An Integrated Biological Approach to Nuclear Receptor Signaling in Physiological Control and Disease

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ABSTRACT: The nuclear receptors (NRs)—vitamin D receptor (VDR); peroxisome proliferator-activated receptor (PPAR) α, δ, γ, and pregnane X receptor (PXR)—act as sensors for various molecules encountered by the body on a daily basis. The effects of these ligands can be understood by the fact that numerous genes involved in the cellular processes, such as general homeostasis, growth, and defense against microbes, are under the control of these five NRs. The target gene and protein expression patterns of VDR, PPARs, and PXR; the resulting changes in metabolite levels; and their physiological consequences create a network that can be monitored by high-throughput methods and analyzed by multimodal approaches, such as systems biology. We suggest that the fine regulation of this NR network is specific to each human individual and depends, in part, on the constellation of regulatory small nucleotide polymorphisms (SNPs) in his or her genome. When regulatory SNPs affect NRs response elements, lifetime exposure to food components will have different accumulative consequences on the expression of the respective NR target genes. These differences will influence the individual's susceptibility to aging-related diseases, such as type 2 diabetes, atherosclerosis, cancer, and osteoporosis. Furthermore, it is anticipated that systems biology methods will also help to identify the most critical genes, proteins, or metabolites in the NR network that will serve as biomarkers for the early detection of these diseases.

KEY WORDS: VDR, PPAR, PXR, transcriptional regulation, nutrigenomics, aging-related diseases, regulatory SNPs, systems biology

I. INTRODUCTION

For homeostasis, growth, and defense against harmful agents the human body has to acquire, absorb, distribute, store, and use energy derived from nutrition.1 The biochemical pathways underlying these processes are based on the actions of enzymes and transporters, whose gene expres-
sion is under precise spatial and temporal coordination. These gene expression patterns are based on numerous regulatory events that mediate both the repression and the activation of target genes or their protein products at the correct time and place. One strategy used by organisms to achieve this coordination is to place sets of genes or proteins involved in a common process under the control of the product of another gene. This master gene must have the qualities of being able to recognize its targets and also be sensitive to specific environmental cues to allow the activation of a particular pathway, when necessary. Living cells have achieved this numerous times during evolution by the development of ligand-dependent transcription factors, which are able both to respond to specific ligands and to recognize discrete, defined DNA sequences found near target genes. These transcription factors also have the ability to attract and interact with other factors that facilitate the recruitment of the basal transcriptional apparatus, thus enabling specific gene activation to occur. Many dietary compounds, such as the micronutrient vitamin D and macronutrient fatty acids, as well as xenobiotic substances that are also ingested by diet, can act—directly or after metabolic conversion—as agonists to different members of the nuclear receptor (NR) superfamily of ligand-modulated transcription factors. These transcription factors must themselves be tightly regulated, because dysfunction or inappropriate activation of NR signaling leads to proliferative, reproductive, and metabolic diseases, such as cancer, infertility, obesity, and type 2 diabetes. In this review, we present an approach to the integrated action of the micronutrient sensor vitamin D receptor (VDR); the three fatty acid sensors peroxisome proliferator-activated receptors (PPARs) α, δ, and γ; and the xenobiotic sensor pregnane X receptor (PXR).

The interrelation of NRs, their diet-derived ligands, and metabolizing enzymes is a central issue in the new discipline nutrigenomics, which is the study of the impact of nutrient-derived compounds on the genome. It also encompasses the effects of food on physiological functions, such as resistance to external assault from opportunistic pathogens. For example, both nutritional overload and undernourishment have implications for immune function; consequently, metabolism and immunity are closely linked. Starvation and malnutrition can suppress immune function and increase susceptibility to infections, whereas obesity is associated with a state of aberrant immune activity and increasing risk for associated inflammatory diseases, including atherosclerosis, type 2 diabetes, airway inflammation, and fatty liver disease, a condition that impairs that organ's role in immunity.

Until the very recent past, approaches to diseases and gene function, in general, have tended to focus on one gene at a time. However, in the last 10 years, high-volume research approaches have allowed scientists to grasp the total information contained within a cell concerning transcriptional activity, protein content, and metabolites and have subsequently generated massive amounts of data, which need to be handled in a different way. One way to monitor and analyze the output of high-throughput methods is systems biology, which aims to both reduce experimental data to meaningful paradigms and to build up *in silico* testable hypotheses. We anticipate that systems biology will be of tremendous benefit for the study of NR biology, not only at the level of describing patterns found within gene expression data derived from ligand treatment but in other aspects, as well. For example, we suggest that the fine regulation of the NR network is specific to each human individual and depends on the constellation of regulatory small nucleotide polymorphisms (SNPs) in his or her genome. It is envisaged that some of these regulatory SNPs will affect the binding of NRs to a subset of response elements (REs) in their target genes. This could determine an individual's susceptibility to aging-related diseases, such as type 2 diabetes, atherosclerosis, cancer, and osteoporosis. Finally, out of the oceans of experimental data, systems biology will also help to identify biomarkers (whether they be genes, proteins, or metabolites) for the early detection of these diseases. To discuss the relevance of systems biology to the field of NR biology, we first must first describe the key concepts of this area of research in more detail.

II. THE NUCLEAR RECEPTOR SUPERFAMILY

NRs represent an important transcription factor family. The 48 human members of this super-
family are the best characterized genes of approximately 3000 mammalian genes that are involved in transcriptional regulation. NRs modulate genes that affect processes as diverse as reproduction, development, inflammation, and general metabolism. They were first recognized as the receptors for the steroid hormones estradiol (ER α and β), progesterone (PR), testosterone (AR), cortisol (GR), and aldosterone (MR); for thyroid hormones (TR α and β); and for the biologically active forms of the fat-soluble vitamins A and D, all-trans retinoic acid (RAR α, β, and γ) and 1α,25-dihydroxyvitamin D3 (1α,25(OH)2D3, VDR). This group of 12 NRs constitutes the classic endocrine NR subgroup. They can be defined functionally as being able to bind their specific ligand with a Kd of 1 nM or less. The 36 remaining NRs are structurally related to the endocrine NRs but were orphans at the time of their cloning because neither their ligands nor their physiological functions were initially known. However, during the last 15 years, natural and synthetic ligands have been identified for nearly half of these receptors, so that they now form the group of adopted orphan NRs. Interestingly, most of the latter group of NRs have as their natural ligands dietary components, such as lipids or exogenously derived compounds that are encountered in the micro- to millimolar concentration range. Consequently, these receptors have activation thresholds (in terms of Kd) in the same molar range. This functionally separates them from the endocrine receptors.

NRs have a modular structure to which certain functions can be ascribed. The amino-terminus is of variable length and sequence in the different family members. It contains a transactivation domain, termed AF-1, which is recognized by co-activator (CoA) proteins and/or other transcription factors. The central DNA-binding domain has two zinc-finger motifs that are common to the nuclear receptors—ERα and β, PR, AR, GR, and MR—bind to DNA as homodimers. Their ligands are the adopted orphans PPAR α, δ, and γ; the oxysterol sensors liver X receptors (LXRs) α and β; the bile acid sensor farnesoid X receptor (FXR); and the xenobiotic sensors PXR and constitutive androstane receptor (CAR)—function as heterodimers with one of the three retinoid X receptors (RXRs) α, δ, and γ. RXR was the first orphan NR for which with the vitamin A derivative 9-cis retinoic acid, an endogenous ligand, was identified. Because of its role as preferred heterodimeric partner, RXR typically does not function alone but serves as master regulator of several other regulatory pathways.

The adopted orphans respond to dietary lipids, and their concentrations cannot be limited by simple negative feedback control systems, as in the case of steroid receptors (Fig. 1). In their function as lipid sensors, they activate a feed-forward, metabolic cascade that maintains nutrient lipid homeostasis by governing the transcription of a common family of genes involved in lipid metabolism, storage, transport, and elimination. On the basis of their high ligand-binding affinity and the control of ligand metabolism, VDR, TRs, and RARs more functionally resemble the other endocrine NRs. However, because their ligands or precursors can derive from external sources, they share the dietary sensing capability of the adopted orphan receptors.
FIGURE 1. The impact of NRs on pharmacogenomics and nutrigenomics. The NRs VDR, PPARs, and PXR are involved in key metabolic processes, which determine the levels of their own and each others’ ligands. Solid, arrow-ended lines define molecular transitions, and dashed lines indicate activation pathways. Key enzymes are shown, and vertical arrows indicate their up- and downregulation. LOX designates lipoxygenases; the 5-lipoxygenase gene is upregulated by 1α,25(OH)₂D₃.
II. NUCLEAR RECEPTOR SIGNALING

The major protein constituents of chromatin are the four different histones that form a nucleosome, onto which DNA is wound (Fig. 2A). Covalent modifications of the lysines at the amino-terminal tails of these histone proteins neutralize their positive charge, and, thus, their attraction for the negatively charged DNA backbone is diminished. Consequently, the association between the histone and the DNA becomes less stable. This influences the degree of chromatin packaging and regulates the access of transcription factors to their potential binding sites. More than 10 specific modifications of histones are known to date, but it has been found that the acetylation of...
the lysine at position 8 of histone 4 correlates most strongly with the activation of chromatin on a promoter preceding the initiation of transcription. Therefore, in most cases, the histones associated with active regions of promoters have a higher degree of acetylation at certain positions than in repressed or silent regions. Presently, most studies on transcriptional regulation have concentrated on isolated promoter regions or proximal promoters, where binding sites of NRs and other transcription factors have been localized. An essential prerequisite for the direct modulation of transcription by NR ligands is the location of at least one activated NR protein close to the transcription start site (TSS) of the respective primary NR target gene. This is achieved, in most cases, through the specific binding of the DNA-binding domain of the NR to the major groove of a hexameric DNA sequence, referred to as the core-binding motif, with the consensus sequence RGKTSA (R = A or G, K = G or T, S = C or G). Heterodimeric RXR-NR complexes bind to two core-binding motifs in a direct repeat (DR)-type orientation with 1–5 intervening nucleotides. The spacing nucleotide number depends on the particular RXR binding partner. For example, PPARs bind to DR1-type REs, whereas VDR binds to DR3-type REs. In addition, VDR also binds to DR4-type REs along with PXR, FXR, TRs, CAR, and LXR. Finally, RARs have been shown to bind to DR5-type REs. It should also be noted that, effective VDR, TR, RAR, and PXR binding has also observed on inverted repeat (ER)-type REs with 6 to 9 nucleotides (ER6, ER7, ER8, ER9).

When NRs are bound to REs in the regulatory regions of their target genes, they recruit positive and negative co-regulatory proteins, referred to as CoAs and co-repressors (CoRs), respectively. In most cases, unliganded NRs preferentially interact with CoRs to mediate repression, whereas agonist-bound NRs recruit CoA proteins and act as transcriptional activators. CoA and CoR proteins both contain multiple, short receptor interaction domains, whose core is composed of the sequence LXXLL (L = leucine, X = any amino acid) in the case of CoAs and LXXXXXX(L/I) in the case of CoRs. The receptor interaction domains containing peptides make contacts with helices 3, 4, and 12 of the NR LBD. This interaction is stabilized by an evolutionary conserved charge clamp that is formed between a glutamate in helix 12 and either a lysine in helix 3 (in the case of CoA binding) or a lysine in helix 4 (for contacting CoR). Because CoAs and CoRs interact with the same hydrophobic groove on the surface of the LBD, their binding is mutually exclusive and, therefore, defines a strict functional switch.

Most co-regulators are not exclusive to NRs but are also used in a similar manner by other transcription factors. On the basis of their mode of action, co-regulators can be classified into two main groups. The first group contains factors that covalently modify histones, such as acetylation/deacetylation and methylation/demethylation, a process that follows the precise and combinatorial histone code. The second group of co-regulators includes ATP-dependent chromatin remodeling factors that modulate promoter accessibility to transcription factors and to the basal transcriptional machinery. The complex network of co-regulators can be viewed as defining a co-factor code that is characterized by distinct patterns of co-regulator recruitment and by their regulated enzymatic activities. The histone code can, therefore, be considered to be in a continuum with the co-factor code, because histones are crucial targets for the enzymatic activities of co-regulators and have a key role in specifying co-regulator recruitment on the basis of the reading of the histone code (Fig. 2A), as well.

In a simplified view of NR signaling, in the absence of ligand the NR interacts with CoR proteins, such as NCoR, SMRT, and Alien, which, in turn, associate with histone deacetylases (HDACs) leading to a locally increased chromatin packaging. The binding of ligand induces the dissociation of the CoR and the association of a CoA of the p160-family, such as SRC-1, TIF2, and RAC3, in complex with more general CoAs, such as CREB-binding protein (CBP). Some CoAs have histone acetylase (HAT) activity or are complexed with proteins harboring HAT activity, resulting in the net effect of causing local chromatin relaxation. In a subsequent step, ligand-activated NRs changes rapidly from interacting with the CoAs of the p160-family to those of mediator complexes, such as thyroid hormone receptor-associated protein.
The mediator complexes, which consist of approximately 15–20 proteins, build a bridge to the basal transcription machinery. In this way, ligand-activated NRs execute two tasks: the modification of chromatin and the regulation of transcription.

Cell- and time-specific expression patterns of some co-regulators can produce distinct modulations of NR transcriptional activity due to differences in the relative CoR and CoA protein levels. This aspect may have some diagnostic and therapeutic value in different types of cancer. However, the switch between gene repression and activation is more complex than a simple alternative recruitment of two different regulatory complexes. Most co-regulators are co-expressed in the same cell type at relatively similar, high levels, which raises the possibility of their concomitant recruitment to a specific promoter. Therefore, repression and activation is more likely achieved by a series of sequential events that are mediated by multiple enzymatic activities that are promoter and cell-type specific. Transcriptional regulation is a highly dynamic event of rapid association and dissociation of proteins and their modification, including degradation and de novo synthesis. A pattern of recruitment and release of cohorts of co-regulatory complexes was demonstrated on a single region of the trefoil factor-1 (TFF1/pS2) promoter in breast cancer cells. This study revealed detailed and coordinated patterns of co-regulator recruitment and preferential selectivity for factors that have similar enzymatic activities. Interestingly, some co-regulators seemed to be redundant and different family members were equally capable of being recruited alternately to the promoter.

Most models still place the gene promoter central recruiting transcription factors and co-regulators where the position of the promoter is stationary relative to visiting transcription factor complexes. However, it also remains possible that cytoskeletal fibers, such as nuclear actin complexes, pull promoters to preassembled transcription factories. In this model, the promoter-specific NR REs could represent specific attachment sites for actin filaments. Therefore, the mode of interaction with DNA, either directly or through other transcription factors, could be an important regulator of specificity.

### III. IN SILICO SCREENING FOR NUCLEAR RECEPTOR RESPONSE ELEMENTS

Statistically, the NR core-binding motif RGKTSA should be found, on average, in every 256 bp of genomic DNA. Furthermore, dimeric assemblies of such hexamers should show up as DRs every 65,536 bp, and as ERs every 32,768 bp in a random sequence. Therefore, an in silico screen of the human genome would identify for every NR, on average, 50,000–100,000 putative REs (Fig. 3). Because NR proteins have an abundance of, at most, a few thousand molecules per cell, a realistic number of NR target genes per cell should be closer to this number. If we also consider the fact that many NR target genes appear to have more than one functional RE for any given NR, it could be expected that the real number of NR target genes in any cell type is much less than the number of NR molecules. These calculations make it obvious that not every putative NR binding site is used in nature in any cell at any given time.

The most obvious reason is that most of these sequences are covered by nucleosomes in some repressed way, so that they are not accessible to the NR. This applies, in particular, to those sequences that are either contained within interspersed sequences, are located isolated from binding sites for other transcription factors, or lie outside of insulator sequences marking the border of chromatin loops. This perspective strongly discourages the idea that isolated, simple NR REs may be functional in vivo. In turn, this idea implies that the more transcription factor binding sites a given promoter region contains and the more transcription factors that are expressed, the higher is the chance that this area of the promoter becomes locally decondensed. An interesting example is the VDRE of the rat osteocalcin gene, which is flanked on both sides by a binding site for the transcription factor Runx2/Cbfa1 acting as a link to the nuclear scaffold. By contacting CoA proteins and HAT’s, Runx2/Cbfa1 seems to mediate the opening of chromatin locally, which allows efficient binding of VDR-RXR heterodimers to this decondensed region.

This abundance of potential REs may also explain why, in numerous microarray experiments, the same ligand (for instance 1α,25(OH)2D3), in
different cell lines, leads to the regulation of only a few genes consistently (CYP24 gene in the case of 1α,25(OH)2D3). The shear abundance of potential REs suggests that cellular context and local chromatin packaging defines whether that particular RE is potentially active. This also implies that it might be possible to selectively induce any particular gene to give rise to potentially therapeutic effects in diseases. Conversely, this knowledge could lead to the identification of inappropriately active REs, which have to be switched off to restore health.

Detailed knowledge about the individual binding-sequence preference of a given NR is of critical importance for the effective prediction of an NR RE’s functionality. Experimental studies, such as a series of gelshifts with a large number of natural REs,51–54 are essential to create position-weight matrices that include information about the relative binding strength of an NR within a dimeric complex. In an NR position-weight matrix, not only its hexameric core-binding sequences but also its preference for 5′-flanking dinucleotides should be taken into account.55,56 Unfortu-
nately, commonly used programs for in silico screening of NR REs, such as NUBI-scan\textsuperscript{57} and NHR-scan,\textsuperscript{58} are unable to identify complex REs.

Multidisciplinary efforts to develop tools and characterize the regulatory component of the human genome, such as the ENCODE project,\textsuperscript{59} are improving conditions. Moreover, programs such as TRANSFAC\textsuperscript{60} have the ability to identify larger numbers of REs for different transcription factors, making the identification of potential complex REs possible. However, even these programs are unable to predict chromatin-condensation states and nucleosome positioning, which is essential information in determining the likelihood that a given RE lies in an accessible region of a promoter. Therefore, general parameters, such as nucleosome positioning, interspecies homology screening of regulatory sequences, as well as the binding sites of all other transcription factors and their cell-specific expression patterns, have to be included in more effective versions of in silico screening software to more efficiently predict the state of chromatin.

IV. THE VDR AND ITS TARGET GENES

The biologically most active vitamin D\textsubscript{3} metabolite, 1α,25(OH)\textsubscript{2}D\textsubscript{3}, mediates its genomic effects via the NR VDR, which is the only nuclear protein that binds the nuclear hormone with high affinity ($K_d = 0.1$ nM).\textsuperscript{10} 1α,25(OH)\textsubscript{2}D\textsubscript{3} is known to be essential for mineral homeostasis and skeletal integrity due to its regulation of Ca\textsuperscript{2+}-translocation, to be essential for mineral homeostasis and skeletal integrity due to its regulation of Ca\textsuperscript{2+}-translocation (Fig. 1).\textsuperscript{67} The first hydroxylation of vitamin D\textsubscript{3} occurs at the C25 position and is catalyzed by vitamin D-25-hydroxylase in the liver to produce 25(OH)D\textsubscript{3}, the major circulating form of vitamin D\textsubscript{3} in mammals. 25(OH)D\textsubscript{3} is the substrate for a second hydroxylase, the renal enzyme 25(OH)D\textsubscript{3}-1-hydroxylase, which is encoded by the gene CYP27B1, resulting in the production of 1α,25(OH)\textsubscript{2}D\textsubscript{3}. Catabolism of vitamin D\textsubscript{3} metabolites is initiated by the widely expressed enzyme 25(OH)D\textsubscript{3}-24-hydroxylase, which is encoded by the gene CYP24. CYP24 is the most responsive primary VDR target, whose steady-state mRNA expression level is very low in the absence of ligand but is induced up to 1000-fold by stimulation with 1α,25(OH)\textsubscript{2}D\textsubscript{3}.\textsuperscript{66} Most other known primary 1α,25(OH)\textsubscript{2}D\textsubscript{3} target genes, such as cyclin C and p21\textsuperscript{(waf1/cip1)}, are much less responsive and often show an inducibility of 2-fold or less after short-term treatment with 1α,25(OH)\textsubscript{2}D\textsubscript{3}.\textsuperscript{69,70} However, both genes have 10,000- to 100,000-fold higher basal expression levels compared to that of the CYP24 gene.

To study the regulation of the CYP24, cyclin C, and p21\textsuperscript{(waf1/cip1)} genes by 1α,25(OH)\textsubscript{2}D\textsubscript{3} in more detail, 7.1 to 8.4 kB of their promoters was studied using chromatin-immunoprecipitation (ChIP) assays, using, in each case, a set of 20–25 overlapping promoter regions.\textsuperscript{68,71,72} The spatiotemporal, 1α,25(OH)\textsubscript{2}D\textsubscript{3}-dependent chromatin changes in the three gene promoters was analyzed by ChIP assays with antibodies against acetylated histone 4, VDR, RXR, and RNA polymerase II. Promising promoter regions were then screened in silico for putative VDREs, whose functionality was analyzed in gel shift, reporter gene, and re-ChIP assays. This approach identified 4 VDREs for both the CYP24 and cyclin C genes and 3 VDRE-containing regions in the p21\textsuperscript{(waf1/cip1)} gene promoter.\textsuperscript{73} However, most of them are simultaneously under the control of other transcription factors, such as p53.
in case of the \( p21^{[\alpha \delta/\alpha \delta]} \) gene and, therefore, possess significant basal levels of transcription. Consequently, the fold induction afforded by 1\( \alpha, 25(\text{OH})_2 \text{D}_3 \) stimulation is much less than for the \( \text{CYP24} \) gene.

An alternative approach was performed with the six members of the insulin-like growth factor binding protein (\( \text{IGFBP} \)) gene family, where, first, an in silico screen was performed, which was then followed by the analysis of candidate 1\( \alpha, 25(\text{OH})_2 \text{D}_3 \)-responsive sequences by gel shift, reporter gene, and re-ChIP assays.\(^7\) Induction of gene expression was confirmed independently using quantitative real-time PCR techniques. By using this approach, the genes \( \text{IGFBP}1, 3, \text{ and } 5 \) were demonstrated to be primary 1\( \alpha, 25(\text{OH})_2 \text{D}_3 \) target genes. The in silico screening of the 174 kb of genomic sequence surrounding all 6 \( \text{IGFBP} \) genes identified 15 candidate VDREs, 10 of which were shown to be functional in ChIP assays in the cell lines used. Importantly, the in silico screening approach was not restricted to regulatory regions that comprise only maximal 2 kb of sequence up- and downstream of the TSS, as in a recent whole genome screen for regulatory elements.\(^7\) Instead, it involved up to 10 kb of flanking sequences, as well as intronic and intergenic sequences. Therefore, this approach revealed candidate VDREs that are located more than 30 kb distant from their target gene TSS. On the basis of the present understanding of enhancers, DNA looping and chromatin units being flanked by insulators or matrix attachment sites, these distances are not limitations.\(^7\) At the next stage, we begin to look at the maximal possible adjacent sequences in our in silico studies, since we cannot rule out the possibility of functionality based on distance from the promoter. Eventually, when technology permits, we envisage the creation of whole-chromosome and whole-genome arrays of functional REs.

Although individual REs have been shown to be able to induce transactivation on their own, multiple, complex VDREs, as now observed in the primary VDR target genes discussed above,\(^6\),\(^7\)\(^1\),\(^7\)\(^2\),\(^7\)\(^4\) seem to favor the prominent response of the gene’s transcription to VDR. Even if the in vitro DNA-binding affinity of VDR-RXR heterodimers to these VDREs differs (compare Refs. 68, 71, 72, and 74), at the chromatin level all VDRE-containing promoter regions show comparable association strength with VDR and RXR. Each of the multiple 1\( \alpha, 25(\text{OH})_2 \text{D}_3 \)-responsive promoter regions is able to independently contact the basal transcriptional machinery. This suggests that the simultaneous communication of the individual promoter regions with the basal transcription machinery occurs through a discrete three-dimensional organization of the promoter within the nucleus of a cell. This arrangement would, therefore, allow the close contact of distant regions.\(^7\)

Recently, it was observed that the human \( \text{PPAR}\delta \) gene is a primary 1\( \alpha, 25(\text{OH})_2 \text{D}_3 \) target with a potent DR3-type VDRE in close proximity to the TSS.\(^7\) \( \text{PPAR}\delta \) and VDR are widely expressed, and an apparent overlap in the physiological action of both receptors is their involvement in the regulation of cellular growth, particularly in neoplasms. Prostate and breast cancer cells display a spectrum of sensitivities to the antiproliferative action of 1\( \alpha, 25(\text{OH})_2 \text{D}_3 \) and the levels of expression of a number of genes, including that of \( \text{VDR} \),\(^8\) \( \text{CYP24} \),\(^8\) and the CoR \( \text{SMRT} \);\(^8\) have been used as markers for the efficacy of the treatment of a cancer with VDR agonists. Epidemiological studies have linked the incidence of prostate and breast cancer to low serum levels of 25(\( \text{OH} \))\( \text{D}_3 \) as a result of diet and environment,\(^8\),\(^3\) and the initiation or progression of these cancers seems to relate to reduced dietary intake and/or cellular resistance to the antiproliferative effects of 1\( \alpha, 25(\text{OH})_2 \text{D}_3 \). High \( \text{PPAR}\delta \) expression in tumor seems to be positive for the prognosis of the respective cancer.\(^8\) In this way, the upregulation of \( \text{PPAR}\delta \) expression can be considered as a supportive action and part of the mechanism of the antiproliferative action of 1\( \alpha, 25(\text{OH})_2 \text{D}_3 \) and its synthetic analogues.

Another potential area of overlap for these two NRs is metabolism and nutrition. PPARs sense fatty acid derivatives, whereas VDR is known to interact with some specific bile acids, oxidized derivatives of cholesterol (Fig. 1). Both NRs are highly expressed in the colon, an organ with an important role in absorbing nutrients from the diet. Therefore, interaction between these two NRs in the gut may have important consequences for the body’s ability to handle material acquired from the diet.
V. PPAR SIGNALING

All three PPAR subtypes are important sensors of cellular levels of fatty acids and fatty-acid derivatives, which are mainly derived from the lipoygenase and cyclooxygenase (COX) pathways (Fig. 1). The large ligand-binding pocket of their LBD enables the PPARs to sense a broad range of fatty acids and their metabolites with relatively low affinity (Kd in the order of 1–10 μM). Polyunsaturated fatty acids activate all three PPAR subtypes with relatively low affinity, whereas fatty-acid derivatives show more selective binding profiles. However, because of their hydrophobic nature, PPAR ligands are most probably delivered to the nucleus and to their receptor by intracellular fatty acid–binding proteins (FABPs).

The leukotriene LTB4, 8(5)-hydroxyeicosatetraenoic acid (HETE), as well as fibrates, such as fenofibrate and gemfibrozil, are PPARα ligands. PPARα is expressed in liver, kidney, intestine, heart, skeletal muscle, brown adipose tissue, adrenal gland, and pancreas and is considered to be a global regulator of fatty acid catabolism. PPARα target genes function together to coordinate the complex metabolic changes necessary to conserve energy during fasting and feeding. In the liver, PPARα upregulates FABP and long-chain acyl-CoA synthetase genes. By increasing β-oxidation, PPARα not only stimulates energy production but also shortens long-chain fatty acids, thus preventing lipid accumulation and toxicity. The mitochondrial HMGCoA synthase is also a target of PPARα and plays a role in the formation of fatty acid–derived ketone bodies, which are used as an alternative energy source for the burning of sugars. Moreover, PPARα upregulates apoA-I and apoA-II and downregulates apoC-III. These effects decrease triglyceride levels and contribute to the beneficial effects of fibrates on lipoprotein levels in hypertriglyceridemic individuals.

Ligands for PPARγ include fatty acids, 15deoxy-prostaglandinJ2 (15d-PGJ2), 15-HETE, 13-hydroxyoctadecadienoic acid, and antiadipogenic drugs, such as the thiazolidinedione rosiglitazone. PPARγ is at the crossroads of obesity, insulin resistance, and cardiovascular disease, since it is a key regulator of adipogenesis, increases the uptake of circulating fatty acids into adipocytes via the activation of lipoprotein lipase, FABP and oxidized LDL receptor 1, and promotes recycling of intracellular fatty acids through upregulation of phosphoenolpyruvate carboxykinase, glycerol kinase, and the glycerol transporter aquaporin 7. PPARγ promotes insulin sensitivity through altering the communication between adipocytes, muscle, and liver cells by inducing the expression of the insulin-sensitizing factor adiponectin.

PPARγ also induces the differentiation of macrophages, which are the effector cells of the innate immune system and combine pathogen-clearing capacities with immune modulatory functions. A dysregulation of the lipid uptake of macrophages can result in massive intracellular cholesterol accumulation, which creates the foamy appearance of macrophages seen in atherosclerotic lesions. PPARγ is involved in the lipid efflux of macrophages, such as reverse cholesterol transport, which prevents atherosclerotic lipid accumulation. Moreover, PPARγ has a role in the regulation of inflammation and immunity through the inhibition of the expression of a number of proinflammatory genes, such as TNF-α, IL-1β, and inducible NO synthase (iNOS). Gene expression profiling studies suggest that the anti-inflammatory actions of PPARγ in macrophages may be relevant to obesity-induced insulin resistance. Adipose tissue macrophages were found to be a major source of inflammatory mediators that are linked to insulin resistance and are subject to counter-regulation by PPARγ. PPARγ also upregulates CYP27A1, which functions as an integrator of the PPAR/LXR cholesterol efflux pathway in macrophages by generating ligands that activate LXRs. Finally, PPARγ is thought to have overall anticarcinogenic effects in many different cell types, due to its antiproliferation, prodifferentiation, and proapoptotic properties.

PPARδ is expressed broadly and has been detected in all of the tissues tested, albeit with varied expression levels. The receptor is required for placental development and is involved in the control of lipid metabolism. The best-characterized function of PPARδ is its role in the control of cell proliferation, differentiation, and survival, especially in keratinocytes. The receptor exerts its antiapoptotic functions through increased expression of ILK and PDK1, which are impor-
tant in signaling pathways that control cell adhesion, proliferation, and survival. ILK and PDK1 phosphorylate and activate the survival factor AKT1. Because all of these proteins are ubiquitously expressed, it is likely that PPARδ also promotes viability in other cell types. PPARδ participates in the regulation of many cell functions that are involved in the development of tumors when uncontrolled. However, the final outcome of PPARδ activation in vivo is impossible to predict on the basis of current knowledge. Indeed, activating PPARδ might have procarcinogenic consequences due to resistance to apoptosis and increased migration properties, as well as antitumorogenic effects derived from its prodifferentiating properties. Both of these would again depend on the particular cell- or tissue-specific context.

Ligands for PPARδ include long-chain fatty acids and carboprostacyclin. Constitutive activation of PPARδ in adipose tissue leads to an improvement in lipid profiles and a reduction of adiposity. PPARδ also has anti-inflammatory properties via the inhibition of COX-2 and iNOS in macrophages and cancer cells. The receptor has been suggested to act as a molecular switch between certain types of pro- and anti-inflammatory contexts that are dependent on interactions with CoRs, such as BCL-6. Since this receptor is widely expressed, PPARδ may also affect lipid metabolism in peripheral tissues.

All three PPAR subtypes recognize DR1-type REs with comparable affinity and nearly identical sensitivity to deviations from the core-binding motif RGGTCA. Whole mouse and human genome expression screens, as well as individual gene analysis, already identified more than one hundred PPAR target genes. However, so far, only a few natural REs for PPARs have been characterized. Moreover, whole promoter/gene screening for PPAR binding in chromatin templates, as performed for VDR target genes, are still missing.

Recently, it was demonstrated that PPARs interact with CoA proteins in the absence of ligands and show high basal activity in reporter gene assays. Therefore, PPARs resemble more the constitutive active receptor CAR than the endocrine NR VDR. A specific PPAR ligand increases the reporter gene activity 2- to 5-fold, whereas VDR agonists show, in a comparable system, a more than 80-fold activation of their receptor. In contrast to endocrine NRs, the position of helix 12 of the PPARs is tightly controlled by four groups of amino acids, so that PPAR agonists may not be able to dramatically improve the interaction of the receptor with CoA proteins. This opens the possibility for the development of new ligands, which may have the properties of antagonists or inverse agonists. The evolutionary perspective to the NR superfamily suggests that the first NRs may have been true orphans, of which a few have acquired ligand binding over time. In molecular terms, this means that during evolution, some orphan NRs have given up the tight control of helix 12 and have learned to control its position primarily by ligand binding.

VI. PXR SIGNALING

The defense against myriad xenobiotics that are ingested in the diet, inhaled, or otherwise absorbed is mediated, to a large extent, by cytochrome P450 enzymes (CYPs) (Fig. 1). These are heme-containing monoxygenases involved in endobiotic and xenobiotic clearance, including the elimination of therapeutic drugs. PXR is expressed predominantly in the liver and is activated by a variety of structurally distinct ligands that are known to induce the expression of CYP genes that are central to drug metabolism. These compounds include phenobarbital, rifampicin, dexamethasone, nifedipine, taxol, and hyperforin (the active agent of the herbal remedy St. John’s wort). As with the PPARs, PXR also has a large ligand-binding pocket, which accommodates various ligands in different orientations. Phase I drug metabolism genes regulated by PXR include several CYPs, such as CYP3A4, CYP2B6, CYP2C8, CYP2C9, and CYP2C19; carboxyl-esterases and dehydrogenases; as well as enzymes involved in heme production and the P450 reaction cycle. Indeed, PXR has been termed the master regulator of the expression of CYP3A4, which metabolizes more than 50% of human prescribed drugs. PXR also controls the expression of the phase II drug metabolism genes encoding UDP-glucuronosyltransferases and glutathione-S-transferases and phase III drug efflux pumps.
such as multidrug resistance 1 and multidrug resistance protein 2. Thus, PXR is an important and efficient regulator of the expression of genes involved in all phases of drug metabolism and excretion. The xenobiotic activation of PXR induces a positive feed-forward loop that aids in the clearance of foreign chemicals and, thereby, resets the xeno-sensor for another round of signaling.

PXR is also activated by a variety of endogenous ligands, including pregnanes, bile acids, steroid hormones, and dietary vitamins (Fig. 1). Activation of mammalian PXR by high concentrations of bile acids, which may accumulate in cholestasis, initiates a coordinated metabolic response to eliminate excess bile acids by the increasing expression of metabolic enzymes and bile acid efflux pumps and decreasing the expression of enzymes involved in bile acid biosynthesis and uptake. Interestingly, lithocholic acid and its metabolites are also activators of VDR, a close relative of PXR in evolutionary terms. Another member of the same NR subfamily is CAR, whose gene probably arose from a duplication of a premammalian xenobiotic-sensing NR gene. CAR does not bind bile acids and responds to a more narrow range of phenobarbital-like inducers than PXR. In addition to overlapping ligand profiles, PXR and CAR also compete for the same type of DNA-binding sites and co-regulators. With such interactions occurring amongst the xeno-sensing NRs, it is perhaps not surprising that their activation often results in the activation of overlapping, but distinct, sets of genes. Examples are the human genes CYP3A4 and iNOS.

Recently, the promoters of the human genes OATP2 and SHP1 were screened in silico and by ChIP assays in hepatocytes for the binding of PXR-RXR heterodimers. Two functional REs were identified for both genes within 10 kb of their promoter sequence. Real-time PCR confirmed the two genes as PXR targets. This study also showed that PXR-RXR heterodimers also occupy their REs in the absence of ligand, which is consistent with a solely nuclear localization for the NR. The SHP protein inhibits CYP7A1 gene activation (whose product is involved in bile acid synthesis) via a protein–protein interaction with the liver homologue receptor, which is another NR involved in the activation of the CYP7A1 gene. Since PXR is also activated by bile acids, this suggests an alternative pathway, in which PXR suppresses the expression of CYP7A1 via increased SHP protein levels. Therefore, the finding that OATP2 and SHP1 genes are primary PXR targets confirms the important role of PXR in bile acid homeostasis in humans.

VII. NUTRIGENOMICS

Micro- and macronutrient-sensing NRs, such as VDR and the PPARs, are central topics in nutrigenomics (Fig. 1). This new discipline attempts to study the genome-wide influences of nutrition. This includes the identification of nutrient-sensing transcription factors and their target genes and the description of gene expression patterns of dietary signals. In metabolically active organs, such as the liver, intestine, and adipose tissue, NRs act as nutrient sensors by modulating the expression levels of their target genes in response to nutrient changes. Since NRs have important roles in the regulation of nutrient metabolism, embryonic development, cell proliferation, and differentiation, nutrients are able to influence a wide array of cellular functions.

Gene-expression profiling can help to identify important genes, proteins, or metabolites that are altered in the pre-disease state and that might, therefore, act as molecular biomarkers of diet-related human diseases, such as type 2 diabetes, obesity, or atherosclerosis. This predisease state is characterized by small metabolic perturbations that slowly progress toward disease. The most specific gene, protein, and metabolite signatures could serve as biomarkers of early dysregulation and susceptibility that are influenced by diet. The metabolic gene network of lipid-sensing NRs transcriptionally regulating metabolizing enzymes and transporters, which in turn use signaling lipids as substrates, represents a positive feed-forward autoregulatory loop to maintain lipid homeostasis. It can be used to study principles of nutrient signaling in normal and disease states and is well-suited to identify appropriate biomarkers.

Nutrigenomics is related to pharmacogenomics, but effects of drugs are that of pure compounds usually being administered in small
doses, whereas food shows high complexity and variability. Nutrients can reach high concentrations (µM to mM) without becoming toxic, and each nutrient can also bind to numerous targets with different affinities and specificities. The molecular structure of micro- and macronutrients determines the specific signaling pathways that it activates. Small changes in structure can have a profound influence, so that closely related nutrients can have different effects on cellular function. For example, the ω-3 polyunsaturated fatty acids have a positive influence on cardiac arrhythmia, and ω-6 unsaturated C18 fatty acids (oleic acid and linoleic acid) decrease plasma levels of low-density lipoprotein cholesterol through specific changes in the expression of genes that are involved in cholesterol metabolism, whereas saturated C16 and C18 fatty acids (stearic acid and palmitic acid) do not show these effects.

Taken together, NRs are exquisitely evolved to manage fuel. Dietary and endogenous fats are handled by PPARs and cholesterol by LXR and FXR. Carbohydrate mobilization is under the direction of GR, whereas TR controls the maintenance of basal metabolic rate. MR in general controls salt concentration, whereas calcium levels are balanced by VDR. Eventually, other xenobiotics are taken care of by PXR and CAR. Finally, since fertility is dependent on nutritional status, it is likely that ER, PR, and AR mediate molecular communication with the above-mentioned relatives in the NR superfamily.

VIII. SYSTEMS BIOLOGY

About 50 years ago, the “one-gene-one-enzyme” hypothesis, which connected genotype and phenotype on a molecular level, was one of the founding principles of molecular biology. At present, we are on the level of annotating the genes that the completion of the human and other genomes provided and characterizing their transcriptional control circuits. With some 25,000 genes, the diversity of the human genome is lower than initially expected. Therefore, we cannot approximate the diversity of functions in a simple “one-gene-one-enzyme” approach. To generate the needed diversity, groups of genes or gene products behave in a coordinated way to perform process-specific networks. The function of these networks, in, for example, embryonic development, depends on multiple factors, such as the tissue-specific expression of gene products, their localization, binding, post-translational modification, and degradation.

Systems biology is the study of the behavior of complex biological organization and processes in terms of their molecular constituents. Therefore, one aspect of systems biology is the development of high-throughput techniques to examine and broadly quantify the level of protein, RNA, and metabolites on a gene-by-gene basis and even the post-translational modification and localization of proteins (Fig. 3). But not every transcriptomics, proteomics, and metabolomics experiment is systems biology. For example, if the purpose of a microarray experiment is to identify a few target genes for an NR and then validate only the “most promising candidates,” this is not systems biology.

The new characteristic of systems biology is its way of thinking, rather than its way of doing. Systems thinking realizes that the phenotype of a system is the emergent property of the interactions among all of the components of the system. Thus, it is neither the scale of the system nor the particular approach used to arrive at a list of its functional components that defines a systems biology approach. In fact, for research driven by this concept to succeed, it may be necessary first to use a reduced system to provide experimentally testable hypotheses. For example, to understand the molecular changes that occur in a cell upon binding of a ligand to an NR, one may first use a well-defined cell culture model, although it doesn’t necessarily reflect the complexity of the in vivo responses. To address this complexity, systems biology is built on molecular biology for its flow of information from DNA via RNA, proteins to metabolites, on physiology for its special concern with adaptive states of the cell and organism, on developmental biology for the importance of defining a succession of physiological states in that process, and on evolutionary biology and ecology for the appreciation that all aspects of the organism are products of selection, which is rarely understood on a molecular level.
IX. NUCLEAR RECEPTORS IN DISEASE

In obesity, adipose tissue becomes inflamed both via infiltration of adipose tissue by macrophages and as a result of adipocytes themselves becoming producers of inflammatory cytokines. Inflammation of adipose tissue is a crucial step in the development of peripheral insulin resistance. In addition, in proatherosclerotic conditions, such as obesity and dyslipidemia, macrophages accumulate lipid to become foam cells. During microbial infection, the inflammatory response defends the body while suppressing appetite and conserving fuel. However, even an ill body is capable of defending itself by releasing adrenal steroids, mobilizing massive amounts of fuel, and transiently suppressing inflammation. In fact, natural and synthetic GR ligands are used primarily as anti-inflammatory agents. Other NRs, such as VDR, PPAR, RAR, and LXR, also protect against inflammation. These receptors have the combined ability to manage energy and inflammation, indicating the important synergism between these two systems.

The duality between inflammatory and metabolic pathways is highlighted by the overlapping biology and function of macrophages and adipocytes in obesity (Fig. 2B). Gene expression of both cell types is highly similar; macrophages express many, if not the majority of "adipocyte" gene products, such as FABP4 and PPARγ, whereas adipocytes can express many "macrophage" proteins, such as TNF-α, IL-6, and matrix metalloproteinases. Inflammatory pathways can be initiated by extracellular mediators, such as cytokines and lipids, or by intracellular stresses, such as endoplasmatic reticulum stress or excess reactive oxygen species production by mitochondria. NRs oppose these inflammatory pathways by promoting nutrient transport and metabolism and antagonizing inflammatory activity. In conditions of overnutrition, this becomes a particular challenge. This commonality between distinct physiologic branches suggests that the NR superfamily should be investigated by systems biology approaches as an intact functional dynamic entity. Recent studies by the NURSA consortium (www.nursa.org) have provided evidence that the NR family should be examined as a whole entity in explaining some phenomena. They have recently examined the expression of all NRs in both macrophages stimulated by the stress ligands lipopolysaccharide and IFN-γ and in adipocytes induced by GR and PPARγ ligands. Remarkably, in both cases, a subset of NRs were readily detectable with some of them, for example, VDR, rising in expression levels at various time points during the induction process. This implies new, presently overlooked roles for these NRs and their ligands. Furthermore, the discovery of increases in expression of certain NRs at intermediate and late time points indicates the importance of a multitude of NRs in the proper execution of complex processes, such as differentiation. These and other observations suggest that larger organizational principles exist on the level of transcription factor factories and of enzyme complexes in the nucleus, the cytoplasm, and membranes that contain NRs or their target gene products. A molecular understanding, how the NR superfamily integrates important physiological aspects, will provide a conceptual basis for the treatment of complex human diseases.

In addition to PPARγ and GR ligands, which are used to treat type 2 diabetes and inflammation, respectively, an enormous pharmacopoeia has been developed to combat disorders that have inappropriate NR signaling as a key pathological determinant. These disorders affect every field of medicine, including reproductive biology, osteoporosis, cancer, cardiovascular disease and obesity (Fig. 2B). Many of these diseases have both aging-related and nutritionally dependent aspects. Therefore, to maintain a normal physiological state, the spatial and temporal activity of NRs must be tightly controlled by tissue-specific expression of the receptors, as well as the availability of their ligands. Over the last decades, obesity has become an epidemic resulting from the unrestricted availability of food to a human population that has become, at the same time, more sedentary. This can be viewed, at the level of nutrient-sensing NRs, as an overabundance of ligands. Because obesity is a major risk factor for other diseases, this free fatty-acid excess also has effects on insulin resistance, hyperglycemia, abnormal blood lipid profiles, and hypertension, all of which contribute to cardiovascular disease, which, ultimately, leads to higher incidences of
stroke and myocardial infarction. In summary, we need to develop multimodal strategies that describe and tackle the role of NRs in sensing components of the diet. Out of this, we anticipate that both preventive measures, as well as effective treatments for acute diseases, will emerge.

X. THE IMPACT OF REGULATORY SNPS

Understanding the genetic basis of disease requires not only a mapping of susceptibility genes but also an analysis of their function in normal and disease phenotypes. Many genes that are essential for critical physiological functions have only small quantitative effects, which are even partially masked or accentuated by other genetic and environmental conditions. Furthermore, small innocuous-seeming effects may amplify themselves over the lifetime of an individual. With this in mind, systematic screening of the human genome for genetic variants has been performed by groups, such as the HapMap consortium. Advances in the fundamental understanding of phenotypic diversity in humans led to the identification of alleles that modify disease risk. The mRNA expression of the two alleles of a given gene is controlled both by cis-acting factors, such as DNA polymorphisms and methylation, in its regulatory region, as well as by trans-acting modulators, such as transcription factors, which themselves may be regulated by other genetic and environmental factors. Heritable expression differences resulting from trans-acting mechanisms appear to be quantitatively more important for the interindividual differences in gene expression, but physiological feedback mechanisms can mask the impact of subtle cis-acting variants on expression levels. Evidence for the medical importance of regulatory SNPs has been provided for common diseases, such as stroke and type 2 diabetes, as well as in individually important genes, such as TNF-α and IL-6.

Most known regulatory SNPs have, so far, been mapped to the promoter regions located 5′-flanking to the TSS. However, since our group and others have detected functional REs elsewhere around genes, intronic sequences and regulatory regions located 3′ of the coding region should also be considered. Moreover, even variations of untranslated exonic sequences can influence mRNA stability or processing efficiency. The classical example of the impact of a regulatory SNP is the common monogenic trait of the lactase persistence phenotype (also called lactose intolerance). Lactase persistence is associated with the T allele in an SNP located approximately 14 kb upstream of the lactase gene. However, even in this straightforward example, epigenetic mechanisms, such as different patterns of cytosine methylation or post-translational histone modifications, can confound the effect of the cis-acting variation.

Allele-specific expression of a transcript can be detected in vitro by monitoring the transcriptional activity of the respective genomic sequence in a transiently transfected reporter construct. However, in vivo monitoring of allelic RNA transcripts in tissues or cells of individuals heterozygous for the respective SNP has the advantage that the alleles are expressed in their normal environment, including genomic and chromatin context, and that the comparison of the alleles is made within the same sample. Current techniques can detect mRNA expression differences as low as 1.2-fold between the alleles. The effects of regulatory SNPs on mRNA expression can be detected by real-time PCR of prespliced heteronuclear RNA or by haplotype-specific ChIP (Haplo-ChIP) for RNA polymerase II association with the respective genomic region (Fig. 3). Genome-wide expression profiling can also be used for the detection of genotype-dependent mRNA expression differences. Parallel in silico screening approaches for transcription factor binding sites carrying an SNP may then permit whole-genome association studies. Indeed, SNPs occurring within a transcription factor binding site, such as an NR RE, show in allele-specific differences in recruiting nuclear transcriptional activators, or repressors in vitro have to be confirmed by HaploChIP assays with samples from living cells or tissues. Moreover, direct allele-specific expression measurements have to prove the correlation of a marker SNP with its effects on the gene expression levels. However, the limiting factor for such a study is often the lack of suitable panels of human tissue samples.
X. CONCLUSION

The nucleus is a cellular compartment that is crucial for coordinating the responses to diverse signals. Receptors in the nucleus, such as the NRs, provide an interesting model to study the specific, as well as the more general, mechanisms of transcriptional regulation; they are highly regulated DNA-binding transcription factors that are directly modulated by ligand binding and can both activate and repress gene expression. This review has explored the concept that NRs, in particular those that sense components of our diet, play an essential role in the regulation of metabolic pathways. The organizational scheme proposed here reveals that NRs function as effective regulators of lipid metabolism by affecting the synthesis of key enzymes that control the intensity, duration, and direction of numerous metabolic steps (Fig. 1). Nutrigenomics should identify the dietary signals, elucidate the dietary sensor mechanisms, characterize the target genes of these sensors, analyze the interaction between these signaling pathways and proinflammatory signaling to search for susceptibility genotypes and find signatures that allow the discrimination of healthy versus unhealthy individuals to enable early dietary intervention. We suggest that a human individual’s susceptibility to aging-related syndromes, such as type 2 diabetes, atherosclerosis, cancer, and osteoporosis, may depend on regulatory SNPs affecting the efficacy of NR signaling, such as the response of the network of PPAR target genes to a life-time exposure of nutritional lipids (Fig. 2).

Nutrient-sensing NRs also regulate pathways of cellular growth and defense. Therefore, NRs are able to integrate various central physiological actions in the human body. NR signaling has to be analyzed by systems biology methodology in terms of (1) changes in chromatin packaging in NR target genes, (2) comparative mRNA expression of NR target genes in various human tissues, (3) analysis of key NR target proteins and metabolites, and (4) physiological consequences of NR signaling (Fig. 3). In this review, we focused on the related NRs VDR, PPARs, and PXR, which will be investigated in a Marie Curie research-training network of 14 research teams (www.uku.fi/nucsys), which is funded by the FP6 program of the European Union.

In future medicine, pharmacological interventions will focus on preventing disease-mediated transitions, as well as reversing or terminating those that have occurred. Specific and selective modulators of NR action—natural, diet-derived, and synthetic ligands, as well as co-regulator-mimicking peptides—will be prime drugs of the future. Their proper development will require a systems biology–based fundamental understanding of what underlies normal biological and pathological processes. Predictive and preventative medicine will lead to a more personalized medicine that will revolutionize health care.

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REFERENCES

11. Rochel N, Wurtz JM, Mitschler A, Klaholz B, Moras...


117. Moore LB, Maglich JM, McKee DD, Wisely B, Willson TM, Kliewer SA, et al. Pregnan X receptor (PXR), constitutive androstane receptor (CAR), and benzoyl X receptor (BXR) define three pharmaco-


131. Dandona P, Aljada A, Bandyopadhyay A. Inflamma-