

## Nuclear receptors and gene expression in liver

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### 1. Overview

Nuclear receptors (NR) are a family of ligand-activated transcriptional regulators of several key aspects of hepatic physiology and pathophysiology. In fact, nuclear receptors control a large variety of metabolic processes including hepatic lipid metabolism, drug disposition, bile acid homeostasis, as well as liver regeneration, inflammation, fibrosis, cell differentiation, and tumor formation. Thus, nuclear receptor ligands are being actively explored as novel therapeutic approaches for a broad range of hepatic disorders.

The primary means by which NRs affect such diverse physiological functions is via

NRs in Liver

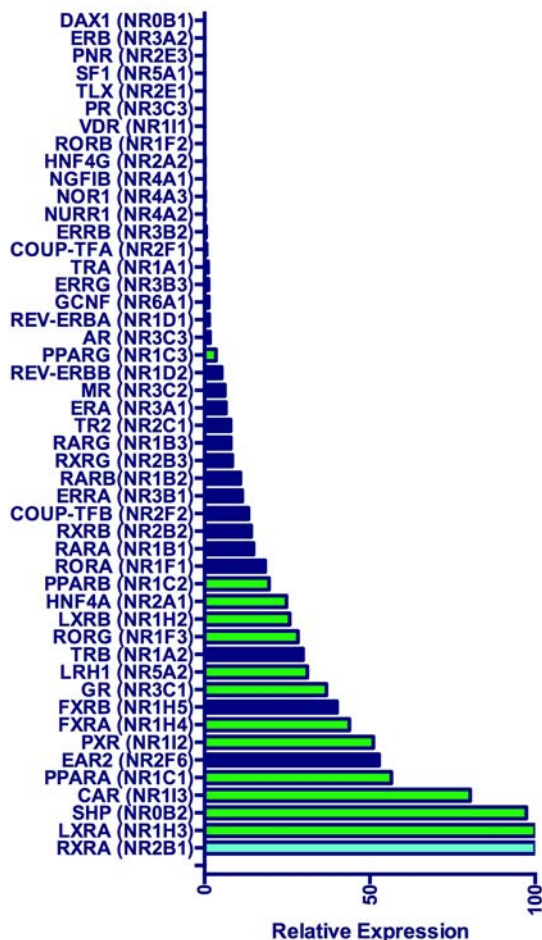


Figure 1. Expression of nuclear receptors in liver (adapted from <https://nursa.org/nursa>). NRs highlighted in green will be discussed in more detail.

alteration of gene transcription. Various NRs sense intracellular molecules, such as bile acids or fatty acids, and regulate intracellular metabolism accordingly. For more information on how NRs regulate gene expression, please consult other White Papers on the NR Resource website ([nrresource.org](http://nrresource.org)). In this document, we will examine NRs that are involved in gene regulation in the liver, in particular those that are involved in liver disease and drug metabolism.

### 2. Expression of NRs in the liver

The primary metabolic cell within the liver is the hepatocyte, although other cells such as Kupffer, Stellate (Ito) and endothelial cells may be involved in responses to diet or xenobiotics. The hepatocyte comprises over 80% of the mass of the liver, and it is the major source of drug and nutrient metabolism. NRs that regulate lipid, glucose, and bile acid homeostasis in the liver are highly expressed in liver (1) (see Figure 1). Of particular note are liver X receptor  $\alpha$  (LXR $\alpha$ ), constitutive androstane receptor (CAR), peroxisome proliferator activated receptor  $\alpha$  (PPAR $\alpha$ ), pregnane X receptor (PXR), farnesoid X receptor (FXR), glucocorticoid receptor (GR), liver receptor homology-1 (LRH-1), retinoid-related orphan receptor  $\gamma$  (ROR $\gamma$ ), hepatic nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ) and the common heterodimer partner Retinoid X receptor  $\alpha$  (RXR $\alpha$ ). Not shown in Figure 1 are two non-NR transcription factors, the Aryl hydrocarbon Receptor (AhR) and Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which also have roles in liver physiology, in particular xenobiotic metabolism. Following a general discussion of how these receptors are involved in physiological functions in the liver, each receptor and its role in gene regulation will be discussed.

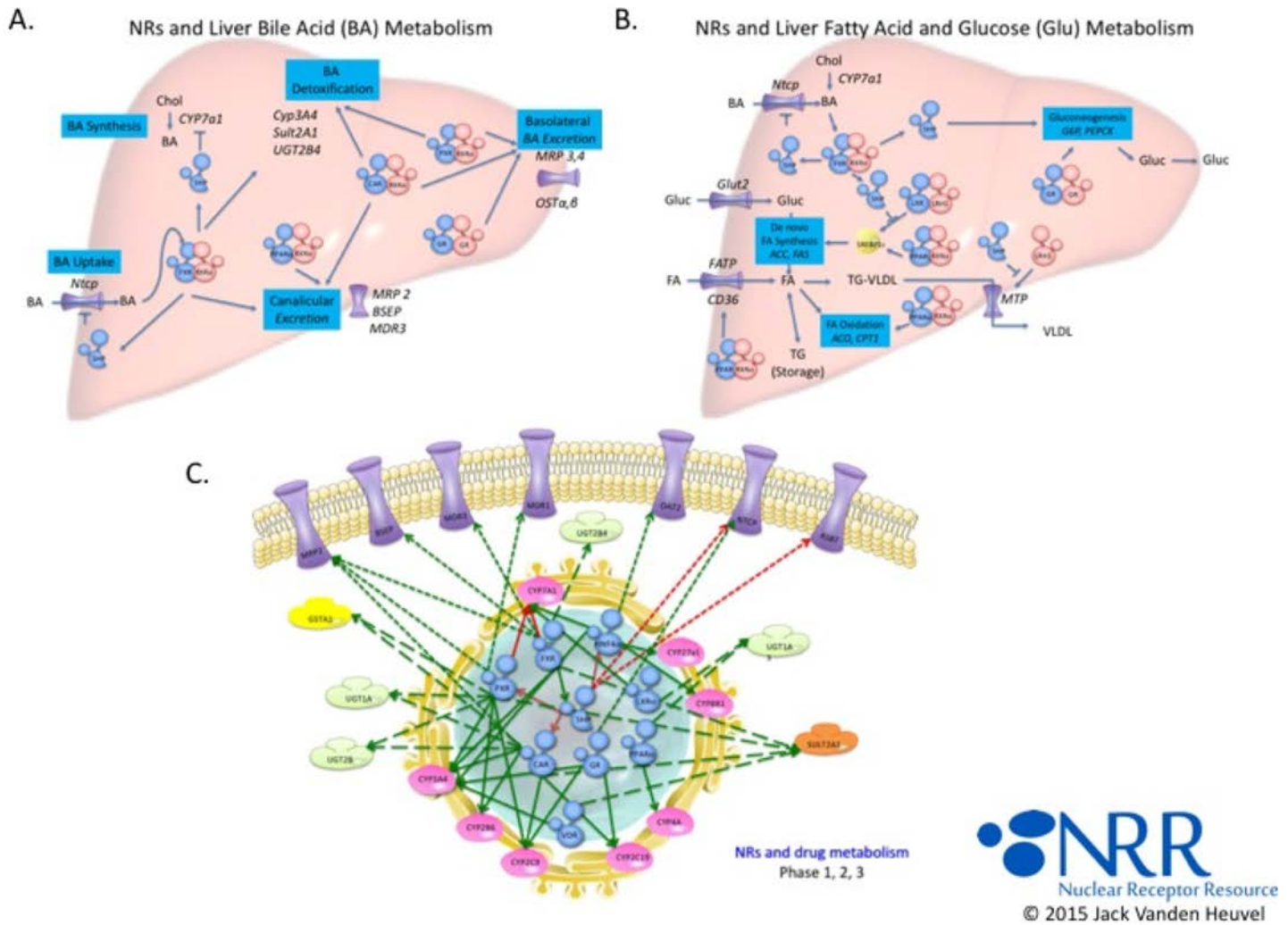
### 3. Role of NRs in Bile Acid Metabolism

NRs play a central role in the regulation of bile acid synthesis, metabolism, and transport (2,3) (Figure 2, panel A). Under cholestatic conditions with high intracellular bile acid load, NRs mediate a coordinated response aimed at protecting hepatocytes from toxic bile acids. Mice lacking the NRs FXR, PXR, and CAR are more vulnerable towards bile acids exposure and cholestatic injury. Genetic variants of FXR may determine susceptibility to gallstones

disease and cholestasis whereas PXR variants have been linked to progression of primary biliary cirrhosis (PBC). Stimulation of the bile acid detoxification machinery with drugs targeting FXR, PXR, and CAR reduces cholestasis and its complications. Such substances are already used in daily clinical practice (e.g., “enzyme inducers” such as rifampicin), whereas others are currently tested in clinical trials and many more are expected to enter clinical trials in the near future. Understanding NR function has therefore not only significantly increased our insights into physiology and pathophysiology of bile acid metabolism but also led to development of NR ligands for the treatment of cholestasis.

### 4. Role of NRs in Hepatic Lipid/Glucose Metabolism

The liver plays a central role in lipid homeostasis and NRs control several aspects of hepatic lipid and glucose metabolism which may be relevant for the pathogenesis and treatment of metabolic syndrome, hepatic insulin resistance, dyslipidemia, atherosclerosis, and nonalcoholic fatty liver disease (NAFLD) (2,3) (Figure 2, panel A). Several endogenous and exogenous lipids such as cholesterol or fatty acids act as physiological NR ligands and their activation frequently promotes metabolism of respective ligands. More specifically, the PPARs and hepatocyte nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ) are activated by various fatty acids, oxidation products of cholesterol such as 24(S)-hydroxycholesterol act as ligands for LXR, and cholesterol metabolites like bile acids act as FXR ligands. Modulation of the activity of these NRs by already available synthetic compounds represents an attractive therapeutic strategy for these metabolic disturbances.



**Figure 2. Role of NRs in bile acid, fatty acid, glucose and xenobiotic metabolism.** **Panel A.** Bile acids (BA) are taken up by the Na<sup>+</sup>/taurocholate cotransporter (NTCP) at the basolateral membrane of hepatocytes and are exported into bile by the canalicular bile salt export pump (BSEP) and the canalicular conjugate export pump (MRP2). The phospholipid export pump (MDR3) mediates excretion of phosphatidylcholine, which forms mixed micelles together with BA and cholesterol in bile and prevents BA-induced bile injury. CYP7A1 is the key enzyme for conversion of cholesterol into bile acids. Under cholestatic conditions, phase I (hydroxylation by way of CYP3A4) and phase II (conjugation by way of UGT2B4 and SULT2A1) bile acid detoxification limits cholestatic liver injury. In addition, MRP3, MRP4, and the heteromeric organic solute transporter OSTα/β at the basolateral membrane of hepatocytes provide an alternative excretion route for BA into the systemic circulation. **Panel B.** Cholesterol (Chol) metabolites (oxysterols) activate LXR, whereas bile acids (BA) stimulate SHP expression by way of FXR (negative feedback inhibition of BA synthesis). SHP inhibits its own expression in a feedback fashion by targeting LRH-1 (not shown). SREBP-1c (induced by insulin, LXR/LRH-1, and PPAR-γ) regulates *de novo* fatty acid (FA) synthesis from glucose (Glc). FXR-induced SHP inhibits LXR/LRH-1-mediated transactivation of SREBP-1c expression, but also indirectly modulates SREBP-1c expression/activity by altering cellular cholesterol content (not shown). Moreover, SHP targets LRH-1-mediated transactivation of MTP expression, required for triglyceride (TG) assembly with apo B as VLDL triglycerides. **Panel C.** Nuclear receptors, as well as AhR and Nrf2 are important transcriptional regulators of drug metabolism enzymes (DMEs). Phase I DMEs consist primarily of the cytochrome P450 (CYP) superfamily of microsomal enzymes. The phase II metabolizing or conjugating enzymes, consisting of many superfamily of enzymes including sulfotransferases (SULT) and UDP-glucuronosyltransferases (UGT). Phase III transporters, including P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) and organic anion transporting polypeptide 2 (OATP2).

### 4. Role of NRs in Xenobiotic Metabolism

Drug metabolizing enzymes (DMEs) play central roles in the metabolism, elimination and detoxification of xenobiotics and drugs introduced into the human body (4)(Figure 2, panel C). Although most tissues contain a wide array of DMEs including phase I, phase II metabolizing enzymes and phase III transporters, the liver is the predominant site of xenobiotic metabolism. Various NRs as well as the aryl hydrocarbon receptor (AhR) and nuclear factor-erythroid-related factor 2 (Nrf2) have been shown to be the key regulators of drug-induced changes in expression of DMEs. For instance, the expression of CYP1 genes can be induced by AhR in response to many polycyclic aromatic hydrocarbon (PAHs). Similarly, the constitutive androstane receptor (CAR) and pregnane X receptor (PXR), both heterodimerize with the retinoid X receptor (RXR), and transcriptionally activate the promoters of CYP2B and CYP3A gene expression by xenobiotics such as phenobarbital-like compounds and dexamethasone and rifampin-type of agents. The peroxisome proliferator activated receptors (PPARs) are activated by lipid lowering, fibrate-type of compounds leading to transcriptional activation of the promoters on CYP4A gene. CYP7A was recognized as the first target gene of the liver X receptor (LXR), in which the elimination of cholesterol depends on CYP7A. Farnesoid X receptor (FXR) was identified as a bile acid receptor, and its activation results in the inhibition of hepatic acid biosynthesis and increased transport of bile acids from intestinal lumen to the liver, and CYP7A is one of its target genes. For the phase II DMEs, phase II gene inducers such as the phenolic compounds butylated hydroxyanisole (BHA), tert-butylhydroquinone (tBHQ) and sulforaphane activate the bZIP transcription

factors Nrf2 and binds to the antioxidant/electrophile response element (ARE) promoter. Phase III transporters, for example, P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and organic anion transporting polypeptide 2 (OATP2) play crucial roles in drug absorption, distribution, and excretion. The NRs PXR and CAR, in particular, are involved in the regulation of these transporters. It appears that in general, exposure to phase I, phase II and phase III gene inducers may trigger a cellular “adaptive” response leading to the increase in their gene expression, which ultimately enhance the elimination and clearance of these xenobiotics. Consequently, this homeostatic response of the liver plays a central role in the protection of the body against environmental insults such as those elicited by exposure to xenobiotics. This in turn is also a major means of potential drug-drug interactions. That is, one drug through its interaction with receptors such as PXR, CAR or AhR and consequent induction of DMEs is able to affect the metabolism of a concurrently administered drug or nutrient.

### 5. Specific nuclear receptors and their role in hepatic gene expression.

As described above, there are several NRs and “xenobiotic-responsive transcription factors” that are highly expressed in the liver. In the following section we will describe the pathways that affect liver disease (steatosis, cholestasis) and drug-drug interactions.

#### a. Liver X Receptors (LXRs)

The transcriptional factor liver X receptors (LXRs, NR1H2, 3) are involved in cholesterol metabolism (2). The LXR gene encodes two distinct products, LXR $\alpha$  and LXR $\beta$ , each with diverse patterns of expression but similar target DNA-binding

## Nuclear Receptor Resource

White Paper

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elements and ligands. The endogenous ligands for LXR are oxysterols (22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 24(S),25-epoxycholesterol, 27-hydroxycholesterol) and fatty acids, but there are several synthetic and antagonists as well. Once activated, LXR induces the expression of a cluster of genes that function in lipid metabolism; these functions are cholesterol absorption, efflux, transport, and excretion (Figure 3).

In addition to its metabolic role, LXRs also modulate immune and inflammatory responses in macrophages (2). LXR is a key regulator of whole-body lipid and bile acid metabolism. LXR regulates a cluster of genes that participate in the transport of excess cholesterol in the form of high-density lipoprotein (HDL) from peripheral tissue to the liver, a process called reverse cholesterol transport (RCT). *In vivo* activation of LXR with a synthetic, high-affinity ligand increases the HDL level and net cholesterol secretion. LXR positively regulates several enzymes involved in lipoprotein metabolism including lipoprotein lipase (LPL), human cholesteryl ester transport protein, and the phospholipid transfer protein. LXR also regulates the crucial bile acid enzyme CYP7A1. The enzymatic activation and conversion of cholesterol to bile acids is one mechanism for handling excess dietary

cholesterol.

### b. Constitutive Androstane Receptor (CAR)

The human constitutive androstane receptor (CAR, NR1I3) regulates the expression of genes involved in xenobiotic metabolism and transport in the liver, including CYP2B and 3A4, UGT1 and MDR (5)(Figure 4). Studies from mouse models show this nuclear receptor is also involved in bile acid, thyroid hormone and HDL homeostasis. The CAR gene uses multiple alternative splicing events during pre-mRNA processing, thereby enhancing the CAR transcriptome. The 348 amino acid long wild-type human CAR (hCAR1) is encoded by 9 exons comprised of a DNA binding domain (DBD), a hinge region, and a ligand binding domain (LBD). hCAR2 contains an additional four amino acids (VSPT) while the predominant variant expressed in the liver, CAR3, contains an additional five amino acids (APYLT); these insertions are found in the ligand binding domain of the receptor. The rat and the mouse CAR sequences are more similar to that of hCAR1 and there is little evidence to support the existence of splice variants in these species. The hCAR2 and hCAR3 transcripts are prominently expressed in human liver and primary hepatocytes, with combined levels ranging up to ~50% of total CAR3. Both CAR2 and CAR3 activate reporters containing response elements derived from the endogenous promoters of the CYP2B6 and CYP3A4 genes. CAR1 is active in the absence of ligand with the unique capability to be further regulated by activators, mainly via inverse agonism. A number of CAR activators, including phenobarbital, do not directly bind to the receptor but affect signaling pathways that impinge on the NR's activity. Unlike CAR1, CAR3 functions as a ligand-dependent receptor, activating transcription in the presence of the human CAR ligand 6-(4-chlorophenyl)imidazo[2,1-b]thiazole-5-carbaldehyde O-3,4-dichloroben-zyl)oxime (CITCO). CAR2 has a ligand binding profile that is distinct from both CAR1 and

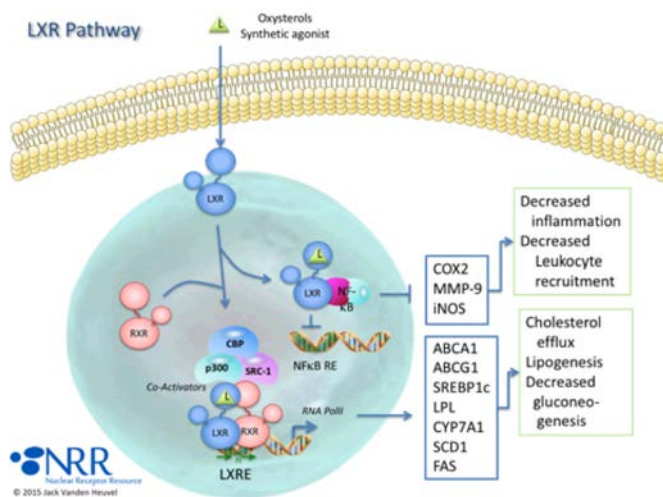


Figure 3. Basic mechanism of action of LXR in liver

CAR3, as evidenced by activation with di-ethylhexyl phthalate (DEHP). In addition to splice variant differences in activation profiles, species differences are seen, as shown with the mouse specific CAR agonist 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP). The existence of splice variants in human CAR and the species differences in activation by xenobiotics certainly makes examining this receptor a challenge.

Activation of the CAR might suppress lipid metabolism and lower serum triglyceride levels by reducing the level of SREBP-1, a master regulator of lipid metabolism.

expressed in the liver, brown adipose tissue, heart, skeletal muscle, kidney, and at lower levels in other organs. PPAR $\gamma$  is highly expressed in adipose tissues and is present in the colon and lymphoid organs. PPAR $\beta/\delta$  is expressed ubiquitously, but its levels vary from tissue-to-tissue. Generally speaking, the endogenous ligands of the PPARs are fatty acids or their metabolites. Whereas PPAR $\alpha$  is regulated by a wide variety of longer-chain length fatty acids including saturated, monounsaturated and polyunsaturated fatty acids (PUFA), PPAR $\gamma$  has more specificity for PUFAs and their oxidized metabolites (6). PPAR $\beta/\delta$  has affinity for PUFAs but also for 4-hydroxynonenal, a toxic lipid (7).

Mechanistically, the PPARs form heterodimers with the RXR and activate transcription by binding to a specific DNA element, termed the peroxisome proliferator response element (PPRE), in the regulatory region of several genes encoding proteins that are involved in lipid metabolism and energy balance (see Figure 5). In the liver, PPAR $\alpha$  promotes fatty acid oxidation. The role of PPAR $\alpha$  in hepatic fatty acid metabolism is especially prominent during fasting where PPAR $\alpha$  target genes such as acyl CoA oxidase (ACO), acyl CoA synthase (ACS) and liver-fatty-acid-binding protein, are involved in breaking down the excess lipid delivered to the liver during this state. PPAR $\alpha$  can also regulate other genes such as LPL, which is involved in the degradation of triglycerides, and APOA1 and APOCIII, which are both decreased by PPAR $\alpha$  agonists. Whereas PPAR $\alpha$  controls lipid catabolism and homeostasis in the liver, PPAR $\gamma$  promotes the storage of lipids in adipose tissue and in liver. In the liver, PPAR $\beta/\delta$  is protective against liver toxicity induced by environmental chemicals, possibly by downregulating the expression of proinflammatory genes. PPAR $\beta/\delta$  regulates glucose utilization and lipoprotein metabolism by promoting reverse cholesterol transport.

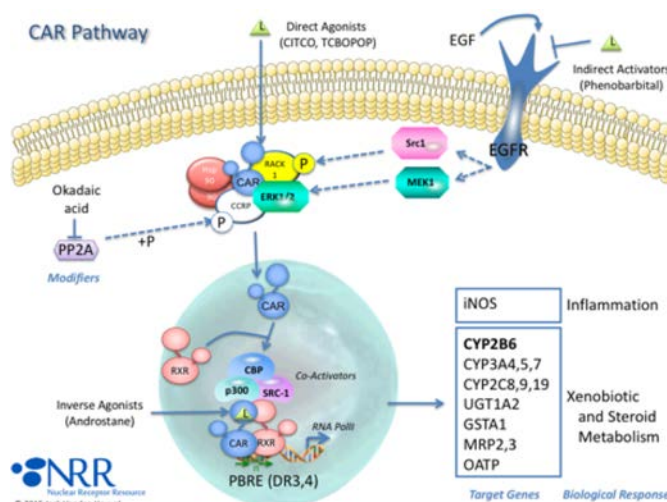


Figure 4. Basic mechanism of action of CAR in liver

### c. Peroxisome Proliferator-Activated Receptors (PPARs)

The peroxisome-proliferator-activated receptors (PPARs; PPAR $\alpha$ , PPAR $\beta/\delta$ , and PPAR $\gamma$ , NR1C1-3) are important metabolic regulators and have been utilized as therapeutic targets for treatment of insulin resistance and impaired fat metabolism (2). PPAR $\alpha$ , PPAR $\beta/\delta$ , and PPAR $\gamma$  exhibit different tissue distribution and functions and, to some extent, different ligand specificities. PPAR $\alpha$  is highly

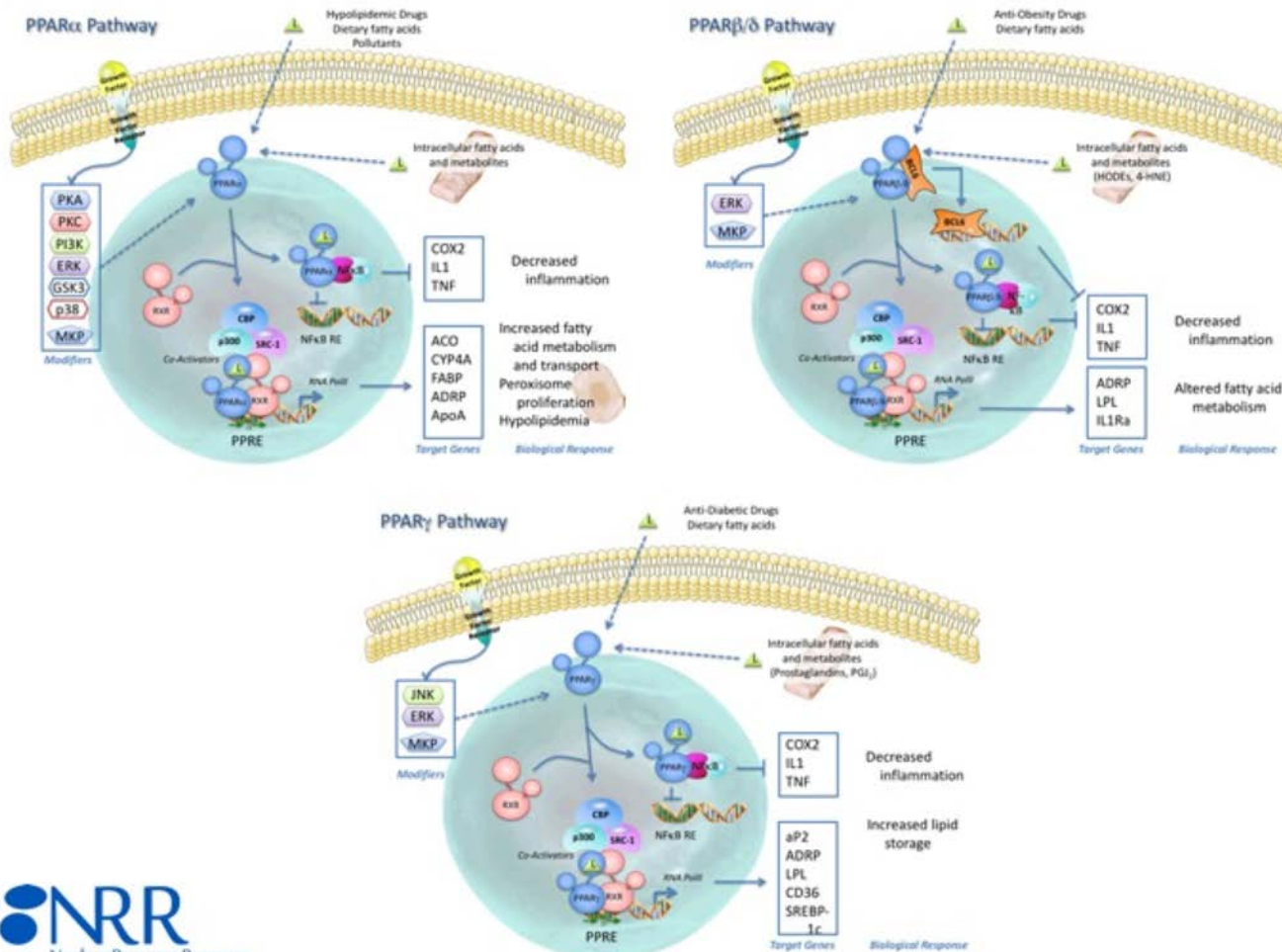


Figure 6. Basic mechanism of action of the PPARs in liver

**d. Pregnane X Receptor (PXR)**

Predominantly expressed in the liver, Pregnane X receptor (PXR, NR1I2) represents one of the most promiscuous receptors among the entire NR superfamily, and can be activated by a structurally diverse collection of chemicals, including both xenobiotics and endogenous chemicals. Xenobiotic mediated activation of PXR is associated with the induction of many target genes including major DMEs and drug transporters (2,5) (Figure 6). These include phase I enzymes such as Cyps, in particular

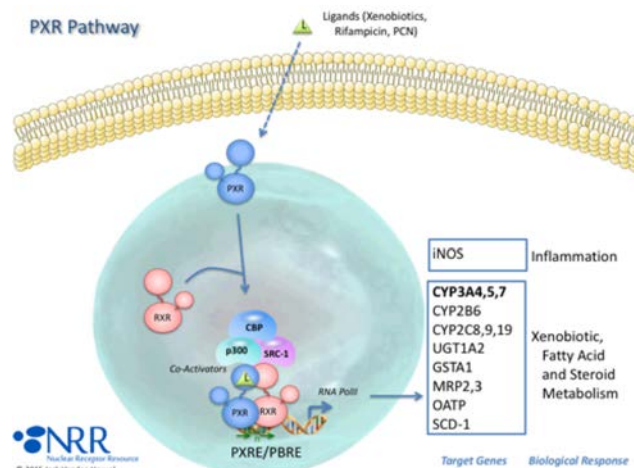


Figure 5. Basic mechanism of action of PXR in liver

Cyp3A4, phase II enzymes such as UGT1A1 and transporters including as the multidrug resistance protein MDR1 P-glycoprotein (Pgp).

In addition to functioning as a xenobiotic receptor and affecting the “adaptive response” to chemical exposure, PXR also influences liver disease. For example, PXR induces lipogenesis in a SREBP-independent manner. Lipid accumulation and marked hepatic steatosis in PXR-transgenic mice are associated with increased expression of the fatty acid translocase CD36 and several accessory lipogenic enzymes, including SCD-1 and long-chain free fatty acid elongase. PXR activation is also associated with suppression of several genes involved in fatty acid  $\beta$ -oxidation, such as PPAR $\alpha$  and thiolase.

### e. Farnesoid X Receptor (FXR)

The main physiological role of the farnesoid X receptor (FXR, NR1H4) is to act as a bile acid sensor in the enterohepatic tissues (2). FXR activation regulates the expression of various transport proteins and biosynthetic enzymes crucial to the physiological maintenance of bile acids and lipid and carbohydrate metabolism (Figure 7). Bile acids bind to and activate this NR with an order of potency of chenodeoxycholic acid > lithocholic acid = deoxycholic acid > cholic acid. In addition to affecting bile acid metabolism, FXR reduces both hepatic lipogenesis and plasma triglyceride and cholesterol levels, induces the genes implicated in lipoprotein metabolism/clearance, and represses hepatic genes involved in the synthesis of triglycerides. FXR promotes reverse transport of cholesterol by increasing hepatic uptake of HDL cholesterol via suppression of hepatic lipase expression and induction of scavenger receptor B1, the HDL uptake transporter in the liver. Activation of the FXR also increases the hepatic expression of receptors such as VLDL receptor and increases the expression of ApoC-II, which coactivates lipoprotein lipase (LPL). Taken together, these data

suggest that FXR activation lowers plasma triglyceride levels via both repressing SREBP1-c and triglyceride secretion and increasing the clearance of triglyceride-rich lipoproteins from the blood. In carbohydrate metabolism, activation of the hepatic FXR regulates gluconeogenesis, glycogen synthesis, and insulin sensitivity.

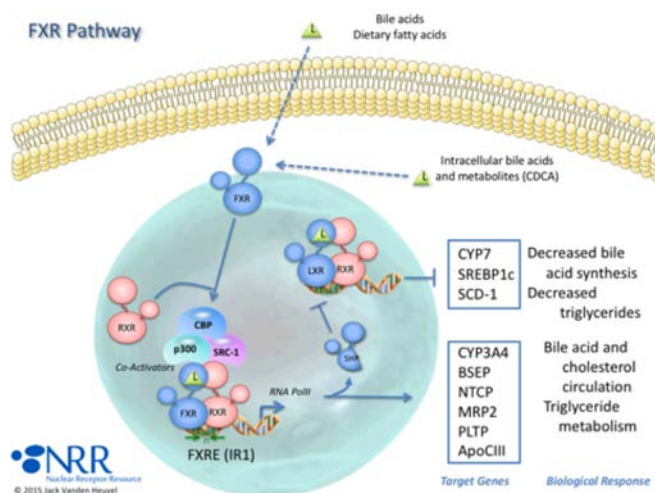


Figure 7. Basic mechanism of action of FXR in liver

### f. Glucocorticoid Receptor (GR)

There is increasing evidence that glucocorticoids (GC) and the glucocorticoid receptor (GR, NR3C1) are important mediators in liver disease (8). The classical activation pathway involves GC binding to the GR causing disassociation of the associated protein complex and subsequent translocation into the cell nucleus (Figure 8). Following translocation, gene transcription is altered by binding of the GR complex to specific DNA sequences known as glucocorticoid response elements (GRE) in the promoter region of target genes. This alteration in gene transcription up-regulates anti-inflammatory protein synthesis and reduces expression of pro-inflammatory cytokines. Multiple mechanisms in both liver and adipose tissue contribute to the development of fatty liver disease associated with GR activity. GCs bind to the GR and affect lipid in both adipose and hepatic tissue. The enzymes 11 $\beta$ -HSD & 5 $\alpha$ R, through their ability to metabolize GC



can affect GR signaling. In addition, the GC-GR interaction is regulated by proteins such as Stat5, FKBP52, HES1, MED1, ANGPTL4 and LXR $\beta$ . In adipose tissue, GCs, in the fasting state, mobilize lipid by increasing Hormone sensitive Lipase (HSL) and Desnutrin (ATGL - which hydrolyses Triglyceride). In the fed state GCs are lipogenic by increasing expression and activity of DGAT. Expression of Lipases, Acyl Co Carboxylases and Fatty Acid Synthases (LPL, ACC and FASN) are also increased. GCs indirectly affect hepatic steatosis by altering the release of NEFAs and adipokines from adipose tissue. GR activation increases *de novo* lipogenesis (DNL) by altering matrix Acyl Co-A Dehydrogenase, as well as PEPCK and glucose-6-phosphate dehydrogenase (G6P). GCs increase TAG accumulation by increasing expression of DGAT and reducing TAG Hydrolase (TGH). NEFAs are re-esterified in the liver into lipid droplets.

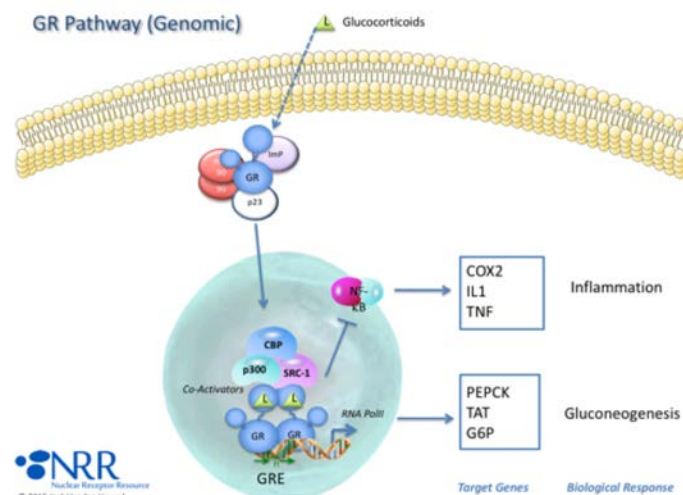


Figure 8. Basic mechanism of action of GR in liver

### g. Liver Receptor Homology-1 (LRH-1)

Liver Receptor Homolog-1 (LRH-1; NR5A2) is a NR that plays vital roles in early development and is important for bile acid synthesis, cholesterol metabolism and steroidogenesis. (9). LRH-1 binds to DNA as monomers to nuclear receptor half site sequences, as shown in Figure 9. LRH-1 was

originally classified as an orphan NR based on its constitutive activity and the lack of known endogenous ligand. This simple view has been in part revisited with the structures of mouse and human LRH-1 LBD proteins. These studies revealed that different phospholipid species, including phosphatidyl glycerol, phosphatidyl ethanolamine and phosphatidyl choline, as well as the second messengers phosphatidyl inositols, can bind to the large ligand binding pocket of human LRH-1 (10).

A key biological function of LRH-1 is the regulation of cholesterol metabolism via its effect on bile acid homeostasis (9).. LRH-1 regulates enterohepatic development and function via the expression of key genes involved in the regulation of bile acid synthesis, cholesterol homeostasis and transport. LRH-1 activates gene transcription of the rate-limiting enzyme in bile acid biosynthesis, cytochrome P450 family 7A1 or Cholesterol 7  $\alpha$ -hydroxylase (Cyp7A1). LRH-1 positively regulates expression of other enzymes and transporters involved in reverse transport of cholesterol and bile acid synthesis pathways. These genes which contain the LRH-1 response element (nuclear receptor half site) in their promoters include cytochrome P450 family 8B1 (Cyp8B1) or Sterol 12 $\alpha$  hydroxylase, multidrug resistance protein 3 (MRP3), cholesteryl ester transfer protein (CETP), scavenger receptor class B type I (SR-BI), mouse apical sodium-dependent bile acid transporter (ASBT) and human Apolipoprotein A1 (ApoA1). Within hepatocytes, cholesterol and cholesteryl esters are converted to bile acids by Cyp7A1 and Cyp8B1 for secretion out of the liver in bile. Furthermore, MRP3 and ASBT are involved in bile acid recycling, indicating that LRH-1 is important for bile acid homeostasis. Many LRH-1 gene targets are involved in the transfer of cholesterol to the liver and subsequent elimination into bile acids, and in bile acid synthesis, highlighting the importance of LRH-1 in cholesterol metabolism.

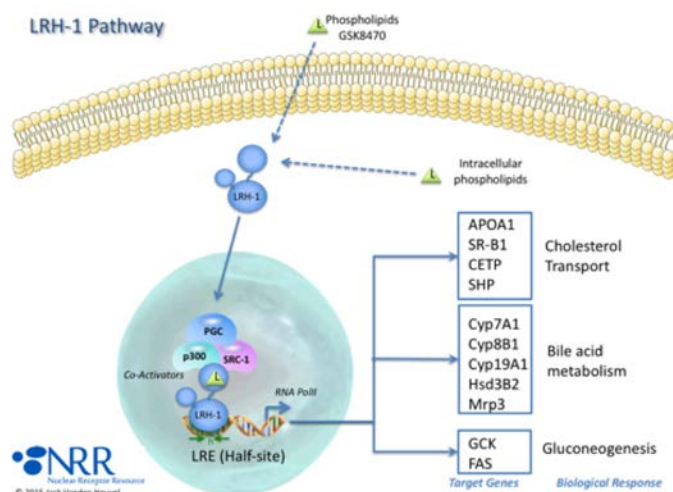


Figure 9. Mechanism of action of LRH-1 in liver

#### h. Retinoid Orphan Receptor $\gamma$ (ROR $\gamma$ )

ROR $\gamma$  constitutes with ROR $\alpha$  and ROR $\beta$ , the retinoic acid-related orphan receptor (ROR; NR1F1–3) subfamily of the nuclear receptors, which regulate transcription by binding as monomers to ROR-responsive elements (ROREs) in the regulatory region of target genes (11)(Figure 10. Through alternative promoter usage, the *ROR $\gamma$*  gene generates 2 isoforms, *ROR $\gamma$ 1* and *ROR $\gamma$ 2* (*ROR $\gamma$ t*), that regulate different physiological functions. *ROR $\gamma$ t* is restricted to several distinct immune cells and is essential for thymopoiesis, lymph node development, and Th17 cell differentiation.

Stearic acid, all-*trans* retinoic acid (ATRA) and the synthetic retinoid ALTA 1550 were identified as putative functional ligands and co-crystallized with ROR $\beta$ . Cholesterol and cholesterol sulfate co-crystallized within the ligand binding pocket of ROR $\alpha$ . Crystal structures of ROR $\gamma$  bound to its agonists 20 $\alpha$ -hydroxycholesterol, 22 $R$ -hydroxycholesterol and 25-hydroxycholesterol have been determined. Ursolic acid (UA), a natural

carboxylic acid ubiquitously present in plants is a strong inverse agonist of ROR $\gamma$ .

ROR $\gamma$  inverse agonists decrease Th17 cell differentiation and may provide a novel therapeutic strategy in the management of several autoimmune diseases. In contrast to ROR $\gamma$ t, relatively little is known about the physiological functions of ROR $\gamma$ 1. The expression of ROR $\gamma$ 1 is highly restricted to tissues that have major functions in metabolism and energy homeostasis, including liver and adipose tissue, and in contrast to ROR $\alpha$  and ROR $\beta$ , ROR $\gamma$  is not expressed in the central nervous system. By using ubiquitous and liver-specific ROR $\gamma$ -deficient mice as models, it was shown that hepatic ROR $\gamma$  modulates daily insulin sensitivity and glucose tolerance. ROR $\gamma$ -target genes in liver include those linked to glucose homeostasis (e.g., G6pase, Pepck, Pklr, Ppard, Gck, Gckr, Glut2, Gys2, Dlat, Pcx, and Klf15). In addition, ROR $\gamma$  regulates the circadian expression of *Insig2a*, *Elovl3*, *Cyp8b1* and other lipid metabolic genes. The circadian regulation of ROR $\gamma$  expression by the clock machinery together with observations that ROR $\gamma$  directly regulates the transcription of a number of lipid metabolic genes, support the hypothesis that ROR $\gamma$  acts downstream of clock proteins and functions as an important link between the circadian clock machinery and its regulation of certain metabolic genes. ROR $\gamma$ -/- mice display normal cholesterol and triglyceride levels but slightly reduced blood glucose levels compared with their wild-type counterparts. These findings suggest that ROR $\gamma$  inverse agonists may hold therapeutic potential for the treatment of metabolic syndrome and associated diseases.

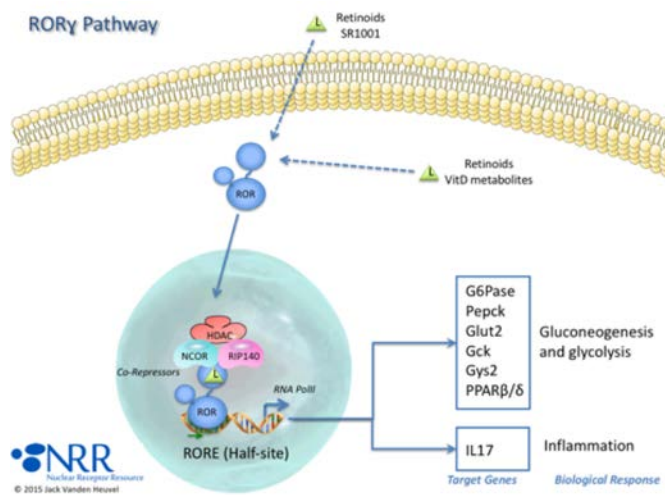


Figure 10. Mechanism of action of ROR $\gamma$  in liver

### i. Hepatocyte Nuclear Factor 4 $\alpha$ (HNF4 $\alpha$ )

HNF4 $\alpha$  (NR2A1), a highly conserved member of the nuclear receptor superfamily (NR) of ligand-dependent transcription factors (TFs), is known as a master regulator of liver-specific gene expression (12). Based on the crystal structure of the HNF4 $\alpha$  ligand binding domain, endogenous ligands may be fatty acids; in fact, linoleic acid (LA, C18:2  $\omega$  6) was found bound to HNF4 $\alpha$  in the crystalized structure and subsequent experiments showed it to be a weak agonist. Several CYP450 genes have been identified as HNF4 $\alpha$  targets with CYP3A4 being arguably one of the most important (Figure 11). HNF4 $\alpha$  is required for both PXR- and CAR-mediated transcriptional activation of CYP3A4. HNF4 $\alpha$  activates the expression of several other Phase I genes, including CYP2C8, CYP2C9, CYP2C19, CYP7A1 and CYP8B1. HNF4 $\alpha$  can bind and activate FMO1 promoter activity in concert with HNF1 $\alpha$ . In terms of Phase II genes, HNF4 $\alpha$  also plays an important role in regulating the basal SULT2A1 promoter, as well as synergizing with PXR and CAR. The expression of the UGT1A6 and UGT1A9 genes were found to be positively correlated with HNF4 $\alpha$  and HNF1 $\alpha$  expression in human liver. Several transporters that are critical for the elimination of

drugs and toxicants (ABCB1, ABCB11, ABCC2, SLCO1B1, SLC22A1), are also HNF4 $\alpha$  target genes.

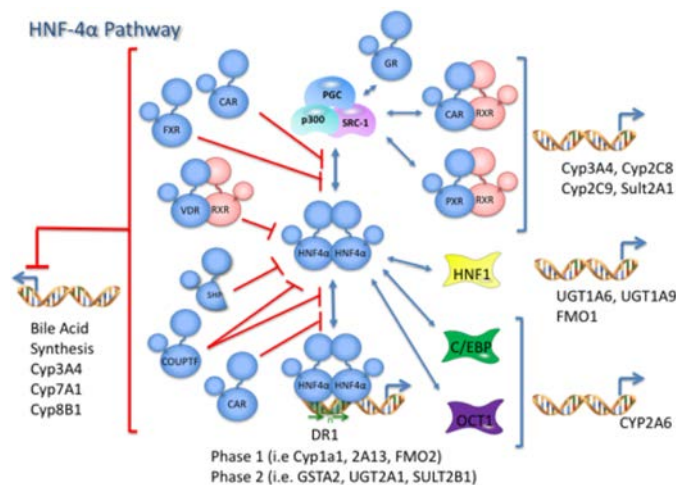


Figure 11. Mechanism of action of HNF4 $\alpha$  in liver

### j. Arylhydrocarbon Receptor (AhR)

The Arylhydrocarbon Receptor (AhR) is a member of the basic-helix-loop-helix (bHLH)- Per-Arnt-Sim (PAS) gene superfamily of transcription factors (4). AhR is known to recognize a range of chemical structures, including non-aromatic and non-halogenated compounds with the prototypical agonist being 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). A number of reports have identified specific dietary constituents that are AHR ligands (13). For example, the flavonoids quercetin, apigenin and kaempferol exhibit AHR agonist and antagonist activity in a cell line specific manner. Cruciferous vegetables contain significant amounts of indole glucosinolates that, upon consumption, are degraded to indole-3-carbinol. This compound then undergoes condensation reactions in the acidic environment of the stomach, creating several products that are capable of activating the AHR, with the compound of highest affinity for the AHR being indolo[3,2-*b*]carbazole. The unliganded AhR exists in the cytosol complexed with a dimer of Hsp90, which maintains the AhR in a ligand-binding

## Nuclear Receptor Resource

White Paper

### Nuclear receptors and gene expression in liver

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conformation and prevents nuclear translocation and/or dimerization with Arnt (Figure 12). The hydrophobic AhR ligands enter the cell by diffusion and are bound by the Hsp90-associated AhR. Ligand binding causes a conformational change resulting in a receptor species with an increased affinity for DNA. The AhR-Arnt complex to xenobiotic response elements (XRE) or Dioxin response elements (DREs) of target genes. Functional XREs, composed of a core pentanucleotide sequence 5'-GCGTG-3', are found in the regulatory regions of CYP1A, CYP1B, and UGT1A genes. DNA microarray studies have established that the AhR either directly or indirectly regulates a myriad of genes. These studies have shown that the AhR regulates genes involved in a wide variety of biochemical pathways, including energy metabolism, lipid and cholesterol synthesis, xenobiotic metabolism and various transporters. A role of AhR in hepatic lipid metabolism has been strengthened by the use of transgenic mice with a constitutively active AhR (CA-AhR) (14). These mice exhibited spontaneous hepatic steatosis, manifested by the accumulation of triglycerides but not cholesterol in the liver. The steatotic effect of AhR was independent of SREBP-1c-mediated de novo fatty acid synthesis. Instead, the lipogenic effect of AhR likely resulted from its activation of CD36 gene expression and the consequent increase of hepatic FFA uptake. In addition to CD36, the expression of FATP1 and FATP2, which can also facilitate hepatic FFA uptake, was increased in CA-AhR transgenic mice.

#### k. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2)

Nuclear erythroid 2-related factor 2 (Nrf2) is a transcription factor that belongs to the Cap-n-collar

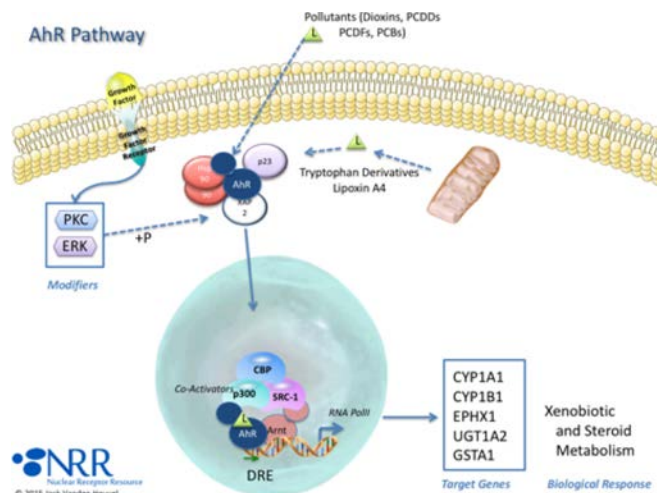


Figure 12. Mechanism of action of AhR in liver

basic leucine zipper family (15). Nrf2 is considered the main mediator of cellular adaptation to redox stress. In its inactive state, Nrf2 is located in the cytoplasm where it interacts with the actin binding protein, Kelch-like ECH associating protein 1, and is rapidly degraded by the ubiquitin-proteasome pathway. KEAP1 has more than 20 free sulfhydryl (-SH) groups in its constituent cysteine residues that act as stress sensors. Various oxidative or electrophilic cellular stresses, including ROS and reactive nitrogen species (RNS), modify KEAP1 cysteine residues, causing their dissociation and subsequent translocation of Nrf2 to the nucleus (Figure 13). In the nucleus, Nrf2 binds to ARE sequences and functions in partnership with other nuclear proteins as a strong transcriptional activator of ARE-responsive genes. ARE-mediated antioxidant proteins and enzymes, such as heme oxygenase-1 (HO-1), NAD(P)H: quinone oxidoreductase 1 (NQO1), glutathione-S-transferases (GST), group C streptococcus (GCS) are involved in the detoxification of increased electrophiles and radicals.

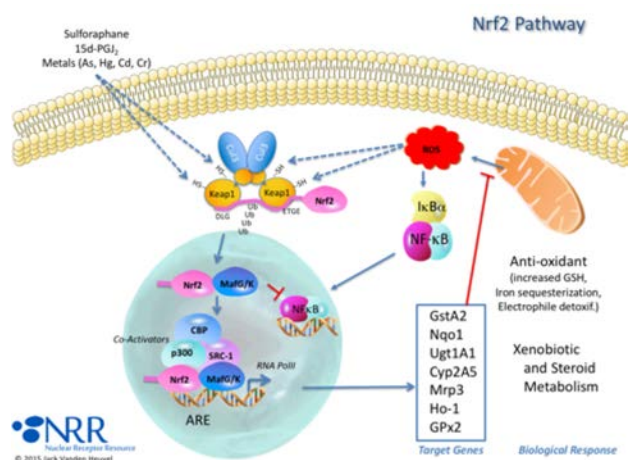


Figure 13. Mechanism of action of Nrf2 in liver

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