Published in final edited form as:
Mol Pharm. 2008 ; 5(1): 17–34.

Cell Signaling and Nuclear receptors: New Opportunities for Molecular Pharmaceuticals in Liver Disease

Jeff L. Staudinger* and Kristin Lichti

University of Kansas, Department of Pharmacology and Toxicology, 1251 Wescoe Hall Dr, 5038 Malott Hall, Lawrence, Kansas, 66045

Abstract

Liver-enriched nuclear receptors (NRs) collectively function as metabolic and toxicological ‘sensors’ that mediate liver-specific gene-activation in mammals. NR-mediated gene-environment interaction regulates important steps in the hepatic uptake, metabolism and excretion of glucose, fatty acids, lipoproteins, cholesterol, bile acids, and xenobiotics. Hence, liver-enriched NRs play pivotal roles in the overall control of energy homeostasis in mammals. While it is well-recognized that ligand-binding is the primary mechanism behind activation of NRs, recent research is revealing that multiple signal transduction pathways modulate NR-function in liver. The interface between specific signal transduction pathways and NRs helps to determine their overall responsiveness to various environmental and physiological stimuli. In general, phosphorylation of hepatic NRs regulates multiple biological parameters including their transactivation capacity, DNA-binding, sub-cellular location, capacity to interact with protein cofactors, and protein stability. Certain pathological conditions including inflammation, morbid obesity, hyperlipidemia, atherosclerosis, insulin resistance, and type-2 diabetes are known to modulate selected signal transduction pathways in liver. This review will focus upon recent insights regarding the molecular mechanisms that comprise the interface between disease-mediated activation of hepatic signal transduction pathways and liver-enriched NRs. This review will also highlight the exciting opportunities presented by this new knowledge to develop novel molecular and pharmaceutical strategies for combating these increasingly prevalent human diseases.

Introduction

General NR Structure and Function

NRs are one of the largest groups of transcription factors with 48 members in the human genome that regulate diverse biological processes including metabolism, homeostasis, development and reproduction ¹. The activity of many NRs is controlled by the binding of small lipophilic molecules such as hormones, fatty acids, bile acids and oxysterols, and xenobiotics.

All members of the NR superfamily share several conserved structural domains that are essential for receptor function ². The C-terminal region encompasses the ligand binding domain (LBD) and includes a region termed activation function 2 (AF-2), which is an important site for co-activator protein-binding. Binding of ligand induces a conformational change that creates a new surface for the recruitment of co-activator proteins in the AF-2 region ³. The LBD is connected to a DNA binding domain (DBD) by a hinge region (H) which contains a nuclear localization signal. The DBD is highly conserved and contains two alpha helices and two zinc fingers that are involved in the specificity of response-element-recognition and in

*To whom correspondence should be addressed: University of Kansas, Department of Pharmacology and Toxicology, 1251 Wescoe Hall Dr., 5038 Malott Hall, Lawrence, Kansas, 66045, Tel 785-864-3951, Fax 785-864-5219, stauding@ku.edu.

receptor dimerization. Most liver-enriched NRs are active as dimers, functioning either as homodimers, or as heterodimers with retinoid x receptor (RXR)⁴. Vertebrate RXR includes at least three distinct genes (RXR α , RXR β , and RXR γ) which give rise to a large number of protein products through differential promoter usage and alternative splicing.

The N-terminal region of NRs is highly variable in sequence and length, but all contain a region termed activation function 1 (AF-1) that acts independently of ligand⁵. The AF-1 domain contains many consensus phosphorylation sites and is therefore, the target of multiple kinases. Although most of the phosphorylation sites identified in NRs are located in the N-terminal domain, many receptors have at least one phosphorylation site in the H region, and there are limited reports of sites located in the LBD and DBD. In addition, there are likely many yet to be identified phosphorylation sites in NRs.

Intracellular Localization

Most NRs are constitutively localized in the nucleus, however, the major proportion of steroid receptors and other a few other exceptional receptors may be located in the cytoplasm in the absence of ligand. Nuclear localization of receptors is mainly regulated by protein-protein interactions such as dimerization with RXRs or co-regulator proteins⁶. In the cytoplasm, NRs are bound to heat shock proteins and this association prevents receptor transportation through the nuclear pores and thus sequesters NRs from binding to DNA⁷. In the nucleus, ligand-mediated activation of NRs causes redistribution of the receptor to chromatin. Recent evidence which will be discussed in more detail has suggested that nuclear localization of some NRs is a cell signaling- and phosphorylation-dependent event.

Co-regulator Proteins

The full activity of NRs depends on a large number of co-regulator proteins that do not bind to DNA directly, but have a pronounced effect on the outcome of gene expression³. In general, non-liganded NRs form a complex with co-repressor proteins which inhibit transcriptional activity, often through the recruitment of other cofactor proteins that contain histone deacetylase (HDAC) activity. HDACs alter chromatin structure by promoting chromatin compaction, thus rendering enhancer regions of genes less accessible to the necessary basal transcriptional machinery. Activation of NRs by ligand-binding or through phosphorylation induces a conformational change which results in the dissociation of the co-repressor multiprotein complexes and subsequent recruitment of co-activator protein complexes that enhance the rate of gene transcription, often through the recruitment multiprotein complexes containing histone acetyltransferase (HAT) activity. Co-regulator proteins thus provide a second level of specificity in the modulation of gene expression by NRs. Most NR-co-activator proteins identified to date preferentially interact with NRs through the C-terminal AF-2 domain via an -LXXLL-motif, which constitutes a prototypical NR-interaction motif. However, in contrast to most co-activator proteins, the peroxisome proliferator activated receptor gamma co-activator 1 alpha ((PPARGC1a/PGC-1 α) interacts not only with the AF-2 region of NRs, but also with the H region of the selected liver-enriched NRs⁸. In addition to NRs, it has also been shown that the intrinsic and recruited enzymatic activities of several NR-associated cofactor proteins are regulated by phosphorylation in a dynamic manner in response to specific signal transduction pathways, and this will be discussed later in this review in more detail.

Liver-Enriched NRs as Targets of Signal Transduction Pathways

The majority of amino acid residues (serine and threonine) identified as being regulated-phosphorylation sites lay within the N-terminal region of NRs that correspond to consensus sites for proline-dependent kinases such as cyclin-dependent kinases (CDK)^{9, 10} and MAP kinases¹¹. For some NRs, such as progesterone receptor (PR) which contains at least 13 sites,

phosphorylation of the N-terminus is quite complex. However, other NRs such as peroxisome proliferator activated receptors (PPARs) contain only one or two phosphorylation sites in the N-terminus¹².

NRs can be phosphorylated constitutively in the absence of ligand, or in response to ligand-mediated activation. Other NRs can be phosphorylated independently of ligand in response to cellular signaling events by MAPKs. For example, growth factors, stress, cytokines, and other signals activate several serine kinase cascade pathways that activate different MAP kinases (Erk, JNK, or p38 MAPK) which can enter the nucleus and phosphorylate NRs. The N-terminal domains of PR^{13, 14}, ER α ^{15, 16}, ER β ^{17, 18}, AR¹⁹, PPARs^{20, 21}, and RAR γ ^{22, 23} have all been reported to be substrates of p42/p44 or p38 MAPK, and that of RXR α is phosphorylated by JNK. In addition to MAPK sites, the N-terminus of many NRs also contains consensus sites for Akt kinase, or PKB, a kinase critical for cell survival and proliferation^{24, 25}. Activated Akt negatively regulates downstream MAP kinases²⁶, and upon nuclear translocation phosphorylates specific NRs including ER α ²⁷ and AR²⁸.

In addition to the N-terminal domain, the LBD and DBD are also targets for protein kinases. Phosphorylation of the LBD can involve the same proline dependent kinases. For example, serine residues contained within the LBD of RXRs are targeted by the stress activated protein kinase- JNK^{29, 30}. Phosphorylation by other kinases such as tyrosine kinases for ER α ^{31, 32} and RXR α ³⁰ or PKA for RAR³³ is also common. Evidence for phosphorylation of the DBD involves either PKA for ER α ³⁴ or PKC for RAR α ³⁵ and VDR^{36, 37}.

While signal transduction pathways and phosphorylation regulate most, if not all NRs in multiple tissue types, this review will focus on the regulation of non-steroid NRs expressed in a liver-enriched manner.

NR2A1, HNF-4 α

Hepatic nuclear factor-4 α (HNF-4 α) is a NR expressed mainly in liver, intestine, and kidney and is critical for development and liver specific gene expression³⁸. HNF-4 α has been implicated in the regulation of many genes in liver such as Cyp7a1³⁹, the constitutive androstane receptor (CAR)⁴⁰, and genes involved in glucose transport and glycolysis⁴¹. Although typically thought of as an orphan receptor, HNF-4 α has been shown to be activated by fatty acyl-CoA thioesters⁴². In addition, the transcriptional activity of HNF-4 α is regulated by phosphorylation of serine, threonine, and tyrosine residues. Phosphorylation of HNF-4 α is required for DNA binding and appropriate subnuclear localization. Violett *et al.* identified a PKA consensus phosphorylation site in the DBD of HNF-4 α and report that HNF-4 α is directly phosphorylated by PKA. PKA-mediated phosphorylation of wild-type HNF-4 α strongly repressed the binding affinity and transcriptional activity of the receptor based on gel-shift assays and reporter gene analysis⁴³. On the other hand, phosphorylation of specific serine and threonine residues in HNF-4 α alters its tertiary structure, which increases the affinity and specificity of DNA-binding in COS-7 cells⁴⁴. In addition to alterations in DNA-binding, tyrosine phosphorylation is required for appropriate sub-nuclear localization and transactivation activity of HNF4 α as evidenced by immunofluorescence, electron microscopy, and reporter gene assay using genistein treatment to inhibit tyrosine phosphorylation⁴⁵.

It has also been recently shown that p38 kinase phosphorylates HNF-4 α at S158 increasing its interaction with co-activator PC4, DNA-binding, and transactivation activity in the presence of IL-1 β and hydrogen peroxide⁴⁶. In addition, inhibition of p38 kinase activity diminishes HNF-4 α nuclear protein levels and its phosphorylation, rendering a less stable protein. Induction of p38 kinase by insulin results in an increase of HNF4 α protein and Cyp7a1 gene expression in primary rat hepatocytes, thus providing a functional link between HNF-4 α phosphorylation and bile acid synthesis⁴⁷. Since HNF-4 α has been shown to activate multiple

genes and interacts with multiple transcription factors and co-regulators in liver such as COUP-TF's, CBP, GRIP-1 SRC-1, p300 and PGC-1 α ^{40, 48, 49}, further study is required to determine the mechanism by which phosphorylation of HNF-4 α modulates protein-protein interactions and differential gene expression.

The NR1C Subfamily- PPARs

The three peroxisome proliferators activated receptor (PPAR) isotypes, PPAR α , β , and γ , form a subfamily of NRs that are mainly involved in lipid and glucose homeostasis, control of inflammation and wound healing, and regulation of food intake and body weight^{50, 51}. PPAR α is expressed in metabolically active tissues including liver, kidney, heart, skeletal muscle and brown fat. PPAR γ is expressed to a high extent in adipose tissue with lower amounts present in kidney, liver, and skeletal and smooth muscle⁵². Fatty acids and fatty acid derivatives are endogenous ligands for PPARs and induce PPAR-dependent gene activation. In addition, PPARs are very important therapeutic targets for the treatment of hyperlipidemia and type-2-diabetes. The hypolipidemic fibrates were the first known synthetic ligands of PPAR α , while thiazolidinediones are the best characterized PPAR γ ligands used in the treatment of type-2-diabetes⁵³. Therefore, understanding the mechanisms that regulate PPAR activity is crucial for effective therapeutic treatment of metabolic diseases.

Insulin treatment enhances PPAR α activity via phosphorylation of S12 and S21 by p42/p44 MAP kinase, but represses PPAR γ activity via phosphorylation of S112^{20, 21}. Furthermore, phosphorylation of the N-terminal domain of PPAR γ has been shown to decrease PPAR γ activity. For example, PDGF treatment decreases PPAR γ transcriptional activity in reporter gene assays, and *in vivo* labeling experiments demonstrated that PPAR γ undergoes EGF-stimulated MAPK-dependent phosphorylation at S82⁵⁴. Further studies indicate that PPAR γ activity is decreased through phosphorylation of the N-terminus at S84 by ERK2 and JNK via TNF4 α and EGF stimulation⁵⁵. In contrast, one study reports an increase in transactivation of PPAR γ via insulin-stimulated ERK2 phosphorylation in CHO cells⁵⁶. Differential modulation of PPAR activity by phosphorylation is likely due to the relationships between specific kinases, serine residues, ligands, receptor isoforms in specific cell types. For example, ERK2 and JNK, but not p38 MAP kinase can phosphorylate PPAR γ on S84⁵⁵, while PPAR α is a substrate for both ERK2 and p38 MAP kinase in a ligand-dependent manner⁵⁷. In addition, phosphorylation of PPAR α by PKA in transient reporter gene assays was shown to have different effects depending on which promoter was used experimentally⁵⁸.

There are multiple mechanisms by which phosphorylation of PPARs modulates their activity. Transfection studies have suggested that phosphorylation of the N-terminus induces the dissociation of co-repressor proteins such as NCoR from PPAR α ²¹. In a similar fashion, phosphorylation of the N-terminus of PPAR α increases co-activation by PGC-1 α ⁵⁷. Phosphorylation can also enhance DNA-binding of PPARs, as is the case with PPAR α phosphorylation by PKA⁵⁸. PPARs may also be modulated through kinase cascades that up-regulate their own expression as shown by the PKC-dependent upregulation of PPAR α gene expression⁵⁹.

MAPKs (ERK1, ERK2, p38, and JNK), PKA, and PKC are the three kinase families that have been implicated in the phosphorylation of PPAR α and γ . Activation of these signaling pathways and phosphorylation of PPARs could affect the endogenous and therapeutic function of PPARs. For example stress or fasting may activate PKA signaling which phosphorylates PPAR α and enhances its activity through the recruitment of PGC-1 α and may affect the function of PPAR α as a drug target.

NR1I2, PXR

Pregnane x receptor (PXR) is a master-regulator of xenobiotic-inducible cytochrome-p450 (CYP) gene expression in liver. The CYPs identified as PXR target-genes encode enzymes responsible for the oxidative metabolism of over 60% of clinically prescribed drugs. In addition, several studies have shown that PXR regulates other genes involved in the metabolism of xenobiotic and endobiotic compounds such as glutathione S-transferases, sulfotransferases, and UDP-glucuronosyltransferases⁶⁰⁻⁶³. PXR also regulates the expression of the drug-transporter genes Oatp2, Mdr1, Mrp2, and Mrp3⁶⁴⁻⁶⁶. PXR is a promiscuous receptor activated by a wide variety of compounds including synthetic and endogenous steroids, bile acids, and a variety of drugs and natural compounds⁶⁷. In this manner, the modulation of PXR activity by ligands and/or signaling pathways represents the basis for an important class of drug-drug interactions.

Drug-inducible CYP gene expression is known to be responsive to cytokine, PKC, and PKA signaling pathways, however the exact mechanism by which these pathways intersect with PXR is unknown. For example, a significant reduction in the hepatic expression of Mdr1 and Mrp3 genes were seen in endotoxin treated mice. Similarly, IL-6-treated mice displayed a 40-70% reduction in the mRNA levels of all Mdr isoforms⁶⁸. Inflammatory cytokines inhibit the inducible expression of Oatp2 during intrahepatic cholestasis⁶⁹. It has also been shown using primary cultures of human hepatocytes that IL-6 markedly decreases the expression of PXR and its close cousin constitutive androstane receptor (CAR). IL-6 also decreases both rifampicin- and phenobarbital-mediated induction of CYP3A and CYP2B gene expression⁷⁰.

Recent evidence has demonstrated crosstalk between PXR and NF- κ B signaling pathways. NF- κ B activation by LPS and TNF4 α repressed PXR association with the CYP3A promoter by disrupting the association of the PXR-RXR protein complex⁷¹. In addition, PXR activation inhibited the activity of NF- κ B and the expression of its target genes. This inhibition was shown to be PXR-dependent and was potentiated by PXR ligands *in vitro* and *in vivo*⁷². PXR activation has also been shown to alleviate the symptoms of inflammatory bowel disease (IBS). Studies using IBS and PXR knockout mouse models have shown that PXR agonist treatment decreased the expression of NF- κ B target gene expression in a PXR-dependent manner⁷³.

In addition to cytokine signaling, CYP3A gene expression is also modulated by PKA and PKC signaling pathways. Co-treatment of primary cultures of rat hepatocytes with phenobarbital and cyclic AMP analogs and PKA activators clearly results in cyclic AMP-associated inhibition of CYP gene expression⁷⁴. However, treatment with the adenyl cyclase activator forskolin and its non-PKA-activating analog 1,9 dideoxyforskolin both resulted in the stimulation of CYP3A gene expression⁷⁵. Ding and Staudinger have shown that both forskolin and 1,9 dideoxyforskolin induce CYP3A expression in primary mouse hepatocytes by functioning as PXR agonists. In addition, activation of PKA signaling potentiated PXR-mediated induction of CYP3A expression and increased the strength of PXR-co-activator protein interactions in mammalian 2-hybrid reporter gene assays. Further kinase assays show that PXR can be a substrate for PKA *in vitro*, suggesting a potential mechanism for PKA-mediated modulation of CYP3A gene expression⁷⁶. It is also of interest that while PKA activation potentiates the expression of CYP3A in mouse hepatocytes, it is a repressive signal in both human and rat hepatocyte cultures (our unpublished data). This suggests a species-specific effect for the modulation of CYP3A by PKA signaling. Differential phosphorylation may be a possible factor for the species-specific responses to PKA signaling. In addition, activation of PKC signaling dramatically represses PXR activity in reporter gene assays and in hepatocytes by increasing the strength of interaction between PXR and the co-repressor NCoR, and by abolishing the ligand-dependent interaction between PXR and co-activator SRC-1⁷⁷.

NR1I3, CAR

Similar to PXR, the NR CAR was first classified as a xenobiotic-sensing transcription factor that regulates numerous hepatic genes in response to a large group of xenobiotics and endobiotics. CAR was originally found to regulate the transcription of genes encoding the CYP2B subfamily⁷⁸. In addition to CYP2B, CAR also regulates the expression of multiple drug and hormone metabolizing enzymes and transporter proteins such as CYP3A, CYP2C, glutathione S-transferases, sulfotransferases, and UDP-glucuronosyltransferases, Oatp2, Mrp2 and Mrp3⁷⁹. Interestingly, treatment of wild type and CAR knockout mice with phenobarbital, the prototypical CAR activator, both induces and represses certain hepatic genes in a CAR-dependent manner suggesting that CAR has diverse roles as both a positive and negative regular of hepatic gene expression in response to phenobarbital⁸⁰. As the function of CAR has expanded, so has interest in the deciphering the molecular mechanism of its activation by drugs.

It is well documented that the phenobarbital-mediated induction of CYP2B genes in cultured hepatocytes is responsive to several serine/threonine protein kinases and phosphatases. However, the mechanism by which these signaling pathways interact with CAR remains poorly understood. For example, activation of PKA signaling negatively impacts the induction of CYP2B expression in primary cultures of rat hepatocytes⁷⁴, and co-treatment with phosphatase inhibitors further potentiates the repressive effects of PKA signaling⁸¹. Serine/threonine-specific protein phosphatases PP1 and PP2A have a positive role in the induction of CYP2B⁸². Pustynnyak *et al.* further reports that rats treated with inhibitors of Ca(2+)/calmodulin-dependent kinase inhibitors exhibited increased gene expression of both CAR and CYP2B, while rats treated with the protein phosphatase PP1 and PP2A inhibitor okadaic acid exhibited the opposite effect⁸³.

In the absence of a ligand or activator, CAR is sequestered in the cytoplasm where it forms a complex with Hsp90 and cytoplasmic CAR retention protein (CCRP). In response to phenobarbital the complex recruits protein phosphatase 2A before translocation of CAR to the nucleus. The protein phosphatase inhibitor okadaic acid represses phenobarbital-induced nuclear translocation of CAR⁸⁴. In addition, dephosphorylation of S202 in mouse CAR is required for its nuclear translocation⁸⁵. The signaling pathway involved in the phosphorylation of S202 remains unknown. Unlike most NRs, CAR translocates to the nucleus without directly binding phenobarbital⁸⁶. Taken together, these data indicate that the phosphorylation status of CAR is intimately involved its cytoplasmic retention and nuclear translocation.

Recent evidence shows that epidermal growth factor (EGF) represses phenobarbital-mediated activation of CAR-dependent transcription⁸⁷, and that the MEK inhibitor U0126 increases the phenobarbital mediated induction of CYP2B in primary rat hepatocytes⁸⁸. In addition, hepatocyte growth factor (HGF) treatment represses induction of cyp2b10 by phenobarbital in primary mouse hepatocytes. HGF treatment increased the phosphorylation of ERK1/2, thus decreasing the nuclear translocation of CAR⁸⁹, however, the exact mechanism by which this occurs is unknown. In addition to MEK/ERK signaling, AMP-activated protein kinase (AMPK) has been suggested to activate CAR in hepatocytes⁹⁰. Additional studies using AMPK knockout mice demonstrate that although AMPK does not regulate the phenobarbital-induced translocation of CAR, it may be involved in the activation of CAR in the nucleus⁹¹. Shindo *et al.*, reported that activation of AMPK resulted in nuclear accumulation of CAR but was not sufficient to induce CYP2B gene expression⁹². Additional studies suggest that phenobarbital targets LKB1 for the activation of AMPK⁹³, adding a proximal target to the elusive sequence of events by which phenobarbital activates transcription of CYP2B. While AMPK appears to be an activating signal for phenobarbital-mediated induction of CYP2B, MEK/ERK seems to be repressive. Further study into the signal-related mechanisms of CAR

activation is required to determine the effect that these pathways might have on the phosphorylation status of CAR or CAR-interacting proteins.

NR1H3, LXR

Liver x receptors (LXR α and LXR β) have emerged as important regulators of cholesterol metabolism and transport, lipid metabolism, glucose homeostasis and inflammation⁹⁴. LXR α is primary expressed in liver, macrophages, and adipose tissue, while LXR β is more ubiquitously expressed⁶⁷. LXR-activating ligands include several oxysterols and 6 α -hydroxy bile acids^{95, 96}. Since the discovery of LXRs, multiple LXR-target genes that are involved in cholesterol and lipid homeostasis have been identified. These include CYP7A1, the rate limiting enzyme in the classical pathway of bile acid synthesis, ATP-binding cassette (ABC) transporters, lipoproteins such as apolipoprotein E (ApoE), lipoprotein lipase (LPL), and lipogenic proteins such as sterol response element binding protein (SREBP)-1C and fatty acid synthase (FAS)⁹⁷⁻¹⁰⁰. Although early reports emphasized the role of LXR in cholesterol homeostasis, recent studies suggest that LXR negatively regulates gluconeogenesis¹⁰¹ and inflammatory responses^{102, 103}.

LXR has been shown to exist as a phosphoprotein in HEK293 cells. Mutational analysis and metabolic labeling indicate that LXR is constitutively phosphorylated at S198 in the hinge region of the receptor at a MAPK consensus site¹⁰⁴. However, the biological significance of this phosphorylation event has yet to be elucidated.

Early studies demonstrate that PKA/PKC modulators such as prostaglandin E2, phorbol esters, 8-bromo-cyclic AMP, and forskolin enhanced the induction of reporter genes by LXR ligands¹⁰⁵. These experiments suggest that transactivation by ligand-activated LXR may be further modulated through kinase signaling. PKA can directly phosphorylate LXR, and has been reported to both increase and decrease transactivation depending on the experimental conditions¹⁰⁶⁻¹⁰⁸. In primary cultures of rat hepatocytes, activation of PKA repressed LXR-mediated SREBP-1C gene expression. Direct phosphorylation of LXR by PKA *in vitro* and *in vivo* at two PKA consensus sites (S195, S196 and S290, S291) located in the LBD was required for trans-repression. PKA-mediated phosphorylation of LXR impaired DNA-binding through the disruption of LXR/RXR dimerization, and decreased transcriptional activity by inhibiting the recruitment of the co-activator protein- SRC-1, and enhancing the recruitment of co-repressor protein- NCoR¹⁰⁸. On the other hand, Tamura et al. have demonstrated that PKA signaling can increase LXR transactivation in reporter gene assays conducted in remal As4.1 mouse cell lines¹⁰⁶.

In addition to inducing genes involved in cholesterol and glucose homeostasis, LXR reciprocally represses a set of inflammatory genes including iNOS, COX-2 and IL-6, MMP-9 after bacterial, LPD, TNF α or IL-1 β stimulation^{102, 103}. Importantly, LXR agonists reduce inflammation *in vivo*. The mechanism by which LXR represses inflammatory genes is not well understood. No LXREs have been identified on the promoters of the repressed genes. In addition to possible competition for co-regulator proteins, recent evidence suggests that inhibition of the NF- κ B pathway is involved likely through trans-repression of NF- κ B in the nucleus¹⁰⁹. In a recent study of trans-repression of the iNOS promoter, SUMOylation of PPAR γ was identified as a mechanism of repression¹¹⁰. It remains to be determined the extent to which post-translational modification of LXR is involved in LXR-mediated trans-repression of inflammatory genes.

NR5A2, LRH-1

The orphan NR liver receptor homolog 1 (LRH-1) functions to regulate the expression of a number of genes involved in bile acid homeostasis and other liver functions. Unlike the

majority of NRs that function as dimers, LRH-1 binds as a monomer to an extended NR half site in the promoter its target genes¹¹¹. LRH-1 is an important regulator of CYP7A1 gene expression^{112, 113}. LRH-1 also regulates the expression of other genes involved in cholesterol and bile acid homeostasis including CYP8B1, MRP3, cholesterol ester transfer protein (CETP), apical sodium-dependent bile acid transporter (ASBT), and apolipoprotein A1^{60, 114-119}.

No ligands for LRH-1 have yet been identified, and the mechanisms that modulate its activity are still unclear. Lee *et al.* have shown that treatment with PMA increases LRH-1 activation in Hela cells. This response is blocked by the ERK1/2 inhibitor U0126. Mutation analysis confirm that phosphorylation of LRH-1 at S238 and S243 in the H domain stimulates LRH-1 transactivation¹²⁰. In contrast, activation of stress-activated protein kinase (JNK) pathways is associated with inhibitory effects on the LRH-1 target CYP7A1; however the role of LRH-1 in this pathway is unclear^{121, 122}.

Treatment of HepG2 cells with the inflammatory cytokine- TNF α , a potent activator of the JNK pathway, increased the expression of LRH-1 and MRP3, and also increased LRH-1-binding to the MRP3 promotor¹¹⁴. Krylova et al., have suggested that phosphatidylinositols, major intracellular signaling molecules, bind to LRH-1, linking phospholipids signaling and gene expression¹²³. However the function role of phosphatidylinositols as modulators of LRH-1 function remains unknown.

NR1H4, FXR

Farnesoid x receptor (FXR) is a NR expressed in liver, intestine, kidney and adipose tissue. FXR has emerged as a key player involved in the maintenance of cholesterol and bile acid homeostasis through its regulation of the expression of genes involved in the synthesis, uptake, and excretion of bile acids¹²⁴. An important breakthrough in the FXR field was the discovery that FXR is directly activated by several bile acids including chenodeoxycholic acid (CDCA), lithocholic acid (LCA), and deoxycholic acid (DCA)¹²⁵⁻¹²⁷. Studies with FXR knockout mice revealed that a number of genes involved in cholesterol homeostasis are also regulated by FXR including Cyp7a1, Cyp8b1, intestinal bile acid binding protein (I-BABP), canalicular bile salt excretory pump (BSEP), phospholipids transfer protein (PLTP), and the hepatic basolateral transporter NTCP¹²⁸. Additional studies reveal that FXR induces expression of the NR- SHP. Increased SHP then represses Cyp7a1 transcription by inhibiting the activity of LRH-1, which is a positive regulator of the Cyp7a1 promoter¹¹².

Although there is no evidence at this time for direct phosphorylation of FXR, the expression and activity of FXR are modulated by signal transduction pathways. For example, FXR is thought to modulate insulin signaling. FXR expression is reduced in streptozotocin (STZ)-induced diabetic rat models, and administration of insulin restores FXR mRNA to normal levels¹²⁹. Additional support for this concept comes from the observation that glucose reduces FXR expression in the liver¹²⁹, while activation of FXR by the synthetic agonist GW4064 reduced plasma glucose levels in mice¹³⁰. Furthermore, loss of FXR disrupts normal glucose homeostasis and leads to the development of insulin resistance in FXR knockout mice¹³¹⁻¹³³. FXR may also play a role in regulating glucose and lipid metabolism during alterations in nutritional status. A recent study reports that FXR expression is induced in mouse liver in response to fasting, a condition during which PKA signaling is enhanced¹³⁴.

Bile acids that are FXR agonist have been shown to activate multiple signal transduction pathways. Treatment with taurocholic acid results in activation of the JNK pathway¹³⁵, and deoxycholic acid treatment activates the Raf-1/MEK/ERK signaling cascade in primary rat hepatocytes¹³⁶. In addition, treatment of HepG2 cells with bile acids results in the activation of PKC, and treatment with PKC inhibitors reduces the bile acid-mediated repression of Cyp7a1 gene expression¹³⁷. While there is evidence for multiple bile acid-responsive

pathways, future research goals include elucidation of the effects that kinase activation has on the phosphorylation status and functional activity of FXR.

NR0B2, SHP

The small heterodimer partner (SHP) is an atypical orphan member of the NR superfamily in that it lacks the conserved DBD. SHP was isolated in a yeast 2-hybrid screen based on its ability to dimerize with other NRs¹³⁸. It is expressed mainly in liver, small intestine, spleen, heart and pancreas¹³⁹. SHP interacts with a variety of NRs in liver including PPAR α ¹⁴⁰, LRH-1^{112,141}, LXR¹⁴², and HNF4 α ¹⁴³. SHP acts as a direct transcriptional repressor and inhibits the activity of most NRs with which it interacts¹⁴⁴. Two notable exceptions are that SHP enhances the transcriptional activation of PPAR α ¹⁴⁵, and PPAR γ ¹⁴⁶ under certain conditions.

It has been shown that SHP expression is regulated by the JNK pathway. Gupta *et al.* provide evidence that bile acids rapidly down-regulate CYP7A1 transcription via activation of the JNK/c-Jun pathway, and that SHP is a direct target-gene of activated c-Jun¹³⁵. Over-expression of c-Jun resulted in increased SHP promoter activity, whereas mutation of the c-Jun response element in the SHP promoter abolished activation induction of reporter gene expression under the control of the SHP promoter. This study provides an alternative mechanism for bile acid-mediated induction of SHP expression that is independent of FXR.

NRs and FGFs

Recent evidence has uncovered several novel NR-dependent mechanisms involving fibroblast growth factors (FGFs). This recent thrust of research has created a new paradigm that particular FGFs function as metabolic hormones and act through yet to be described signal transduction cascades to elicit specific physiological responses. FGFs function in processes such as development and wound healing. However, three member of the FGF family, FGF19 (FGF15 in mouse), FGF21, and FGF23 have recently emerged as novel metabolic hormones.

As mentioned earlier in this review, activation of FXR by bile acids down-regulates Cyp7a1 gene expression in mice through an indirect mechanism involving the induction and activation of the negative regulator- SHP. Recently, an additional FXR-dependent mechanism involving FGF19 has been described. FGF19 binds to its cell surface receptor, FGFR4, and increases JNK-dependent signaling¹⁴⁷. In primary cultures of human hepatocytes, FXR activation induces expression of FGF19. FGF19 then modulates bile acid biosynthesis by reducing the expression of CYP7A1 through a JNK-dependent pathway without affecting SHP expression¹⁴⁸. It has also been shown that over-expression of FGF19, using either a transgenic approach or with chronic FGF19 treatment, improves insulin sensitivity and glucose homeostasis in diet-induced obese mice, in part through increased metabolic rate and fatty acid oxidation^{149,150}. Conversely, activation of the JNK signaling pathway in the liver has been shown to increase insulin resistance¹⁵¹, however there is currently no explanation for this discrepancy.

FGFR4 deficient mice exhibit reduced JNK activity, an increased bile acid pool, and enhanced expression of CYP7A1¹⁵². On the other hand, transgenic mice expressing constitutively active FGFR4 exhibit increased JNK activity, a reduced bile acid pool, and reduced expression of CYP7A1¹⁵³. The expression of FGF15, the mouse ortholog of FGF19, is induced by FXR activation in the small intestine, but not in the liver. FGF15 expression then represses CYP7A1 in liver through a mechanism that involves FGFR4 and SHP¹⁵⁴. In addition, mice lacking FGF15 have increased hepatic Cyp7a1 expression and activity corresponding to increased bile acid excretion¹⁵⁴. Taken together, these studies define FGF19 in humans and FGF15 in mice as pivotal components of a novel signaling pathway that cooperates with FXR and SHP to maintain bile acid homeostasis.

PPAR α , a fatty acid-activated NR, regulates the utilization of fat during the starvation response. Recently, a PPAR α -dependent role for FGF 21 in the adaptive response to starvation has been described. FGF21 has been observed to have a variety of beneficial effects on metabolic parameters. Treatment of obese and leptin-deficient mice with FGF21 decreases serum glucose and triglyceride concentrations, and increases insulin sensitivity and glucose clearance. Moreover, mice that over-expressed FGF21 are resistant to diet induced obesity¹⁵⁵. Similar results were observed in FGF21 treatment of diabetic rhesus monkeys¹⁵⁶. While these studies show that the administration of FGF21 has important metabolic effects, recent studies have provided insight to the physiological role of FGF21. Inagaki *et al.* and Badman *et al.*, show that FGF21 expression in the liver of fasted mice is induced following activation of PPAR α ^{157, 158}. Adenoviral knockdown of endogenous FGF21 resulted in fatty liver, increased serum triglyceride levels, and decreased serum ketone levels in mice fed a low carbohydrate, high fat diet¹⁵⁷. This was associated with the decreased expression of fatty acid oxidizing enzymes and key enzymes in ketone body production that are known PPAR α -target genes¹⁵⁷. In transgenic mice over-expressing FGF21, ketogenesis and ketone body concentration in serum was increased several fold. Interestingly, recombinant FGF21 treatment rescued defective ketone body production in PPAR α knockout mice¹⁵⁸. Over-expression of FGF21 in mice also produced decreased body temperature and locomotor activity during fasting and increased lipolysis in white adipose tissue¹⁵⁸. These studies make it evident that FGF21 signaling collaborates with PPAR α , and together they function in liver as master regulators of energy balance. The precise molecular mechanisms that are downstream of these FGF signaling pathways in liver remain to be elucidated.

Co-Regulator Proteins as Targets of Signal Transduction Pathways

Interaction of NRs with co-regulator proteins provides a second level of regulation in target gene activation. The association of co-regulator proteins with NRs is clearly controlled at the level of ligand binding. In addition, the activation of cell signaling events and/or protein kinases directly regulates the association of NRs with co-regulator proteins. Numerous examples cited above illustrate how phosphorylation of NRs can result in increased or decreased strength of interaction between the receptor and co-activator or co-repressor multiprotein complexes. Realization that the specificity and activity of co-regulator proteins may also be regulated by signal transduction and phosphorylation is relatively new concept for which much less is known.

NCoR and SMRT Co-repressor Proteins

The most extensively characterized co-repressor proteins for NRs are NR co-repressor (NCoR) and silencing-mediator for retinoid and thyroid hormone receptors (SMRT)^{159, 160}. NCoR and SMRT interact with and mediate the repression of overlapping sets of NRs. NCoR and SMRT do not have intrinsic enzymatic activity; instead they have conserved modular domains that interact with HDACs. These co-repressor proteins can bind to NRs at their conserved C-terminal receptor interacting domain (RID) in the presence or absence of ligand and are regulated by a variety of signal transduction pathways¹⁶¹.

It had been previously observed that activation of tyrosine kinases negatively regulates the interaction between transcription factors and SMRT¹⁶². Further studies reveal that phosphorylation of SMRT in the C-terminal RID by the MAP kinase-kinase MEK-1 and MEK-1 kinase (MEKK-1) inhibits the interaction between SMRT and NRs¹⁶³. In addition, introduction of MEK-1 and MEKK-1 signaling into transfected cells led to the redistribution of SMRT from the nucleus to the perinucleus or cytoplasm¹⁶³. In contrast, phosphorylation of SMRT by casein kinase 2 (CK2) on S1492 stabilizes SMRT-NR interactions¹⁶⁴. Therefore, different signaling pathways can modulate different transcriptional outcomes via SMRT phosphorylation.

In contrast to SMRT, NCoR is refractory to MEKK1 phosphorylation, does not release from NR partners and does not change its sub-cellular distribution in response to MEKK1 signaling¹⁶⁵. These results indicate that the closely related SMRT and NCoR are regulated by distinct kinase signaling pathways. Although NCoR is fully refractory to MEKK-1 signaling, it is partially inhibited by EGF receptor signaling, indicating that NCoR may respond to an as yet undefined secondary pathway activated by EGF signaling¹⁶⁵. Recent evidence suggests that this differential response may be determined by alternative mRNA splicing of SMRT and NCoR¹⁶⁶. NCoR has been shown to be phosphorylated by Akt at S401, leading to the reversal of NCoR-mediated repression and nuclear export of NCoR. However, SMRT possesses an alanine residue at position 401 and is resistant to the actions of Akt¹⁶⁷.

Finally, since SMRT and NCoR exist in co-repressor multiprotein complexes, their activity may be affected by activation of signaling cascades that result in the phosphorylation of an HDAC, or other proteins in the complex. For example, phosphorylation of HDAC4 by ERK1 and ERK2 enhances its nuclear accumulation, whereas phosphorylation of HDAC1 and HDAC2 alters their interactions with co-repressor complexes¹⁶⁸⁻¹⁷⁰. IL-1 β has been reported to inhibit NCoR through an indirect pathway resulting in the MEKK-1 phosphorylation of a TAB2 subunit present in a subset of NCoR-HDAC3 complexes, whereas SMRT is resistant to this pathway¹⁷¹.

p160/SRC Co-activator Proteins

Steroid receptor co-activators (SRC) proteins are widely expressed and co-activate most NRs as well as many general transcription factors. The C-terminal region of SRC contains HAT activity, albeit relatively weak^{172, 173}. Furthermore, SRCs recruit other co-activator proteins such as CBP/p300 and pCAF to a larger multiprotein complex that participates in chromatin remodeling¹⁷⁴. There are three members of the SRC family, all of which contain conserved centrally located -LXXLL- motifs that are responsible for ligand-dependent interaction with NR through the AF-2 domain¹⁷⁵.

Seven phosphorylation sites for SRC-1 and six for SRC-3 have been identified^{176, 177}. All seven of the sites identified in SRC-1 contained consensus-phosphorylation sequences for serine/threonine-proline directed kinases, and two contained perfect consensus sequences for the MAPK family and are phosphorylated by ERK-2. Phosphorylation of SRCs can be induced by a variety of environmental stimuli including EGF, cyclic AMP, cytokines, and steroid hormones¹⁷⁶⁻¹⁸⁰. In addition, the phosphorylation of SRCs induced by these agents is required for optimal co-activator activity. For example, ERKs can phosphorylate SRC-2 at S736 and treatment of cells with EGF increases the transcriptional activity of GAL4-GRIP1¹⁸¹. In addition, SRC-1 phosphorylation at S1185 and T1179 is induced by cyclic AMP, and phosphorylation at these sites enhances the ligand-dependent and -independent activity of PR¹⁷⁹. PKA did not phosphorylate these sites *in vitro*, but blockage of PKA activity in COS-1 cells prevented cyclic AMP mediated phosphorylation of these sites¹⁷⁹. This phosphorylation event was also shown to be required for the interaction of SRC with pCAF or CBP¹⁷⁹. In a similar manner, EGF-stimulated phosphorylation of SRC-3 by MAPK stimulates the recruitment of p300 and enhances ligand-dependent ER activity¹⁷⁸. Phosphorylation of SRC-3 was shown to selectively affect the interactions with NRs, NF- κ B, and CBP¹⁷⁷. These data suggest that the phosphorylation of SRCs seems to be involved in the regulation of protein-protein interactions, however, it remains to be seen whether phosphorylation can affect other aspects of SRC-function.

All three SRCs contain both redundant and distinct functions which may be modulated by the signal transduction pathways that interact with SRCs and result in their phosphorylation. For example, SRC-3 contains several distinct patterns of phosphorylation. Phosphorylation of all six sites of SRC-3 is shown to be induced by estrogen and androgen hormones and is required

for co-activation of estrogen and androgen receptors¹⁷⁷. However, phosphorylation of only five of the six sites is induced by TNF α and is required for co-activation of NF- κ B¹⁷⁷. Further evidence shows that SRC-3, but not SRC-1 was co-purified in complex with I κ B kinase (IKK), and consequently, phosphorylation of SRC-3, but not SRC-1 is enhanced in response to TNF α stimulation¹⁸⁰. Thus, it appears that phosphorylation provides a molecular basis that determines the ability of SRCs to distinguish among various transcription factor families, and helps to provide specific responses to various upstream signaling pathways.

In addition to SRC, phosphorylation of other proteins in the co-activator complex can modulate transactivation potential of NRs. CBP/p300 can be phosphorylated *in vivo* and participates in cyclic AMP-regulated gene expression¹⁸². Kinase activities are also found to be associated with CBP/p300. Activation of cellular Ras with insulin treatment stimulated the recruitment of S6 kinase pp90_{RSK} to CBP¹⁸³. Binding of pp90_{RSK} to CBP repressed the transcription of cyclic AMP-responsive genes via CREB¹⁸³. CBP also contains a signal-regulated transcriptional activation domain that is controlled by calcium/calmodulin-dependent protein kinase IV and by cyclic AMP¹⁸⁴. Signal transduction pathways may also influence acetyltransferase activities and substrate preferences. An example of this is best illustrated with the POU homeodomain transcription factor Pit-1. Pit-1 function requires CBP/p300 and pCAF and is positively regulated by cyclic AMP and MAPK signal transduction pathways¹⁸⁵. Interestingly, stimulation of Pit-1 activity by cyclic AMP requires the intrinsic HAT activity of CBP, whereas stimulation of Pit-1 activity by the MAP kinase pathway requires the HAT activity of pCAF¹⁸⁵. It is thus plausible that activation of different signaling pathways could influence the group of co-activators that are required for NR mediated transactivation.

The PGC Family of Co-integrator Proteins

There are three members of the PPAR γ co-activator (PGC) family, PGC-1 α , PGC-1 β , and PGC-1 related co-activator (PRC). However, PGC-1 α is the most extensively characterized member of the family. Like many protein cofactors, PGC1 α co-activates multiple NRs. PGC-1 α is selectively expressed mainly in skeletal muscle, cardiac muscle, white fat, and liver¹⁸⁶. PGC-1 α binds to NR-LBDs with high affinity and, similar to SRCs, contains a triplet – LXXLL- motif for binding to NRs through their AF-2 domains. PGC-1 α does not have intrinsic HAT activity, and serves as a molecular scaffold that recruits additional factors such as CBP/p300¹⁸⁷. The physiological role of PGC-1 α has been well characterized as a master regulator of energy homeostasis in fat, liver and muscle¹⁸⁸. Specifically in liver, PGC1- α plays a prominent role in the regulation of genes involved in energy metabolism and glucose homeostasis. PGC-1 α is induced in liver by fasting and up-regulates the expression of key genes that participate in gluconeogenesis¹⁸⁹, fatty acid oxidation¹⁹⁰, and bile acid synthesis¹⁹¹.

PGC-1 α interacts with a multitude of signaling pathways that affect both its expression and/or phosphorylation status. Agents that increase cyclic AMP signaling such glucagon, catecholamines, and glucocorticoids induce PGC-1 α expression in liver¹⁸⁹. This cyclic AMP/PKA-dependent induction of PGC-1 α is mediated by phosphorylation and activation of the transcription factor CREB which directly regulates the *PGC-1 α* promoter¹⁹². On the other hand, LKB1/AMPK signaling appears to regulate the repression PGC-1 α gene expression. In LKB1 deficient liver, transducer of regulated CREB activity 2 (TORC2), a transcriptional co-activator of CREB, was de-phosphorylated and entered the nucleus, driving expression of PGC-1 α ¹⁹³.

In insulin stimulated skeletal muscle, PGC-1 α gene expression is down-regulated by Akt-mediated phosphorylation and nuclear exclusion of FOXO1¹⁹⁴. In addition to insulin, obesity and saturated fatty acids decrease PGC-1 α gene expression and function via p38 MAPK-dependent transcriptional pathways¹⁹⁵. Moreover, palmitate, a common saturated fatty acid,

reduces PGC-1 α expression in skeletal muscle through a mechanism involving MAPK-ERK and NF- κ B activation ¹⁹⁶.

It is worth noting that the most prominent PGC-1 α post-translational modification in terms of control of its activity and physiological output is acetylation ^{197, 198}. Methylation can also enhance PGC-1 α activity ¹⁹⁹. However, this review will focus on the effect of phosphorylation of PGC-1 α . Cytokines such as IL-1 α , IL-1 β , and TNF α have been shown to activate the transcriptional activity of PGC-1 α in muscle through direct phosphorylation by p38 MAPK resulting in increased stability and half-life and activation of PGC-1 α protein ²⁰⁰. p38 MAPK phosphorylates PGC-1 α at three residues (T262, S265, and T298) that occur in a region previously shown to interact with NRs, however, it remains to be seen whether phosphorylation of PGC-1 α affects NR docking. Further studies performed in primary hepatocytes confirm that PGC-1 α phosphorylation by p38 MAPK is necessary for free fatty acid induced activation of PEPCK, a PGC-1 α -target gene involved in gluconeogenesis ²⁰¹. The precise mechanism by which p38 MAPK-mediated phosphorylation of PGC-1 α alters the amount and activity of PGC-1 α will likely provide important physiological, and perhaps therapeutic, insight. Kralli's group has also shown that activation of p38 MAPK leads to and increase in PGC-1 α activity. They propose that a repressor binds to the PGC-1 α -LXXLL- motif and that the interaction is terminated upon activation of p38 MAPK ²⁰². This suggests a model where the repressor and NRs compete to recruit PGC-1 α to an inactive or active state, and that cellular signaling such as ligand or kinase signaling can shift the equilibrium between the two states.

In addition to p38 MAPK, two recent reports show that PGC-1 α is phosphorylated by AMPK and Akt/PKB. AMPK activation in muscle increases the expression of genes required for glucose uptake, fatty acid oxidation, and mitochondrial biogenesis. Using primary muscle cells and PGC-1 α knockout mice, Jager *et al.* demonstrated that the effect of AMPK mediated gene expression is dependent on PGC-1 α function. Furthermore, AMPK phosphorylates PGC-1 α at T177 and S583, which is required for PGC-1 α dependent induction of the PGC-1 α promoter ²⁰³. In liver, the mechanism by which insulin regulates lipid synthesis and degradation are largely unknown. Insulin treatment, through protein kinase Akt2/protein kinase B (PKB) resulted in the phosphorylation and inhibition of PGC-1 α ²⁰⁴. Akt phosphorylates PGC-1 α at S570 which prevents the recruitment of PGC-1 α to its target promoters ²⁰⁴. Repression of PGC-1 α activity by phosphorylation impairs its ability to promote gluconeogenesis and fatty acid oxidation in liver. PGC-1 α has an additional role in the regulation of this pathway. PGC-1 α co-activates PPAR α in the expression of tribbles homolog TRB-3, a fasting inducible inhibitor of Akt/PKB ²⁰⁵. This mechanism by which insulin signaling regulates PGC-1 α activity could provide insight into alternative drug targets for the treatment of type-2-diabetes.

Therapeutic Obstacles and Opportunities

NRs control many aspects of biology including development, reproduction, and homeostasis through target gene activation. The ability to modulate by their activity using fat-soluble molecules makes them extremely attractive drug targets. As our understanding of NR signaling increases, so does our appreciation of the complexity of their regulation. It is possible that management of diseases in the future will include therapies that not only target NRs, but also co-regulator proteins and signaling pathways that are critical in the modulation of their function.

PPARs are the targets of some commonly used drugs in the treatment of hyperlipidemia and type-2-diabetes. Activation of PPAR α by fibrates causes the up-regulation of genes involved in the β -oxidation of fatty acids. This results in the decreased synthesis of triglycerides and decreased LDL secretion by the liver ⁶⁷. Glitazones such as rosiglitazone and pioglitazone are PPAR γ agonists. PPAR γ is known to regulate glucose homeostasis and adipogenesis,

making it an attractive target for the treatment of type-2-diabetes. However, recent evidence has indicated an increased risk of heart attacks with rosiglitazone (marketed as Avandia) and the FDA released a safety alert on the drug in May 2007²⁰⁶. Further research surrounding the signaling events and co-regulator proteins that affect PPAR γ activity in multiple tissues may be useful in separating the therapeutic effects from the toxic effects of drugs like rosiglitazone.

One therapeutic challenge and opportunity in development of drugs that target NRs are selective therapeutic modulators (SRMs). SRMs are NR ligands that exhibit agonistic or antagonistic activity in a cell- or tissue-dependent manner. The classic SRM is tamoxifen, which can selectively activate or inhibit estrogen receptor and is commonly used in the treatment of breast cancer. Tamoxifen exhibits agonist (estrogen-like) activity in uterus and antagonist (anti-estrogen-like) activity in breast²⁰⁷. SRM-induced alterations in the conformation of NRs may affect the ability of the receptor to bind to co-regulators or to be phosphorylated. The expression profile of specific co-activator proteins and co-repressor proteins in a given cell type may affect the relative agonist –vs-antagonist activity of SRMs. However, as evidenced in this review, it is likely that cellular signaling events contribute to SRM activity due to altered activation, binding, and localization of co-regulator proteins, as well as NRs. Increased understanding of the effect of cellular signaling on NRs and their co-regulator proteins has the potential to aid in the process of discovery of novel SRMs and the development of new and more effective drug therapeutics.

Most NRs regulate a myriad of target genes that control multiple processes. One of the challenges in designing NR agonists is separating the desired therapeutic effects from the undesirable side effects. For example, the functional ability of LXRs to promote reverse cholesterol transport, improve glucose tolerance, and alleviate inflammation makes them attractive drug targets for the treatment of metabolic and inflammatory diseases. However, the finding that first generation synthetic ligands of LXR increase hepatic lipogenesis and plasma triglyceride levels is a therapeutic obstacle that needs to be overcome²⁰⁸. The increase in hepatic lipogenesis has been attributed to the LXR mediated induction of SREBP-1c, therefore an agonist designed to increase reverse cholesterol transport but not to induce SREBP-1c may be a more effective therapy. Loss of LXR results in the increased expression of ABCA1 and decreased expression of SREBP-1c suggesting that LXRs interact differentially with the transcriptional machinery on either promoter. Better understanding of the differential mechanisms and signaling pathways that interface with LXR during activation of specific target genes may provide insight in to the design of a selective agonist or may present new drug targets.

Understanding of the signaling mechanisms that interface with NRs could also be useful in modulating the effect of a receptor without directly targeting it, or in the development of therapeutic molecules that only induce specific NR-target genes. There are multiple areas of potential therapeutic usefulness for FXR modulators such as cholestatic disorders, fatty liver disorder, or metabolic and inflammatory diseases. However, activation of FXR induces a complex physiological response that may lead to undesirable side effects in addition to the beneficial response. For example, the use of an FXR agonist in the regulation of glucose homeostasis may also result in the inhibition of bile acid synthesis and impact cholesterol excretion. Therefore, the identification of selective bile acid receptor modulators (SBARMs) may be necessary to target specific groups of genes modulated by FXR. Additionally, the identification of how signaling pathways intersect with and modulate FXR may provide additional therapeutic opportunities that don't target FXR itself. For example, FGF19 or FGFR4 may prove to have interesting therapeutic potential in cholesterol and bile acid regulation.

The xenobiotic receptors PXR and CAR may have useful implications in the treatment of cholestatic liver disease. However, it has been hypothesized that unwanted activation of PXR

is responsible for nearly 60% of all drug-drug interactions. Due to their promiscuous nature PXR and CAR are capable of modulating a number of genes in response to many different ligands. PXR and CAR activation by a specific drug results in the increased metabolism of not only that drug, but other drugs that may be in the system as well. In order for PXR and CAR to be effective therapeutic targets, the activation of a potential therapeutic-target gene must be separated from the activation of genes involved in drug metabolism. A better understanding of the co-regulator proteins and signaling pathways that interface with PXR and CAR may provide alternative drug therapies toward that end. In addition, pharmaceutical companies commonly screen for PXR activation by drug candidates in rodent and human species in order to avoid future drug-drug interactions. However, there is a significant species-specific response of PXR- and CAR-target genes with respect to activating ligands and signaling pathways. Understanding the signaling pathways that affect these two receptors may also be useful in the development of more accurate activation assays in order to predict and prevent unwanted and potentially lethal drug-drug interactions.

Conclusion

It is clear that multiple signaling pathways and phosphorylation events affect NR-mediated signaling. They modulate protein-protein interactions, sub-cellular localization, DNA-binding, protein stability, and transactivation capacity. The situation is further complicated by the fact that many NR cofactor proteins are themselves modulated by signaling pathways and phosphorylation events that affect their intrinsic and recruited enzymatic activities. Further investigation into the role of cell signaling pathways in NR-mediated transcription, and into signaling pathway crosstalk will be necessary to fully understand the functional implication of these signaling events. In addition, further characterization of these processes will likely lead to the development of novel and selective therapeutic molecules for a multitude of indications.

References

1. Maglich JM, Sluder A, Guan X, Shi Y, McKee DD, Carrick K, Kamdar K, Willson TM, Moore JT. *Genome Biol* 2001;2(8):29.1–29.7.
2. Kumar R, Johnson BH, Thompson EB. *Essays Biochem* 2004;40:27–39. [PubMed: 15242337]
3. Glass CK, Rosenfeld MG. *Genes Dev* 2000;14(2):121–41. [PubMed: 10652267]
4. Mangelsdorf DJ, Evans RM. *Cell* 1995;83(6):841–50. [PubMed: 8521508]
5. Nagpal S, Friant S, Nakshatri H, Chambon P. *Embo J* 1993;12(6):2349–60. [PubMed: 8389696]
6. Baumann CT, Maruvada P, Hager GL, Yen PM. *J Biol Chem* 2001;276(14):11237–45. [PubMed: 11152480]
7. DeFranco DB, Ramakrishnan C, Tang Y. *J Steroid Biochem Mol Biol* 1998;65(16):51–8. [PubMed: 9699857]
8. Tcherepanova I, Puigserver P, Norris JD, Spiegelman BM, McDonnell DP. *J Biol Chem* 2000;275(21):16302–8. [PubMed: 10748020]
9. Morgan DO. *Annu Rev Cell Dev Biol* 1997;13:261–91. [PubMed: 9442875]
10. Morgan DO. *Nature* 1995;374(6518):131–4. [PubMed: 7877684]
11. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. *Endocr Rev* 2001;22(2):153–83. [PubMed: 11294822]
12. Rochette-Egly C. *Cell Signal* 2003;15(4):355–66. [PubMed: 12618210]
13. Lange CA, Shen T, Horwitz KB. *Proc Natl Acad Sci U S A* 2000;97(3):1032–7. [PubMed: 10655479]
14. Shen T, Horwitz KB, Lange CA. *Mol Cell Biol* 2001;21(18):6122–31. [PubMed: 11509655]
15. Bunone G, Briand PA, Miksicek RJ, Picard D. *Embo J* 1996;15(9):2174–83. [PubMed: 8641283]
16. Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H, Metzger D, Chambon P. *Science* 1995;270(5241):1491–4. [PubMed: 7491495]
17. Driggers PH, Segars JH, Rubino DM. *J Biol Chem* 2001;276(50):46792–7. [PubMed: 11579095]

18. Tremblay A, Tremblay GB, Labrie F, Giguere V. *Mol Cell* 1999;3(4):513–9. [PubMed: 10230404]
19. Yeh S, Lin HK, Kang HY, Thin TH, Lin MF, Chang C. *Proc Natl Acad Sci U S A* 1999;96(10):5458–63. [PubMed: 10318905]
20. Hu E, Kim JB, Sarraf P, Spiegelman BM. *Science* 1996;274(5295):2100–3. [PubMed: 8953045]
21. Juge-Aubry CE, Hammar E, Siegrist-Kaiser C, Pernin A, Takeshita A, Chin WW, Burger AG, Meier CA. *J Biol Chem* 1999;274(15):10505–10. [PubMed: 10187842]
22. Gianni M, Bauer A, Garattini E, Chambon P, Rochette-Egly C. *Embo J* 2002;21(14):3760–9. [PubMed: 12110588]
23. Gianni M, Kopf E, Bastien J, Oulad-Abdelghani M, Garattini E, Chambon P, Rochette-Egly C. *J Biol Chem* 2002;277(28):24859–62. [PubMed: 12032135]
24. Alessi DR, Cohen P. *Curr Opin Genet Dev* 1998;8(1):55–62. [PubMed: 9529606]
25. Scheid MP, Woodgett JR. *Nat Rev Mol Cell Biol* 2001;2(10):760–8. [PubMed: 11584303]
26. Datta SR, Brunet A, Greenberg ME. *Genes Dev* 1999;13(22):2905–27. [PubMed: 10579998]
27. Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H. *J Biol Chem* 2001;276(13):9817–24. [PubMed: 11139588]
28. Lin HK, Yeh S, Kang HY, Chang C. *Proc Natl Acad Sci U S A* 2001;98(13):7200–5. [PubMed: 11404460]
29. Adam-Stitah S, Penna L, Chambon P, Rochette-Egly C. *J Biol Chem* 1999;274(27):18932–41. [PubMed: 10383391]
30. Lee HY, Suh YA, Robinson MJ, Clifford JL, Hong WK, Woodgett JR, Cobb MH, Mangelsdorf DJ, Kurie JM. *J Biol Chem* 2000;275(41):32193–9. [PubMed: 10938283]
31. Arnold SF, Obourn JD, Jaffe H, Notides AC. *Mol Endocrinol* 1995;9(1):24–33. [PubMed: 7539106]
32. Migliaccio A, Pagano M, Auricchio F. *Oncogene* 1993;8(8):2183–91. [PubMed: 7687761]
33. Rochette-Egly C, Oulad-Abdelghani M, Staub A, Pfister V, Scheuer I, Chambon P, Gaub MP. *Mol Endocrinol* 1995;9(7):860–71. [PubMed: 7476969]
34. Chen D, Pace PE, Coombes RC, Ali S. *Mol Cell Biol* 1999;19(2):1002–15. [PubMed: 9891036]
35. Delmotte MH, Tahayato A, Formstecher P, Lefebvre P. *J Biol Chem* 1999;274(53):38225–31. [PubMed: 10608897]
36. Hsieh JC, Jurutka PW, Galligan MA, Terpening CM, Haussler CA, Samuels DS, Shimizu Y, Shimizu N, Haussler MR. *Proc Natl Acad Sci U S A* 1991;88(20):9315–9. [PubMed: 1656468]
37. Hsieh JC, Jurutka PW, Nakajima S, Galligan MA, Haussler CA, Shimizu Y, Shimizu N, Whitfield GK, Haussler MR. *J Biol Chem* 1993;268(20):15118–26. [PubMed: 8392065]
38. Hayhurst GP, Lee YH, Lambert G, Ward JM, Gonzalez FJ. *Mol Cell Biol* 2001;21(4):1393–403. [PubMed: 11158324]
39. Stroup D, Chiang JY. *J Lipid Res* 2000;41(1):1–11. [PubMed: 10627496]
40. Ding X, Lichti K, Kim I, Gonzalez FJ, Staudinger JL. *J Biol Chem* 2006;281(36):26540–51. [PubMed: 16825189]
41. Stoffel M, Duncan SA. *Proc Natl Acad Sci U S A* 1997;94(24):13209–14. [PubMed: 9371825]
42. Wan YJ, Cai Y, Cowan C, Magee TR. *Cancer Lett* 2000;154(1):19–27. [PubMed: 10799735]
43. Viollet B, Kahn A, Raymondjean M. *Mol Cell Biol* 1997;17(8):4208–19. [PubMed: 9234678]
44. Jiang G, Nepomuceno L, Yang Q, Sladek FM. *Arch Biochem Biophys* 1997;340(1):1–9. [PubMed: 9126270]
45. Ktistaki E, Ktistakis NT, Papadogeorgaki E, Talianidis I. *Proc Natl Acad Sci U S A* 1995;92(21):9876–80. [PubMed: 7568236]
46. Guo H, Gao C, Mi Z, Zhang J, Kuo PC. *J Biochem (Tokyo)* 2007;141(5):635–40. [PubMed: 17317687]
47. Xu Z, Tavares-Sanchez OL, Li Q, Fernando J, Rodriguez CM, Studer EJ, Pandak WM, Hylemon PB, Gil G. *J Biol Chem*. 2007
48. Wang JC, Stafford JM, Granner DK. *J Biol Chem* 1998;273(47):30847–50. [PubMed: 9812974]
49. Yoshida E, Aratani S, Itou H, Miyagishi M, Takiguchi M, Osumi T, Murakami K, Fukamizu A. *Biochem Biophys Res Commun* 1997;241(3):664–9. [PubMed: 9434765]
50. Desvergne B, Wahli W. *Endocr Rev* 1999;20(5):649–88. [PubMed: 10529898]

51. Escher P, Wahli W. *Mutat Res* 2000;448(2):121–38. [PubMed: 10725467]
52. Auboeuf D, Rieusset J, Fajas L, Vallier P, Frering V, Riou JP, Staels B, Auwerx J, Laville M, Vidal H. *Diabetes* 1997;46(8):1319–27. [PubMed: 9231657]
53. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. *J Biol Chem* 1995;270(22):12953–6. [PubMed: 7768881]
54. Camp HS, Tafuri SR. *J Biol Chem* 1997;272(16):10811–6. [PubMed: 9099735]
55. Adams M, Reginato MJ, Shao D, Lazar MA, Chatterjee VK. *J Biol Chem* 1997;272(8):5128–32. [PubMed: 9030579]
56. Zhang B, Berger J, Zhou G, Elbrecht A, Biswas S, White-Carrington S, Szalkowski D, Moller DE. *J Biol Chem* 1996;271(50):31771–4. [PubMed: 8943212]
57. Barger PM, Browning AC, Garner AN, Kelly DP. *J Biol Chem* 2001;276(48):44495–501. [PubMed: 11577087]
58. Lazennec G, Canaple L, Saugy D, Wahli W. *Mol Endocrinol* 2000;14(12):1962–75. [PubMed: 11117527]
59. Yaacob NS, Norazmi MN, Gibson GG, Kass GE. *Toxicol Lett* 2001;125(13):133–41. [PubMed: 11701232]
60. Chen F, Ma L, Dawson PA, Sinal CJ, Sehayek E, Gonzalez FJ, Breslow J, Ananthanarayanan M, Shneider BL. *J Biol Chem* 2003;278(22):19909–16. [PubMed: 12456679]
61. Maglich JM, Stoltz CM, Goodwin B, Hawkins-Brown D, Moore JT, Kliewer SA. *Mol Pharmacol* 2002;62(3):638–46. [PubMed: 12181440]
62. Sonoda J, Xie W, Rosenfeld JM, Barwick JL, Guzelian PS, Evans RM. *Proc Natl Acad Sci U S A* 2002;99(21):13801–6. [PubMed: 12370413]
63. Wei P, Zhang J, Dowhan DH, Han Y, Moore DD. *Pharmacogenomics J* 2002;2(2):117–26. [PubMed: 12049174]
64. Geick A, Eichelbaum M, Burk O. *J Biol Chem* 2001;276(18):14581–7. [PubMed: 11297522]
65. Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM, Edwards PA. *J Biol Chem* 2002;277(4):2908–15. [PubMed: 11706036]
66. Staudinger JL, Madan A, Carol KM, Parkinson A. *Drug Metab Dispos* 2003;31(5):523–7. [PubMed: 12695338]
67. Francis GA, Fayard E, Picard F, Auwerx J. *Annu Rev Physiol* 2003;65:261–311. [PubMed: 12518001]
68. Hartmann G, Kim H, Piquette-Miller M. *Int Immunopharmacol* 2001;1(2):189–99. [PubMed: 11360920]
69. Hartmann G, Cheung AK, Piquette-Miller M. *J Pharmacol Exp Ther* 2002;303(1):273–81. [PubMed: 12235261]
70. Pascussi JM, Gerbal-Chaloin S, Pichard-Garcia L, Daujat M, Fabre JM, Maurel P, Vilarem MJ. *Biochem Biophys Res Commun* 2000;274(3):707–13. [PubMed: 10924340]
71. Gu X, Ke S, Liu D, Sheng T, Thomas PE, Rabson AB, Gallo MA, Xie W, Tian Y. *J Biol Chem* 2006;281(26):17882–9. [PubMed: 16608838]
72. Zhou C, Tabb MM, Nelson EL, Grun F, Verma S, Sadatrafiei A, Lin M, Mallick S, Forman BM, Thummel KE, Blumberg B. *J Clin Invest* 2006;116(8):2280–2289. [PubMed: 16841097]
73. Shah YM, Ma X, Morimura K, Kim I, Gonzalez FJ. *Am J Physiol Gastrointest Liver Physiol* 2007;292(4):G1114–22. [PubMed: 17170021]
74. Sidhu JS, Omiecinski CJ. *J Biol Chem* 1995;270(21):12762–73. [PubMed: 7759530]
75. Sidhu JS, Omiecinski CJ. *J Pharmacol Exp Ther* 1996;276(1):238–45. [PubMed: 8558437]
76. Ding X, Staudinger JL. *J Pharmacol Exp Ther* 2005;312(2):849–56. [PubMed: 15459237]
77. Ding X, Staudinger JL. *Biochem Pharmacol* 2005;69(5):867–73. [PubMed: 15710363]
78. Honkakoski P, Zelko I, Sueyoshi T, Negishi M. *Mol Cell Biol* 1998;18(10):5652–8. [PubMed: 9742082]
79. Pascussi JM, Dvorak Z, Gerbal-Chaloin S, Assenat E, Maurel P, Vilarem MJ. *Drug Metab Rev* 2003;35(4):255–68. [PubMed: 14705859]
80. Ueda A, Hamadeh HK, Webb HK, Yamamoto Y, Sueyoshi T, Afshari CA, Lehmann JM, Negishi M. *Mol Pharmacol* 2002;61(1):1–6. [PubMed: 11752199]

81. Sidhu JS, Omiecinski CJ. *J Pharmacol Exp Ther* 1997;282(2):1122–9. [PubMed: 9262383]
82. Joannard F, Galisteo M, Corcos L, Guillouzo A, Lagadic-Gossmann D. *Cell Biol Toxicol* 2000;16(5):325–37. [PubMed: 11201056]
83. Pustyl'nyak VO, Gulyaeva LF, Lyakhovich VV. *Toxicology* 2005;216(23):147–53. [PubMed: 16153763]
84. Kawamoto T, Sueyoshi T, Zelko I, Moore R, Washburn K, Negishi M. *Mol Cell Biol* 1999;19(9):6318–22. [PubMed: 10454578]
85. Hosseinpour F, Moore R, Negishi M, Sueyoshi T. *Mol Pharmacol* 2006;69(4):1095–102. [PubMed: 16377764]
86. Swales K, Negishi M. *Mol Endocrinol* 2004;18(7):1589–98. [PubMed: 14988430]
87. Bauer D, Wolfram N, Kahl GF, Hirsch-Ernst KI. *Mol Pharmacol* 2004;65(1):172–80. [PubMed: 14722249]
88. Joannard F, Rissel M, Gilot D, Anderson A, Orfila-Lefevre L, Guillouzo A, Atfi A, Lagadic-Gossmann D. *Toxicol Lett* 2006;161(1):61–72. [PubMed: 16154717]
89. Koike C, Moore R, Negishi M. *Mol Pharmacol* 2007;71(5):1217–21. [PubMed: 17314319]
90. Rencurel F, Stenhouse A, Hawley SA, Friedberg T, Hardie DG, Sutherland C, Wolf CR. *J Biol Chem* 2005;280(6):4367–73. [PubMed: 15572372]
91. Rencurel F, Foretz M, Kaufmann MR, Stroka D, Looser R, Leclerc I, da Silva Xavier G, Rutter GA, Viollet B, Meyer UA. *Mol Pharmacol* 2006;70(6):1925–34. [PubMed: 16988011]
92. Shindo S, Numazawa S, Yoshida T. *Biochem J* 2007;401(3):735–41. [PubMed: 17032173]
93. Blattler SM, Rencurel F, Kaufmann MR, Meyer UA. *Proc Natl Acad Sci U S A* 2007;104(3):1045–50. [PubMed: 17213310]
94. Beaven SW, Tontonoz P. *Annu Rev Med* 2006;57:313–29. [PubMed: 16409152]
95. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. *Nature* 1996;383(6602):728–31. [PubMed: 8878485]
96. Song C, Liao S. *Endocrinology* 2000;141(11):4180–4. [PubMed: 11089551]
97. Chiang JY, Kimmel R, Stroup D. *Gene* 2001;262(12):257–65. [PubMed: 11179691]
98. Costet P, Luo Y, Wang N, Tall AR. *J Biol Chem* 2000;275(36):28240–5. [PubMed: 10858438]
99. Mak PA, Laffitte BA, Desrumaux C, Joseph SB, Curtiss LK, Mangelsdorf DJ, Tontonoz P, Edwards PA. *J Biol Chem* 2002;277(35):31900–8. [PubMed: 12032151]
100. Ulven SM, Dalen KT, Gustafsson JA, Nebb HI. *J Lipid Res* 2004;45(11):2052–62. [PubMed: 15292368]
101. Cao G, Liang Y, Broderick CL, Oldham BA, Beyer TP, Schmidt RJ, Zhang Y, Staybrook KR, Suen C, Otto KA, Miller AR, Dai J, Foxworthy P, Gao H, Ryan TP, Jiang XC, Burris TP, Eacho PI, Etgen GJ. *J Biol Chem* 2003;278(2):1131–6. [PubMed: 12414791]
102. Castrillo A, Joseph SB, Marathe C, Mangelsdorf DJ, Tontonoz P. *J Biol Chem* 2003;278(12):10443–9. [PubMed: 12531895]
103. Joseph SB, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P. *Nat Med* 2003;9(2):213–9. [PubMed: 12524534]
104. Chen M, Bradley MN, Beaven SW, Tontonoz P. *FEBS Lett* 2006;580(20):4835–41. [PubMed: 16904112]
105. Huang CJ, Feltkamp D, Nilsson S, Gustafsson JA. *Biochem Biophys Res Commun* 1998;243(3):657–63. [PubMed: 9500983]
106. Tamura K, Chen YE, Horiuchi M, Chen Q, Daviet L, Yang Z, Lopez-Illasaca M, Mu H, Pratt RE, Dzau VJ. *Proc Natl Acad Sci U S A* 2000;97(15):8513–8. [PubMed: 10890879]
107. Tamura K, Chen YE, Tanaka Y, Sakai M, Tsurumi Y, Koide Y, Kihara M, Pratt RE, Horiuchi M, Umemura S, Dzau VJ. *Mol Cell Endocrinol* 2004;224(12):11–20. [PubMed: 15353176]
108. Yamamoto T, Shimano H, Inoue N, Nakagawa Y, Matsuzaka T, Takahashi A, Yahagi N, Sone H, Suzuki H, Toyoshima H, Yamada N. *J Biol Chem* 2007;282(16):11687–95. [PubMed: 17296605]
109. Terasaka N, Hiroshima A, Ariga A, Honzumi S, Koieyama T, Inaba T, Fujiwara T. *Febs J* 2005;272(6):1546–56. [PubMed: 15752369]

110. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK. *Nature* 2005;437(7059):759–63. [PubMed: 16127449]
111. Lu TT, Repa JJ, Mangelsdorf DJ. *J Biol Chem* 2001;276(41):37735–8. [PubMed: 11459853]
112. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, Wilson JG, Lewis MC, Roth ME, Maloney PR, Willson TM, Kliewer SA. *Mol Cell* 2000;6(3):517–26. [PubMed: 11030332]
113. Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ. *Mol Cell* 2000;6(3):507–15. [PubMed: 11030331]
114. Bohan A, Chen WS, Denson LA, Held MA, Boyer JL. *J Biol Chem* 2003;278(38):36688–98. [PubMed: 12837754]
115. del Castillo-Olivares A, Gil G. *J Biol Chem* 2000;275(23):17793–9. [PubMed: 10747975]
116. Delerive P, Galardi CM, Bisi JE, Nicodeme E, Goodwin B. *Mol Endocrinol* 2004;18(10):2378–87. [PubMed: 15218078]
117. Inokuchi A, Hinoshita E, Iwamoto Y, Kohno K, Kuwano M, Uchiumi T. *J Biol Chem* 2001;276(50):46822–9. [PubMed: 11590139]
118. Luo Y, Liang CP, Tall AR. *J Biol Chem* 2001;276(27):24767–73. [PubMed: 11331284]
119. Schoonjans K, Annicotte JS, Huby T, Botrugno OA, Fayard E, Ueda Y, Chapman J, Auwerx J. *EMBO Rep* 2002;3(12):1181–7. [PubMed: 12446566]
120. Lee YK, Choi YH, Chua S, Park YJ, Moore DD. *J Biol Chem* 2006;281(12):7850–5. [PubMed: 16439367]
121. Li T, Jahan A, Chiang JY. *Hepatology* 2006;43(6):1202–10. [PubMed: 16729332]
122. Stroup D. *Front Biosci* 2005;10:1678–92. [PubMed: 15769658]
123. Krylova IN, Sablin EP, Moore J, Xu RX, Waitt GM, MacKay JA, Juzumiene D, Bynum JM, Madauss K, Montana V, Lebedeva L, Suzawa M, Williams JD, Williams SP, Guy RK, Thornton JW, Fletterick RJ, Willson TM, Ingraham HA. *Cell* 2005;120(3):343–55. [PubMed: 15707893]
124. Fiorucci S, Rizzo G, Donini A, Distrutti E, Santucci L. *Trends Mol Med* 2007;13(7):298–309. [PubMed: 17588816]
125. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B. *Science* 1999;284(5418):1362–5. [PubMed: 10334992]
126. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, Lehmann JM. *Science* 1999;284(5418):1365–8. [PubMed: 10334993]
127. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. *Mol Cell* 1999;3(5):543–53. [PubMed: 10360171]
128. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. *Cell* 2000;102(6):731–44. [PubMed: 11030617]
129. Duran-Sandoval D, Mautino G, Martin G, Percevault F, Barbier O, Fruchart JC, Kuipers F, Staels B. *Diabetes* 2004;53(4):890–8. [PubMed: 15047603]
130. Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, Willson TM, Edwards PA. *Proc Natl Acad Sci U S A* 2006;103(4):1006–11. [PubMed: 16410358]
131. Cariou B, van Harmelen K, Duran-Sandoval D, van Dijk TH, Grefhorst A, Abdelkarim M, Caron S, Torpier G, Fruchart JC, Gonzalez FJ, Kuipers F, Staels B. *J Biol Chem* 2006;281(16):11039–49. [PubMed: 16446356]
132. Duran-Sandoval D, Cariou B, Percevault F, Hennuyer N, Grefhorst A, van Dijk TH, Gonzalez FJ, Fruchart JC, Kuipers F, Staels B. *J Biol Chem* 2005;280(33):29971–9. [PubMed: 15899888]
133. Ma K, Saha PK, Chan L, Moore DD. *J Clin Invest* 2006;116(4):1102–9. [PubMed: 16557297]
134. Zhang Y, Castellani LW, Sinal CJ, Gonzalez FJ, Edwards PA. *Genes Dev* 2004;18(2):157–69. [PubMed: 14729567]
135. Gupta S, Stravitz RT, Dent P, Hylemon PB. *J Biol Chem* 2001;276(19):15816–22. [PubMed: 11278771]
136. Rao YP, Studer EJ, Stravitz RT, Gupta S, Qiao L, Dent P, Hylemon PB. *Hepatology* 2002;35(2):307–14. [PubMed: 11826403]

137. Stravitz RT, Vlahcevic ZR, Gurley EC, Hylemon PB. *J Lipid Res* 1995;36(6):1359–69. [PubMed: 7666012]
138. Seol W, Choi HS, Moore DD. *Science* 1996;272(5266):1336–9. [PubMed: 8650544]
139. Lee HK, Lee YK, Park SH, Kim YS, Park SH, Lee JW, Kwon HB, Soh J, Moore DD, Choi HS. *J Biol Chem* 1998;273(23):14398–402. [PubMed: 9603951]
140. Masuda N, Yasumo H, Tamura T, Hashiguchi N, Furusawa T, Tsukamoto T, Sadano H, Osumi T. *Biochim Biophys Acta* 1997;1350(1):27–32. [PubMed: 9003453]
141. Lee YK, Moore DD. *J Biol Chem* 2002;277(4):2463–7. [PubMed: 11668176]
142. Brendel C, Schoonjans K, Botrugno OA, Treuter E, Auwerx J. *Mol Endocrinol* 2002;16(9):2065–76. [PubMed: 12198243]
143. Lee YK, Dell H, Dowhan DH, Hadzopoulou-Cladaras M, Moore DD. *Mol Cell Biol* 2000;20(1):187–95. [PubMed: 10594021]
144. Seol W, Chung M, Moore DD. *Mol Cell Biol* 1997;17(12):7126–31. [PubMed: 9372944]
145. Kassam A, Capone JP, Rachubinski RA. *Mol Cell Endocrinol* 2001;176(12):49–56. [PubMed: 11369442]
146. Nishizawa H, Yamagata K, Shimomura I, Takahashi M, Kuriyama H, Kishida K, Hotta K, Nagaretani H, Maeda N, Matsuda M, Kihara S, Nakamura T, Nishigori H, Tomura H, Moore DD, Takeda J, Funahashi T, Matsuzawa Y. *J Biol Chem* 2002;277(2):1586–92. [PubMed: 11696534]
147. Xie MH, Holcomb I, Deuel B, Dowd P, Huang A, Vagts A, Foster J, Liang J, Brush J, Gu Q, Hillan K, Goddard A, Gurney AL. *Cytokine* 1999;11(10):729–35. [PubMed: 10525310]
148. Holt JA, Luo G, Billin AN, Bisi J, McNeill YY, Kozarsky KF, Donahee M, Wang DY, Mansfield TA, Kliewer SA, Goodwin B, Jones SA. *Genes Dev* 2003;17(13):1581–91. [PubMed: 12815072]
149. Fu L, John LM, Adams SH, Yu XX, Tomlinson E, Renz M, Williams PM, Soriano R, Corpuz R, Moffat B, Vandlen R, Simmons L, Foster J, Stephan JP, Tsai SP, Stewart TA. *Endocrinology* 2004;145(6):2594–603. [PubMed: 14976145]
150. Tomlinson E, Fu L, John L, Hultgren B, Huang X, Renz M, Stephan JP, Tsai SP, Powell-Braxton L, French D, Stewart TA. *Endocrinology* 2002;143(5):1741–7. [PubMed: 11956156]
151. Nakatani Y, Kaneto H, Kawamori D, Hatazaki M, Miyatsuka T, Matsuoka TA, Kajimoto Y, Matsuhisa M, Yamasaki Y, Hori M. *J Biol Chem* 2004;279(44):45803–9. [PubMed: 15331609]
152. Yu C, Wang F, Kan M, Jin C, Jones RB, Weinstein M, Deng CX, McKeehan WL. *J Biol Chem* 2000;275(20):15482–9. [PubMed: 10809780]
153. Yu C, Wang F, Jin C, Huang X, McKeehan WL. *J Biol Chem* 2005;280(18):17707–14. [PubMed: 15750181]
154. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, Luo G, Jones SA, Goodwin B, Richardson JA, Gerard RD, Repa JJ, Mangelsdorf DJ, Kliewer SA. *Cell Metab* 2005;2(4):217–25. [PubMed: 16213224]
155. Kharitonov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB. *J Clin Invest* 2005;115(6):1627–35. [PubMed: 15902306]
156. Kharitonov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT, Hansen BC, Shanafelt AB, Etgen GJ. *Endocrinology* 2007;148(2):774–81. [PubMed: 17068132]
157. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. *Cell Metab* 2007;5(6):426–437. [PubMed: 17550778]
158. Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, Li Y, Goetz R, Mohammadi M, Esser V, Elmquist JK, Gerard RD, Burgess SC, Hammer RE, Mangelsdorf DJ, Kliewer SA. *Cell Metab* 2007;5(6):415–25. [PubMed: 17550777]
159. Chen JD, Evans RM. *Nature* 1995;377(6548):454–7. [PubMed: 7566127]
160. Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK, et al. *Nature* 1995;377(6548):397–404. [PubMed: 7566114]
161. Privalsky ML. *Annu Rev Physiol* 2004;66:315–60. [PubMed: 14977406]
162. Hong SH, Wong CW, Privalsky ML. *Mol Endocrinol* 1998;12(8):1161–71. [PubMed: 9717842]
163. Hong SH, Privalsky ML. *Mol Cell Biol* 2000;20(17):6612–25. [PubMed: 10938135]

164. Zhou Y, Gross W, Hong SH, Privalsky ML. *Mol Cell Biochem* 2001;220(12):1–13. [PubMed: 11451368]
165. Jonas BA, Privalsky ML. *J Biol Chem* 2004;279(52):54676–86. [PubMed: 15491994]
166. Jonas BA, Varlakhanova N, Hayakawa F, Goodson M, Privalsky ML. *Mol Endocrinol*. 2007
167. Hermanson O, Jepsen K, Rosenfeld MG. *Nature* 2002;419(6910):934–9. [PubMed: 12410313]
168. Galasinski SC, Louie DF, Gloor KK, Resing KA, Ahn NG. *J Biol Chem* 2002;277(4):2579–88. [PubMed: 11709551]
169. Tsai SC, Seto E. *J Biol Chem* 2002;277(35):31826–33. [PubMed: 12082111]
170. Zhou X, Richon VM, Wang AH, Yang XJ, Rifkind RA, Marks PA. *Proc Natl Acad Sci U S A* 2000;97(26):14329–33. [PubMed: 11114188]
171. Baek SH, Ohgi KA, Rose DW, Koo EH, Glass CK, Rosenfeld MG. *Cell* 2002;110(1):55–67. [PubMed: 12150997]
172. Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, Evans RM. *Cell* 1997;90(3):569–80. [PubMed: 9267036]
173. Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai MJ, O'Malley BW. *Nature* 1997;389(6647):194–8. [PubMed: 9296499]
174. Xu J, O'Malley BW. *Rev Endocr Metab Disord* 2002;3(3):185–92. [PubMed: 12215713]
175. Heery DM, Kalkhoven E, Hoare S, Parker MG. *Nature* 1997;387(6634):733–6. [PubMed: 9192902]
176. Rowan BG, Weigel NL, O'Malley BW. *J Biol Chem* 2000;275(6):4475–83. [PubMed: 10660621]
177. Wu RC, Qin J, Yi P, Wong J, Tsai SY, Tsai MJ, O'Malley BW. *Mol Cell* 2004;15(6):937–49. [PubMed: 15383283]
178. Font de Mora J, Brown M. *Mol Cell Biol* 2000;20(14):5041–7. [PubMed: 10866661]
179. Rowan BG, Garrison N, Weigel NL, O'Malley BW. *Mol Cell Biol* 2000;20(23):8720–30. [PubMed: 11073973]
180. Wu RC, Qin J, Hashimoto Y, Wong J, Xu J, Tsai SY, Tsai MJ, O'Malley BW. *Mol Cell Biol* 2002;22(10):3549–61. [PubMed: 11971985]
181. Lopez GN, Turck CW, Schaufele F, Stallcup MR, Kushner PJ. *J Biol Chem* 2001;276(25):22177–82. [PubMed: 11301320]
182. Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR, Goodman RH. *Nature* 1993;365(6449):855–9. [PubMed: 8413673]
183. Nakajima T, Fukamizu A, Takahashi J, Gage FH, Fisher T, Blenis J, Montminy MR. *Cell* 1996;86(3):465–74. [PubMed: 8756728]
184. Chawla S, Hardingham GE, Quinn DR, Bading H. *Science* 1998;281(5382):1505–9. [PubMed: 9727976]
185. Xu L, Lavinsky RM, Dasen JS, Flynn SE, McInerney EM, Mullen TM, Heinzel T, Szeto D, Korzus E, Kurokawa R, Aggarwal AK, Rose DW, Glass CK, Rosenfeld MG. *Nature* 1998;395(6699):301–6. [PubMed: 9751061]
186. Larrouy D, Vidal H, Andreelli F, Laville M, Langin D. *Int J Obes Relat Metab Disord* 1999;23(12):1327–32. [PubMed: 10643692]
187. Puigserver P, Spiegelman BM. *Endocr Rev* 2003;24(1):78–90. [PubMed: 12588810]
188. Spiegelman BM, Heinrich R. *Cell* 2004;119(2):157–67. [PubMed: 15479634]
189. Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM. *Nature* 2001;413(6852):131–8. [PubMed: 11557972]
190. Vega RB, Huss JM, Kelly DP. *Mol Cell Biol* 2000;20(5):1868–76. [PubMed: 10669761]
191. Shin DJ, Campos JA, Gil G, Osborne TF. *J Biol Chem* 2003;278(50):50047–52. [PubMed: 14522988]
192. Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M. *Nature* 2001;413(6852):179–83. [PubMed: 11557984]
193. Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, Montminy M, Cantley LC. *Science* 2005;310(5754):1642–6. [PubMed: 16308421]
194. Southgate RJ, Bruce CR, Carey AL, Steinberg GR, Walder K, Monks R, Watt MJ, Hawley JA, Birnbaum MJ, Febbraio MA. *Faseb J* 2005;19(14):2072–4. [PubMed: 16203862]

195. Crunkhorn S, Dearie F, Mantzoros C, Gami H, da Silva WS, Espinoza D, Faucette R, Barry K, Bianco AC, Patti ME. *J Biol Chem* 2007;282(21):15439–50. [PubMed: 17416903]
196. Coll T, Jove M, Rodriguez-Calvo R, Eyre E, Palomer X, Sanchez RM, Merlos M, Laguna JC, Vazquez-Carrera M. *Diabetes* 2006;55(10):2779–87. [PubMed: 17003343]
197. Lerin C, Rodgers JT, Kalume DE, Kim SH, Pandey A, Puigserver P. *Cell Metab* 2006;3(6):429–38. [PubMed: 16753578]
198. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. *Nature* 2005;434(7029):113–8. [PubMed: 15744310]
199. Teyssier C, Ma H, Emter R, Kralli A, Stallcup MR. *Genes Dev* 2005;19(12):1466–73. [PubMed: 15964996]
200. Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, Krauss S, Mootha VK, Lowell BB, Spiegelman BM. *Mol Cell* 2001;8(5):971–82. [PubMed: 11741533]
201. Collins QF, Xiong Y, Lupo EG Jr, Liu HY, Cao W. *J Biol Chem* 2006;281(34):24336–44. [PubMed: 16803882]
202. Knutti D, Kressler D, Kralli A. *Proc Natl Acad Sci U S A* 2001;98(17):9713–8. [PubMed: 11481440]
203. Jager S, Handschin C, St-Pierre J, Spiegelman BM. *Proc Natl Acad Sci U S A*. 2007
204. Li X, Monks B, Ge Q, Birnbaum MJ. *Nature* 2007;447(7147):1012–6. [PubMed: 17554339]
205. Koo SH, Satoh H, Herzig S, Lee CH, Hedrick S, Kulkarni R, Evans RM, Olefsky J, Montminy M. *Nat Med* 2004;10(5):530–4. [PubMed: 15107844]
206. Nissen SE, Wolski K. *N Engl J Med* 2007;356(24):2457–71. [PubMed: 17517853]
207. Smith CL, O'Malley BW. *Endocr Rev* 2004;25(1):45–71. [PubMed: 14769827]
208. Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, Lustig KD, Shan B. *Genes Dev* 2000;14(22):2831–8. [PubMed: 11090131]

Figure 1. Activation of signaling pathways modulates nuclear receptor transcriptional activity
Activation of MAPK signaling cascades (p38, JNK, and ERK), FGF signaling, and GPCR signaling results in phosphorylation-dependent modulation of NR activity. Signaling pathways and phosphorylation events affect nuclear receptors or nuclear receptor cofactors through the modulation of protein–protein interactions, subcellular localization, DNA-binding, protein stability, and transactivation capacity. The interface between signal transduction pathways and NRs is critical in the responsiveness of the system to environmental and physiological stimuli.