

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

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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 reveals 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, subcellular 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.

Keywords: Nuclear receptors; signal transduction; phosphorylation; liver; therapeutic agents

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 coactivator protein binding. Binding of ligand induces a conformational change that creates a new surface for the

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recruitment of coactivator proteins in the AF-2 region.³ The LBD is connected to a DNA binding domain (DBD) by a hinge region (H) that contains a nuclear localization signal. The DBD is highly conserved and contains two α helices and two zinc fingers that are involved in the specificity of response-element recognition and in 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) that 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 ligand.⁵ 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 a few other exceptional receptors may be located in the cytoplasm in the absence of ligand. Nuclear localization of NRs is mainly regulated by protein-protein interactions such as dimerization with RXRs or coregulator proteins.⁶ 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 DNA.⁷ 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 coregulator proteins that do not bind to DNA directly but have a pronounced effect on the outcome of gene expression.³ In general, nonliganded NRs form a complex with corepressor proteins that 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 corepressor multiprotein complexes and subsequent recruitment of coactivator protein complexes that enhance the rate of gene transcription, often thought the recruitment of multiprotein complexes containing histone acetyltransferase (HAT) activity. Coregulator proteins thus provide a second level of specificity in the modulation of gene expression by NRs. Most NR-coactivator 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 coactivator proteins, the peroxisome proliferator activated receptor γ coactivator 1 α (PGC-1 α) interacts not only with the AF-2 region of NRs but also with the H region of the selected liver-enriched NRs.⁸ 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

Figure 1 outlines the interface between NRs and the signal transduction pathways discussed in this review. While tyrosine phosphorylation of selected NRs has been observed, the functional significance of this type of phosphorylation is unknown. The majority of amino acid residues identified as being regulated-phosphorylation sites are serine and threonine residues, and many lay within the N-terminal AF-1 region of NRs and correspond to consensus sites for proline-dependent kinases such as cyclin-dependent kinases^{9,10} and MAP kinases (MAPKs).¹¹ 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

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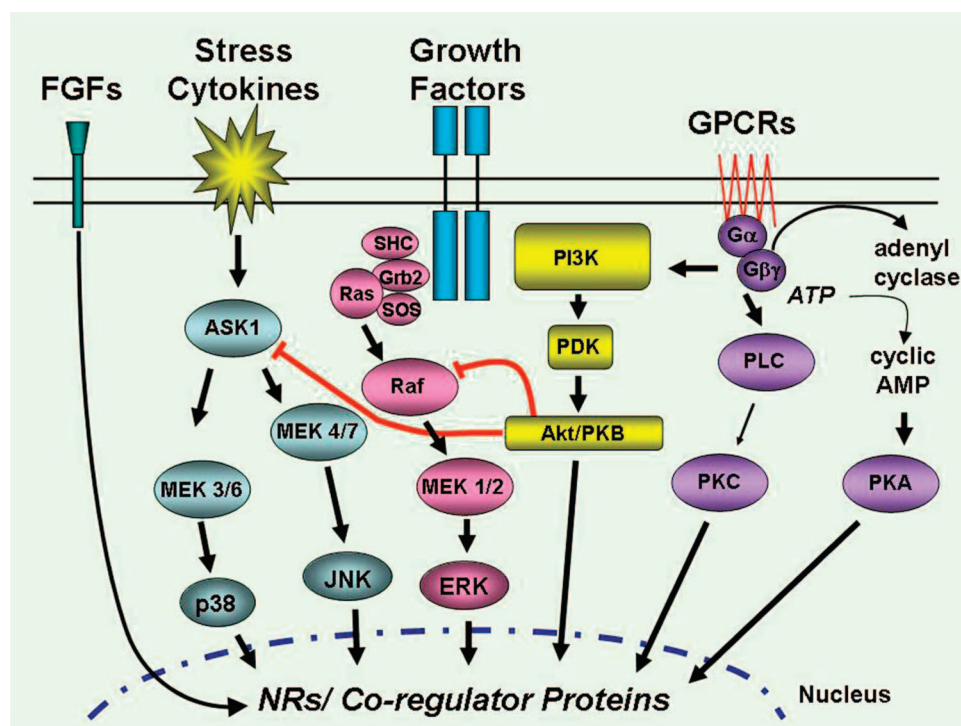


Figure 1. Activation of signaling pathways modulates nuclear receptor transcriptional activity. Activation of MAPK signaling cascades (p38, JNK, and ERK), FGF signaling, and GPCR signaling results in phosphorylation-dependent modulation of NR activity. Signaling pathways and phosphorylation events affect 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.

receptors (PPARs) contain only one or two phosphorylation sites in the N-terminus.¹²

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, including extracellular signal-regulated kinase (ERK), Jun-N-terminal kinase (JNK), or p38 MAPK, which can enter the nucleus and phosphorylate NRs. The N-terminal domains of PR,^{13,14} estrogen receptor alpha

(ER α),^{15,16} estrogen receptor β (ER β),^{17,18} androgen receptor,¹⁹ PPARs,^{20,21} and retinoic acid receptor γ (RAR γ)^{22,23} have all been reported to be substrates of ERK or p38

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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 protein kinase B, a kinase critical for cell survival and proliferation.^{24,25} Activated Akt negatively regulates downstream MAP kinases,²⁶ and upon nuclear translocation phosphorylates specific NRs including ER α ²⁷ and AR.²⁸

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 cyclic-AMP-dependent protein kinase (PKA) for RAR α ³³ is also common. Evidence for

phosphorylation of the DBD involves either PKA for ER α ³⁴ or PKC for RAR α ³⁵ and vitamin D receptor.^{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 nonsteroid 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 expression.³⁸ HNF-4 α has been implicated in the regulation of many genes in liver such as Cyp7a1,³⁹ the constitutive androstane receptor (CAR),⁴⁰ and genes involved in glucose transport and glycolysis.⁴¹ Although typically thought of as an orphan receptor, HNF-4 α has been shown to be activated by fatty acyl-CoA thioesters.⁴² 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

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binding affinity and transcriptional activity of the receptor based on gel-shift assays and reporter gene analysis.⁴³ 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 cells.⁴⁴ In addition to alterations in DNA-binding, tyrosine phosphorylation is required for appropriate sub-nuclear localization and transactivation activity of HNF-4 α as evidenced by immunofluorescence, electron microscopy, and reporter gene assay using genistein treatment to inhibit tyrosine phosphorylation.⁴⁵

It has also been recently shown that p38 kinase phosphorylates HNF-4 α at S158 increasing its interaction with coactivator PC4, DNA-binding, and transactivation activity in the presence of IL-1 β and hydrogen peroxide.⁴⁶ 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 HNF-4 α protein and Cyp7a1 gene expression in primary rat hepatocytes, thus providing a functional link between HNF-4 α phosphorylation and bile acid synthesis.⁴⁷ Since HNF-4 α has been shown to activate multiple genes and interacts with multiple transcription factors and coregulators in liver such as COUP-TFs, steroid receptor coactivator (SRC) proteins, CBP, 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 muscle.⁵² 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-diabetes.⁵³ 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 S82.⁵⁴ 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 stimulation.⁵⁵ In contrast, another study reports an increase in transactivation of PPAR γ

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via insulin-stimulated ERK2 phosphorylation in CHO cells.⁵⁶ Differential modulation of PPAR activity by phosphorylation is likely due to the relationships between specific kinases, serine residues, ligands, and receptor isoforms in specific cell types. For example, ERK2 and JNK, but not p38 MAP kinase, can phosphorylate PPAR γ on S84,⁵⁵ while PPAR α is a substrate for both ERK2 and p38 MAP kinase in a ligand-dependent manner.⁵⁷ 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 experimentally.⁵⁸

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 corepressor proteins such as nuclear receptor corepressor (NCoR) from PPAR α .²¹ In a similar fashion, phosphorylation of the N-terminus of PPAR α increases coactivation by PGC-1 α .⁵⁷ Phosphorylation can also enhance DNA-binding of PPARs, as is the case with PPAR α phosphorylation by PKA.⁵⁸ 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 expression.⁵⁹

MAPKs, PKA, and PKC are 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, sul-

fotransferases, and UDP-glucuronosyltransferases.^{60–63} PXR also regulates the expression of the drug-transporter genes organic anion transporting polypeptide 2, multidrug-resistance 1, multidrug resistance-associated protein 2, and multidrug resistance-associated protein 3.^{64–66} 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 compounds.⁶⁷ 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 was seen in endotoxin treated mice. Similarly, IL-6-treated mice displayed a 40–70% reduction in the mRNA levels of all Mdr isoforms.⁶⁸ Inflammatory cytokines inhibit the inducible expression of Oatp2 during intrahepatic cholesta-

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sis.⁶⁹ It has also been shown using primary cultures of human hepatocytes that IL-6 markedly decreases the expression of PXR and its close cousin CAR. IL-6 also decreases both rifampicin- and phenobarbital-mediated induction of CYP3A and CYP2B gene expression.⁷⁰

Recent evidence has demonstrated crosstalk between PXR and NF- κ B signaling pathways. NF- κ B activation by lipopolysaccharide and tumor necrosis factor 4 α inhibited PXR association with the CYP3A promoter by disrupting the interaction between the PXR-RXR protein complex.⁷¹ 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. Studies using inflammatory bowel disease 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 manner.⁷³

In addition to cytokine signaling, CYP3A gene expression is also modulated by PKA and PKC signaling pathways. Cotreatment of primary cultures of rat hepatocytes with phenobarbital and cyclic AMP analogues and PKA activators clearly results in cyclic AMP-associated inhibition of CYP gene expression.⁷⁴ However, treatment with the adenylyl cyclase activator forskolin and its non-PKA-activating analogue 1,9-dideoxyforskolin both resulted in the stimula-

tion of CYP3A gene expression.⁷⁵ 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-coactivator 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 expression.⁷⁶ 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 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 corepressor NCoR, and by abolishing the ligand-dependent interaction between PXR and coactivator SRC-1.⁷⁷

NR1I3, CAR. Similar to PXR, the NR superfamily member 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 subfamily.⁷⁸ 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 Mrp3.⁷⁹ 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 regulator of

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hepatic gene expression in response to phenobarbital.⁸⁰ 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 hepatocytes,⁷⁴ and cotreatment with phosphatase inhibitors further potentiates the repressive effects of PKA signaling.⁸¹ Serine/threonine-specific protein phosphatases PP1 and PP2A have a positive role in the induction of CYP2B.⁸² Pustyl'nyak et al. further report 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 effect.⁸³

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. 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 CAR.⁸⁴ In addition, dephosphorylation of S202 in mouse CAR is required for its nuclear translocation.⁸⁵ The signaling pathway involved in the phosphorylation of S202 remains unknown. Unlike most NRs, CAR translocates to the nucleus without directly binding phe-

nobarbital.⁸⁶ Taken together, these data indicate that the phosphorylation status of CAR is intimately involved in its cytoplasmic retention and nuclear translocation.

Recent evidence shows that epidermal growth factor represses phenobarbital-mediated activation of CAR-dependent transcription⁸⁷ and that the MEK inhibitor U0126 increases the phenobarbital mediated induction of CYP2B in primary rat hepatocytes.⁸⁸ In addition, hepatocyte growth factor treatment represses induction of cyp2b10 by phenobarbital in primary mouse hepatocytes. Hepatocyte growth factor treatment increased the phosphorylation of ERK1/2, thus decreasing the nuclear translocation of CAR;⁸⁹ 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 hepatocytes.⁹⁰ 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 nucleus.⁹¹ Shindo et al. reported that activation of AMPK resulted in nuclear accumulation of CAR but was not sufficient to induce CYP2B gene expression.⁹² Additional studies suggest that phenobarbital targets LKB1 for the activation of AMPK,⁹³ adding a proximal target to the elusive sequence of events by which phenobarbital activates transcription of CYP2B.

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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 inflammation.⁹⁴ LXR α is primarily expressed in liver, macrophages, and adipose tissue, while LXR β is more ubiquitously expressed.⁶⁷ 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 transporters, lipoproteins such as apolipoprotein E, lipoprotein lipase, and lipogenic proteins such as sterol response element binding protein-1C and fatty acid synthase.^{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 site.¹⁰⁴ 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 ligands.¹⁰⁵ 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 conditions.^{106–108} In primary cultures of rat hepatocytes, activation of PKA repressed LXR-mediated sterol response element binding protein-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 coactivator protein- SRC-1, and enhancing the recruitment of corepressor protein-NCoR.¹⁰⁸ On the other hand, Tamura et al. have demonstrated that PKA signaling can increase LXR transactivation in reporter gene assays conducted in renal As4.1 mouse cell lines.¹⁰⁶

In addition to inducing genes involved in cholesterol and glucose homeostasis, LXR reciprocally represses a set of

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inflammatory genes including inducible-nitric oxide synthase, cyclooxygenase-2, interleukin (IL)-6, and matrix metalloproteinase-9 after bacterial, LPS, 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 coregulator proteins, recent evidence suggests that inhibition of the NF- κ B pathway is involved likely through trans-repression of NF- κ B in the nucleus.¹⁰⁹ In a recent study of trans-repression of the iNOS promoter, SUMOylation of PPAR γ was identified as a mechanism of repression.¹¹⁰ In a similar manner, SUMOylation of LXR has been shown to negatively regulate a subset of proinflammatory genes by preventing the removal of NCoR corepressor complexes from the promoter.¹¹¹ 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 homologue 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 of its target genes.¹¹² LRH-1 is an important regulator of CYP7A1 gene expression.^{113,114} LRH-1 also regulates the expression of other genes involved in cholesterol and bile acid homeostasis including CYP8B1, Mrp3,

cholesterol ester transfer protein, apical sodium-dependent bile acid transporter, and apolipoprotein A1.^{60,115–120}

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. The ERK1/2 inhibitor U0126 blocks this response. Mutation analysis confirm that phosphorylation of LRH-1 at S238 and S243 in the H domain stimulates LRH-1 transactivation.¹²¹ 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.^{122,123}

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 promoter.¹¹⁵ Krylova et al. have suggested that phosphatidylinositols, major intracellular signaling molecules, bind to LRH-1, linking phospholipids signaling and gene expression.¹²⁴ 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,

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and excretion of bile acids.¹²⁵ An important breakthrough in the FXR field was the discovery that FXR is directly activated by several bile acids including chenodeoxycholic acid, lithocholic acid, and deoxycholic acid.^{126–128} 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, canalicular bile salt excretory pump, phospholipid transfer protein, and the hepatic basolateral transporter Na (+)-taurocholate-cotransporting polypeptide.¹²⁹ Additional studies reveal that FXR induces expression of the NR superfamily member—small heterodimeric partner (SHP). Increased SHP then represses Cyp7a1 transcription by inhibiting the activity of LRH-1, which is a positive regulator of the Cyp7a1 promoter.¹¹³

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-induced diabetic rat models, and administration of insulin restores FXR mRNA to normal levels.¹³⁰ Additional support for this concept comes from the observation that glucose reduces FXR expression in the liver,¹³⁰ while activation of FXR by the synthetic agonist GW4064 reduced plasma glucose levels in mice.¹³¹ Further-

more, loss of FXR disrupts normal glucose homeostasis and leads to the development of insulin resistance in FXR knockout mice.^{132–134} 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 enhanced.¹³⁵

Bile acids that are FXR agonists have been shown to activate multiple signal transduction pathways. Treatment with taurocholic acid results in activation of the JNK pathway,¹³⁶ and deoxycholic acid treatment activates the Raf-1/MEK/ERK signaling cascade in primary rat hepatocytes.¹³⁷ 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 expression.¹³⁸ 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 NR 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 NRs.¹³⁹ It is expressed mainly

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in liver, small intestine, spleen, heart, and pancreas.¹⁴⁰ SHP interacts with a variety of NRs in liver including PPAR α ,¹⁴¹ LRH-1,^{113,142} LXR,¹⁴³ and HNF4 α .¹⁴⁴ SHP acts as a direct transcriptional repressor and inhibits the activity of most NRs with which it interacts.¹⁴⁵ Two notable exceptions are that SHP enhances the transcriptional activation of PPAR α ¹⁴⁶ and PPAR γ ¹⁴⁷ 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 pathway and that SHP is a direct target-gene of activated c-Jun.¹³⁶ Overexpression 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 members of the FGF family, FGF19 (FGF15 in mouse), FGF21, and FGF23, have recently emerged as novel metabolic hormones. The expression of FGF23 plays a role in calcium and phosphate homeostasis and is regulated by the vitamin D receptor (VDR), which is expressed mainly in bone, kidney and intestine and therefore will not be discussed in this review.^{148,149}

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, FGF-receptor 4, and increases JNK-dependent signaling.¹⁵⁰ 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 expression.¹⁵¹ It has also been shown that overexpression 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.^{152,153}

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Conversely, activation of the JNK signaling pathway in the liver has been shown to increase insulin resistance;¹⁵⁴ however, there is currently no explanation for this discrepancy.

FGF-receptor 4 deficient mice exhibit reduced JNK activity, an increased bile acid pool, and enhanced expression of CYP7A1.¹⁵⁵ On the other hand, transgenic mice expressing constitutively active FGFR4 exhibit increased JNK activity, a reduced bile acid pool, and reduced expression of CYP7A1.¹⁵⁶ 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 FGF-receptor 4 and SHP.¹⁵⁷ In addition, mice lacking FGF15 have increased hepatic Cyp7a1 expression and activity corresponding to increased bile acid excretion.¹⁵⁷ 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 overexpressed FGF21 are resistant to diet induced obesity.¹⁵⁸ Similar results were observed in FGF21 treatment

of diabetic rhesus monkeys.¹⁵⁹ 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 .^{160,161} 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 diet.¹⁶⁰ 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 genes.¹⁶⁰ In transgenic mice overexpressing 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 mice.¹⁶¹ Overexpression of FGF21 in mice also produced decreased body temperature and locomotor activity during fasting and increased lipolysis in white adipose tissue.¹⁶¹ 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 coregulator proteins provides a second level of regulation in target gene activation. The association of coregulator 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 coregulator proteins. Numerous examples cited above illustrate how phosphorylation of NRs can result in increased or decreased strength of interaction between the receptor and coactivator or corepressor multiprotein complexes. Realization that the specificity and activity of coregulator proteins may also be regulated by signal transduction and phosphorylation is relatively new concept for which much less is known.

NCoR and SMRT Corepressor Proteins. The most extensively characterized corepressor proteins for NRs are

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NR corepressor (NCoR) and silencing-mediator for retinoid and thyroid hormone receptors (SMRT).^{162,163} 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 corepressor proteins can bind to NRs at their conserved C-terminal receptor interacting domain in the presence or absence of ligand and are regulated by a variety of signal transduction pathways.¹⁶⁴

It had been previously observed that activation of tyrosine kinases negatively regulates the interaction between transcription factors and SMRT.¹⁶⁵ Further studies reveal that phosphorylation of SMRT in the C-terminal receptor-interaction domain by the MAP kinase-kinase MEK-1 and MEK-1 kinase (MEKK-1) inhibits the interaction between SMRT and NRs.¹⁶⁶ 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 cytoplasm.¹⁶⁶ In contrast, phosphorylation of SMRT by casein kinase 2 (CK2) on S1492 stabilizes SMRT-NR interactions.¹⁶⁷ 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 subcellular distribution in response to MEKK1 signaling.¹⁶⁸ 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 signaling.¹⁶⁸ Recent

evidence suggests that this differential response may be determined by alternative mRNA splicing of SMRT and NCoR.¹⁶⁹ 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 Akt.¹⁷⁰

Finally, since SMRT and NCoR exist in corepressor 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 corepressor complexes.^{171–173} IL-1 β has been reported to inhibit NCoR through an indirect pathway resulting in the MEK kinase-1-mediated phosphorylation of the transforming growth factor-beta-activated kinase 1-binding protein 2 subunit present in a subset of NCoR-HDAC3 complexes, whereas SMRT is resistant to this pathway.¹⁷⁴

p160/Steroid Receptor Co-Activator Proteins

Steroid receptor coactivators (SRC) proteins are widely expressed and coactivate most NRs as well and many general transcription factors. The C-terminal region of SRC contains HAT activity, albeit relatively weak.^{175,176} Furthermore, SRCs recruit other coactivator proteins such as CBP, p300, and histone acetyltransferase p300/CBP-associated factor (pCAF) to a larger multiprotein complex that participates in

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chromatin remodeling.¹⁷⁷ 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 domain.¹⁷⁸

Seven phosphorylation sites for SRC-1 and six for SRC-3 have been identified.^{179,180} 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 epidermal growth factor, cyclic AMP, cytokines, and steroid hormones.^{179–183} In addition, the phosphorylation of SRCs induced by these agents is required for optimal coactivator activity. For example, ERKs can phosphorylate SRC-2 at S736 and treatment of cells with EGF increases the transcriptional activity of GAL4-GRIP1.¹⁸⁴ 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 PR.¹⁸² PKA did not phosphorylate these sites *in vitro*, but blockage of PKA activity in COS-1 cells prevented cyclic AMP-mediated phosphorylation of these sites.¹⁸² This phosphorylation event was also shown to be required for the interaction of SRC with pCAF or CBP.¹⁸² In a similar manner, EGF-stimulated phosphorylation of SRC-3 by MAPK stimulates the recruitment of p300 and enhances ligand-dependent ER activity.¹⁸¹ Phosphorylation of SRC-3 was shown to selectively affect

the interactions with NRs, NF- κ B, and CBP.¹⁸⁰ 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 coactivation of estrogen and androgen receptors.¹⁸⁰ However, phosphorylation of only five of the six sites is induced by TNF α and is required for coactivation of NF- κ B.¹⁸⁰ Further evidence shows that SRC-3, but not SRC-1, was copurified in complex with I κ B kinase, and consequently, phosphorylation of SRC-3, but not SRC-1, is enhanced in response to TNF α stimulation.¹⁸³ 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 coactivator complex can modulate transactivation potential of NRs. CBP and p300 can be phosphorylated *in vivo* and participates in cyclic AMP-regulated gene expression.¹⁸⁵ Kinase activities are also found to be associated with CBP and p300. Activation of cellular Ras with insulin treatment stimulated the recruitment of S6 kinase pp90_{RSK} to CBP.¹⁸⁶ Binding of pp90_{RSK} to CBP repressed the transcription of cyclic AMP-responsive genes via CREB.¹⁸⁶ CBP also contains a signal-regulated transcriptional activation domain that is controlled by calcium/calmodulin-dependent protein kinase IV and by cyclic AMP.¹⁸⁷ 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 pathways.¹⁸⁸ 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

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pathway requires the HAT activity of pCAF.¹⁸⁸ It is thus plausible that activation of different signaling pathways could influence the group of coactivators that are required for NR mediated transactivation.

PGC Family of Co-Integrator Proteins

There are three members of the PPAR γ coactivator (PGC) family, PGC-1 α , PGC-1 β , and the PGC-1-related coactivator PRC. However, PGC-1 α is the most extensively characterized member of the family. Like many protein cofactors, PGC1 α coactivates multiple NRs. PGC-1 α is selectively expressed mainly in skeletal muscle, cardiac muscle, white fat, and liver.¹⁸⁹ 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 or p300.¹⁹⁰ The physiological role of PGC-1 α has been well characterized as a master regulator of energy homeostasis in fat, liver and muscle.¹⁹¹ 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 gluconeogenesis,¹⁹² fatty acid oxidation,¹⁹³ and bile acid synthesis.¹⁹⁴

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 liver.¹⁹² 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 promoter.¹⁹⁵ 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 coactivator of CREB, was dephosphorylated and entered the nucleus, driving expression of PGC-1 α .¹⁹⁶

In insulin-stimulated skeletal muscle, PGC-1 α gene expression is down-regulated by Akt-mediated phosphorylation and nuclear exclusion of FOXO1.¹⁹⁷ In addition to insulin, obesity and saturated fatty acids decrease PGC-1 α gene expression and function via p38 MAPK-dependent transcriptional pathways.¹⁹⁸ 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.¹⁹⁹

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.^{200,201} Methylation can also enhance PGC-1 α activity.²⁰² 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

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and half-life and activation of PGC-1 α protein.²⁰³ 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.²⁰⁴ 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 an 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.²⁰⁵ 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.²⁰⁶ In liver, the mechanism by which insulin regulates lipid synthesis and degradation are largely unknown. Insulin treatment, through protein kinase Akt/protein kinase B (PKB) resulted in the phosphorylation and inhibition of PGC-1 α .²⁰⁷ Akt phosphorylates PGC-1 α at S570 which prevents the recruitment of PGC-1 α to its target promoters.²⁰⁷ 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 coactivates PPAR α in the expression of tribbles homo-

logue TRB-3, a fasting inducible inhibitor of Akt/PKB.²⁰⁸ 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 coregulator 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 2007.²⁰⁹ Further research surrounding the signaling events and coregulator 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 ERs and is commonly used in the treatment of breast cancer. Tamoxifen exhibits agonist (estrogen-like) activity in uterus and antagonist (antiestrogen-like) activity in breast.²¹⁰ SRM-induced alterations in the conformation of NRs may affect the ability of the receptor to bind to coregulators or to be phosphorylated. The expression profile of specific coactivator proteins and corepressor 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,

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binding, and localization of coregulator proteins, as well as NRs. Increased understanding of the effect of cellular signaling on NRs and their coregulator 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 myriad 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 overcome.²¹¹ 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 do not 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 coregulator 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, subcellular 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.

Abbreviations Used

NRs, nuclear receptors; LBD, ligand-binding domain; AF-2, activation function 2; DBD, DNA-binding domain; H, hinge region; RXR, retinoid x receptor; AF-1, activation function 1; HDAC, histone deacetylase; HAT, histone acetyl transferase; PGC-1 α , peroxisome proliferator activated receptor gamma co-activator 1 alpha; PPAR, peroxisome proliferator activated receptor; PR, progesterone receptor; ER α , estrogen receptor alpha; ER β , estrogen receptor beta; PKA, cyclic-AMP-dependent protein kinase; ERK, extracellular regulated kinase; JNK, Jun-kinase; PKC, protein kinase C; RAR, retinoic acid receptor; HNF4 α , hepatocyte nuclear factor 4 alpha; PXR, pregnane x receptor; CAR, constitutive androstane receptor; CYP, cytochrome P450; LXR, liver x receptor; NCoR, nuclear receptor co-repressor; SMRT, silencing-mediator for retinoid and thyroid hormone receptors; SRC, steroid receptor co-activator; LRH-1, liver receptor homologue 1; FXR, farnesoid x receptor; FGF, fibroblast growth factor; SHP, small heterodimeric partner.

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