

8. Jordan VC, Brodie AMH. Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer. Steroids. 2007;72(1):7-25.

9. Sonoda J, Pei L, Evans RM. Nuclear receptors: Decoding metabolic disease. FEBS Lett. 2008;582(1):2-9.

10. Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z, et al. Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages. J Biol Chem. 1998;273(40):25573.

11. Berg JM, Merkle DL. On the metal ion specificity of zinc finger proteins. J Am Chem Soc. 1989;111(10):3759-61.

12. Klug A, Schwabe J. Protein motifs 5. zinc fingers. The FASEB Journal. 1995;9(8):597.

13. Wurtz JM, Bourguet W, Renaud JP, Vivat V, Chambon P, Moras D, et al. A canonical structure for the ligand-binding domain of nuclear receptors. Nature Structural & Molecular Biology. 1996;3(1):87-94.

14. Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D. Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-a. Nature. 1995;375(6530):377-82.

15. Subramaniam M, Hefferan T, Tau K, Peus D, Pittelkow M, Jalal S, et al. Tissue, cell type, and breast cancer stage-specific expression of a TGF-ß inducible early transcription factor gene. J Cell Biochem. 1998;68(2):226-36.

16. Chandran UR, Warren BS, Baumann CT, Hager GL, DeFranco DB. The glucocorticoid receptor is tethered to DNA-bound oct-1 at the mouse gonadotropin-releasing hormone distal negative glucocorticoid response element. J Biol Chem. 1999;274(4):2372.

17. Martens C, Bilodeau S, Maira M, Gauthier Y, Drouin J. Protein-protein interactions and transcriptional antagonism between the subfamily of NGFI-B/Nur77 orphan nuclear receptors and glucocorticoid receptor. Molecular Endocrinology. 2005;19(4):885.

18. Gilmore T. Introduction to NF-κB: Players, pathways, perspectives. Oncogene. 2006;25(51):6680-4.

19. Kumar R, Thompson EB. Gene regulation by the glucocorticoid receptor: Structure: Function relationship. J Steroid Biochem Mol Biol. 2005;94(5):383-94.

20. Ronacher K, Hadley K, Avenant C, Stubsrud E, Simons Jr SS, Louw A, et al. Ligand-selective transactivation and transrepression via the glucocorticoid receptor: Role of cofactor interaction. Mol Cell Endocrinol. 2009;299(2):219-31.

21. Wang YD, Chen WD, Huang W. FXR, a target for different diseases. Histol Histopathol. 2008 May;23(5):621-7.

22. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. Science. 1999;284(5418):1362.

23. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, et al. Bile acids: Natural ligands for an orphan nuclear receptor. Science. 1999;284(5418):1365.

24. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell. 1999;3(5):543-53.

25. Lee FY, Lee H, Hubbert ML, Edwards PA, Zhang Y. FXR, a multipurpose nuclear receptor. Trends Biochem Sci. 2006;31(10):572-80.

26. Cariou B, Staels B. FXR: A promising target for the metabolic syndrome? Trends Pharmacol Sci. 2007;28(5):236-43.

27. Fiorucci S, Rizzo G, Donini A, Distrutti E, Santucci L. Targeting farnesoid X receptor for liver and metabolic disorders. Trends Mol Med. 2007;13(7):298-309.

28. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell. 2000;6(3):517-26.

29. Suchy FJ, Ananthanarayanan M. Bile salt excretory pump: Biology and pathobiology. J Pediatr Gastroenterol Nutr. 2006;43(1):S10.

30. Moschetta A, Bookout AL, Mangelsdorf DJ. Prevention of cholesterol gallstone disease by FXR agonists in a mouse model. Nat Med. 2004;10(12):1352-8.

31. Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. J Biol Chem. 2001;276(31):28857.

32. Wong MH, Oelkers P, Dawson PA. Identification of a mutation in the ileal sodium-dependent bile acid transporter gene that abolishes transport activity. J Biol Chem. 1995;270(45):27228.

33. Grober J, Zaghini I, Fujii H, Jones SA, Kliewer SA, Willson TM, et al. Identification of a bile acid-responsive element in the human ileal bile acid-binding protein gene. J Biol Chem. 1999;274(42):29749.

34. Dawson PA, Hubbert M, Haywood J, Craddock AL, Zerangue N, Christian WV, et al. The heteromeric organic solute transporter α-ß, ostα-ostß, is an ileal basolateral bile acid transporter. J Biol Chem. 2005;280(8):6960.

35. Dutton GJ. Glucuronidation of drugs and other compounds. . 1980.

36. Barbier O, Torra IP, Sirvent A, Claudel T, Blanquart C, Duran-Sandoval D, et al. FXR induces the UGT2B4 enzyme in hepatocytes: A potential mechanism of negative feedback control of FXR activity. Gastroenterology. 2003;124(7):1926-40.

37. Mano N, Nishimura K, Narui T, Ikegawa S, Goto J. Characterization of rat liver bile acid acyl glucuronosyltransferase. Steroids. 2002;67(3-4):257-62.

38. van Mil SWC, Milona A, Dixon PH, Mullenbach R, Geenes VL, Chambers J, et al. Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. Gastroenterology. 2007;133(2):507-16.

39. Trauner M, Arrese M, Lee H, Boyer JL, Karpen SJ. Endotoxin downregulates rat hepatic ntcp gene expression via decreased activity of critical transcription factors. J Clin Invest. 1998;101(10):2092.

40. Liu Y, Binz J, Numerick MJ, Dennis S, Luo G, Desai B, et al. Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intra-and extrahepatic cholestasis. J Clin Invest. 2003;112(11):1678-87.

41. Fiorucci S, Clerici C, Antonelli E, Orlandi S, Goodwin B, Sadeghpour BM, et al. Protective effects of 6-ethyl chenodeoxycholic acid, a farnesoid X receptor ligand, in estrogen-induced cholestasis. J Pharmacol Exp Ther. 2005;313(2):604.

42. Zhang Y, Kast-Woelbern HR, Edwards PA. Natural structural variants of the nuclear receptor farnesoid X receptor affect transcriptional activation. J Biol Chem. 2003;278(1):104.

43. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev. 2009;89(1):147.

44. Wang YD, Chen WD, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes nuclear factor κB in hepatic inflammatory response. Hepatology. 2008;48(5):1632-43.

45. Gadaleta RM, Oldenburg B, Willemsen ECL, Spit M, Murzilli S, Salvatore L, et al. Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating NF-[kappa] B signalling in the intestine. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2011.

46. Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. Science. 2006;312(5771):233.

47. Yang F, Huang X, Yi T, Yen Y, Moore DD, Huang W. Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor. Cancer Res. 2007;67(3):863.

48. Modica S, Murzilli S, Salvatore L, Schmidt DR, Moschetta A. Nuclear bile acid receptor FXR protects against intestinal tumorigenesis. Cancer Res. 2008;68(23):9589.

49. Maran RRM, Thomas A, Roth M, Sheng Z, Esterly N, Pinson D, et al. Farnesoid X receptor deficiency in mice leads to increased intestinal epithelial cell proliferation and tumor development. J Pharmacol Exp Ther. 2009;328(2):469.

50. Jantzen HM, Strahle U, Gloss B, Stewart F, Schmid W, Boshart M, et al. Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. Cell. 1987;49(1):29-38.

51. Danesch U, Gloss B, Schmid W, Schütz G, Schüle R, Renkawitz R. Glucocorticoid induction of the rat tryptophan oxygenase gene is mediated by two widely separated glucocorticoid-responsive elements. EMBO J. 1987;6(3):625.

52. Li J, Pircher PC, Schulman IG, Westin SK. Regulation of complement C3 expression by the bile acid receptor FXR. J Biol Chem. 2005;280(9):7427.

53. Song CS, Echchgadda I, Baek BS, Ahn SC, Oh T, Roy AK, et al. Dehydroepiandrosterone sulfotransferase gene induction by bile acid activated farnesoid X receptor. J Biol Chem. 2001;276(45):42549.

54. Laffitte BA, Kast HR, Nguyen CM, Zavacki AM, Moore DD, Edwards PA. Identification of the DNA binding specificity and potential target genes for the farnesoid X-activated receptor. J Biol Chem. 2000;275(14):10638.

55. Newton R, Holden NS. Separating transrepression and transactivation: A distressing divorce for the glucocorticoid receptor? Mol Pharmacol. 2007;72(4):799.

56. Hofmann TG, Schmitz ML. The promoter context determines mutual repression or synergism between NF-κB and the glucocorticoid receptor. Biol Chem. 2002;383(12):1947-51.

57. Wang Y, Zhang J, Dai W, Lei K, Pike JW. Dexamethasone potently enhances phorbol ester-induced IL-1beta gene expression and nuclear factor NF-kappaB activation. The Journal of Immunology. 1997;159(2):534.

58. Webster JC, Huber RM, Hanson RL, Collier PM, Haws TF, Mills JK, et al. Dexamethasone and tumor necrosis factor-α act together to induce the cellular inhibitor of apoptosis-2 gene and prevent apoptosis in a variety of cell types. Endocrinology. 2002;143(10):3866.

59. Chong HK, Infante AM, Seo YK, Jeon TI, Zhang Y, Edwards PA, et al. Genome-wide interrogation of hepatic FXR reveals an asymmetric IR-1 motif and synergy with LRH-1. Nucleic Acids Res. 2010;38(18):6007.

60. Claudel T, Sturm E, Duez H, Torra IP, Sirvent A, Kosykh V, et al. Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-1 transcription via a negative FXR response element. J Clin Invest. 2002;109(7):961-72.

61. Shen H, Zhang Y, Ding H, Wang X, Chen L, Jiang H, et al. Farnesoid X receptor induces GLUT4 expression through FXR response element in the GLUT4 promoter. Cellular Physiology and Biochemistry. 2008;22(1-4):001-14.

62. Takeda T, Kurachi H, Yamamoto T, Nishio Y, Nakatsuji Y, Morishige K, et al. Crosstalk between the interleukin-6 (IL-6)-JAK-STAT and the glucocorticoid-nuclear receptor pathway: Synergistic activation of IL-6 response element by IL-6 and glucocorticoid. J Endocrinol. 1998;159(2):323.

63. Lerner L, Henriksen MA, Zhang X, Darnell JE. STAT3-dependent enhanceosome assembly and disassembly: Synergy with GR for full transcriptional increase of the α2-macroglobulin gene. Genes Dev. 2003;17(20):2564.

64. Dostert A, Heinzel T. Negative glucocorticoid receptor response elements and their role in glucocorticoid action: Transcription factors as targets of novel therapeutic approaches of autoimmune diseases. Curr Pharm Des. 2004;10(23):2807-16.

65. Pascual G, Glass CK. Nuclear receptors versus inflammation: Mechanisms of transrepression. Trends in Endocrinology & Metabolism. 2006;17(8):321-7.

66. Wagner EF, Eferl R. Fos/AP-1 proteins in bone and the immune system. Immunol Rev. 2005;208(1):126-40.

67. Surjit M, Ganti KP, Mukherji A, Ye T, Hua G, Metzger D, et al. Widespread negative response elements mediate direct repression by agonist-liganded glucocorticoid receptor. Cell. 2011;145(2):224-41.

68. Schmitz G, Langmann T, Heimerl S. Role of ABCG1 and other ABCG family members in lipid metabolism. J Lipid Res. 2001;42(10):1513.

69. Gaubatz J, Heideman C, Gotto A, Morrisett J, Dahlen GH. Human plasma lipoprotein [a]. structural properties. J Biol Chem. 1983;258(7):4582.

70. Kostner G, Avogaro P, Cazzolato G, Marth E, Bittolo-Bon G, Qunici G. Lipoprotein lp (a) and the risk for myocardial infarction* 1. Atherosclerosis. 1981;38(1-2):51-61.

71. Rhoads GG, Dahlen G, Berg K, Morton NE, Dannenberg AL. Lp (a) lipoprotein as a risk factor for myocardial infarction. JAMA: The Journal of the American Medical Association. 1986;256(18):2540.

72. Chennamsetty I, Claudel T, Kostner KM, Baghdasaryan A, Kratky D, Levak-Frank S, et al. Farnesoid X receptor represses hepatic human APOA gene expression. J Clin Invest. 2011.

73. Rosenfeld MG, Lunyak VV, Glass CK. Sensors and signals: A coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. Genes Dev. 2006;20(11):1405.

74. Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, et al. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell. 1996;85(3):403-14.

75. Sheppard KA, Phelps KM, Williams AJ, Thanos D, Glass CK, Rosenfeld MG, et al. Nuclear integration of glucocorticoid receptor and nuclear factor-κB signaling by CREB-binding protein and steroid receptor coactivator-1. J Biol Chem. 1998;273(45):29291.

76. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS. Role of transcriptional activation of IκBα in mediation of immunosuppression by glucocorticoids. Science. 1995;270(5234):283.

77. Heck S, Bender K, Kullmann M, Göttlicher M, Herrlich P, Cato ACB. IκBα-independent downregulation of NF-κB activity by glucocorticoid receptor. EMBO J. 1997;16(15):4698-707.

78. Staels B, Koenig W, Habib A, Merval R, Lebret M, Torra IP, et al. Activation of human aortic smooth-muscle cells is inhibited by PPARα but not by PPARγ activators. Nature. 1998;393(6687):790-3.

79. Wissink S, Van Heerde E, Van der Burg B, Van der Saag P. A dual mechanism mediates repression of NF-κB activity by glucocorticoids. Molecular Endocrinology. 1998;12(3):355.

80. Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen ECL, Renooij W, Murzilli S, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut. 2011;60(4):463.

81. Gadaleta RM, van Mil SWC, Oldenburg B, Siersema PD, Klomp LWJ, van Erpecum KJ. Bile acids and their nuclear receptor FXR: Relevance for hepatobiliary and gastrointestinal disease. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2010;1801(7):683-92.

82. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. The Journal of Immunology. 2009;183(10):6251.

83. Kim MS, Shigenaga J, Moser A, Feingold K, Grunfeld C. Repression of farnesoid X receptor during the acute phase response. J Biol Chem. 2003;278(11):8988.

84. Zhou C, Tabb MM, Nelson EL, Grun F, Verma S, Sadatrafiei A, et al. Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. J Clin Invest. 2006;116(8):2280.

85. Nissen RM, Yamamoto KR. The glucocorticoid receptor inhibits NFκB by interfering with serine-2 phosphorylation of the RNA polymerase II carboxy-terminal domain. Genes Dev. 2000;14(18):2314.

86. Gineste R, Sirvent A, Paumelle R, Helleboid S, Aquilina A, Darteil R, et al. Phosphorylation of farnesoid X receptor by protein kinase C promotes its transcriptional activity. Molecular Endocrinology. 2008;22(11):2433.

87. Frankenberg T, Miloh T, Chen FY, Ananthanarayanan M, Sun AQ, Balasubramaniyan N, et al. The membrane protein ATPase class I type 8B member 1 signals through protein kinase C zeta to activate the farnesoid X receptor. Hepatology. 2008;48(6):1896-905.

88. Neimark E, Chen F, Li X, Shneider BL. Bile acid–induced negative feedback regulation of the human ileal bile acid transporter. Hepatology. 2004;40(1):149-56.

89. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. The Journal of Immunology. 2009;183(10):6251.

90. Kemper JK, Xiao Z, Ponugoti B, Miao J, Fang S, Kanamaluru D, et al. FXR acetylation is normally dynamically regulated by p300 and SIRT1 but constitutively elevated in metabolic disease states. Cell metabolism. 2009;10(5):392-404.

91. Kemper JK. Regulation of FXR transcriptional activity in health and disease: Emerging roles of FXR cofactors and post-translational modifications. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2010.

92. Lipford JR, Deshaies RJ. Diverse roles for ubiquitin-dependent proteolysis in transcriptional activation. Nat Cell Biol. 2003;5(10):845-50.

93. GALON J, FRANCHIMONT D, HIROI N, FREY G, BOETTNER A, EHRHART-BORNSTEIN M, et al. Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. The FASEB journal. 2002;16(1):61.

94. Joseph SB, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. Nat Med. 2003;9(2):213-9.

95. Ogawa S, Lozach J, Benner C, Pascual G, Tangirala RK, Westin S, et al. Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. Cell. 2005;122(5):707-21.

96. Welch JS, Ricote M, Akiyama TE, Gonzalez FJ, Glass CK. PPARγ and PPARδ negatively regulate specific subsets of lipopolysaccharide and IFN-γ target genes in macrophages. Proceedings of the National Academy of Sciences. 2003;100(11):6712.

97. Shimizu M, Yamashita D, Yamaguchi T, Hirose F, Osumi T. Aspects of the regulatory mechanisms of PPAR functions: Analysis of a bidirectional response element and regulation by sumoylation. Mol Cell Biochem. 2006;286(1):33-42.

98. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, et al. A sumoylation-dependent pathway mediating transrepression of inflammatory response genes by PPARγ. Nature. 2005;437(7059):759.

99. Ghisletti S, Huang W, Ogawa S, Pascual G, Lin ME, Willson TM, et al. Parallel SUMOylation-dependent pathways mediate gene-and signal-specific transrepression by LXRs and PPAR [gamma]. Mol Cell. 2007;25(1):57-70.

100. Hwang EJ, Lee JM, Jeong J, Park JH, Yang Y, Lim JS, et al. SUMOylation of ROR [alpha] potentiates transcriptional activation function. Biochem Biophys Res Commun. 2009;378(3):513-7.

101. Sentis S, Le Romancer M, Bianchin C, Rostan MC, Corbo L. Sumoylation of the estrogen receptor α hinge region regulates its transcriptional activity. Molecular Endocrinology. 2005;19(11):2671.

102. Floyd ZE, Stephens JM. Control of peroxisome proliferator-activated receptor &ggr; 2 stability and activity by SUMOylation* &ast. Obesity. 2004;12(6):921-8.

103. Yamashita D, Yamaguchi T, Shimizu M, Nakata N, Hirose F, Osumi T. The transactivating function of peroxisome proliferator-activated receptor γ is negatively regulated by SUMO conjugation in the amino-terminal domain. Genes to Cells. 2004;9(11):1017-29.

104. Davies L, Karthikeyan N, Lynch JT, Sial EA, Gkourtsa A, Demonacos C, et al. Cross talk of signaling pathways in the regulation of the glucocorticoid receptor function. Molecular Endocrinology. 2008;22(6):1331.

105. Venteclef N, Jakobsson T, Ehrlund A, Damdimopoulos A, Mikkonen L, Ellis E, et al. GPS2-dependent corepressor/SUMO pathways govern anti-inflammatory actions of LRH-1 and LXRß in the hepatic acute phase response. Genes Dev. 2010;24(4):381.

106. Treuter E, Venteclef N. Transcriptional control of metabolic and inflammatory pathways by nuclear receptor SUMOylation. Biochim Biophys Acta. 2011 Aug;1812(8):909-18.

107. Yoh SM, Privalsky ML. Transcriptional repression by thyroid hormone receptors. J Biol Chem. 2001;276(20):16857.

108. Kerscher O. SUMO junction—what's your function? EMBO Rep. 2007;8(6):550-5.

109. Jakobsson T, Venteclef N, Toresson G, Damdimopoulos AE, Ehrlund A, Lou X, et al. GPS2 is required for cholesterol efflux by triggering histone demethylation, LXR recruitment, and coregulator assembly at the ABCG1 locus. Mol Cell. 2009;34(4):510-8.

110. Heck S, Kullmann M, Gast A, Ponta H, Rahmsdorf H, Herrlich P, et al. A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. EMBO J. 1994;13(17):4087.

111. Cole TJ, Blendy JA, Monaghan AP, Krieglstein K, Schmid W, Aguzzi A, et al. Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. Genes Dev. 1995;9(13):1608.

112. Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, et al. DNA binding of the glucocorticoid receptor is not essential for survival. Cell. 1998;93(4):531-41.

113. Reichardt HM, Tuckermann JP, Göttlicher M, Vujic M, Weih F, Angel P, et al. Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. EMBO J. 2001;20(24):7168-73.

114. Pérez E, Bourguet W, Gronemeyer H, de Lera AR. Modulation of RXR function through ligand design. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2011.

115. Natoli G, De Santa F. Shaping alternative NF-κB-dependent gene expression programs: New clues to specificity. Cell Death & Differentiation. 2006;13(5):693-6.

116. Vanden Berghe W, Ndlovu MN, Hoya-Arias R, Dijsselbloem N, Gerlo S, Haegeman G. Keeping up NF-[kappa] B appearances: Epigenetic control of immunity or inflammation-triggered epigenetics. Biochem Pharmacol. 2006;72(9):1114-31.

117. De Bosscher K, Vanden Berghe W, Haegeman G. The interplay between the glucocorticoid receptor and nuclear factor-κB or activator protein-1: Molecular mechanisms for gene repression. Endocr Rev. 2003;24(4):488.