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# Vitamin A regulation of gene expression: molecular mechanism of a prototype gene

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**Abstract**

Vitamin A regulation of gene expression is a well-characterized example of direct nutrient regulation of gene expression. The downstream metabolites of retinol, all-*trans* and 9-*cis* retinoic acids are the bioactive components that bind and activate their cognate nuclear receptors to regulate target genes. There are multiple retinoid receptor subtypes that are encoded by separate genes and each subtype has different isoforms. These receptors are Class II members of the thyroid/retinoid/vitamin D superfamily of nuclear receptors. The characterization of the retinoid receptors and the DNA response elements of target genes that bind these receptors have vastly expanded our knowledge of the mechanism of retinoid regulation of target genes. The basic regulatory mechanism of retinoids interacting with their cognate receptors is further complicated by the interaction of coactivators and corepressors, nuclear proteins that are involved in activation or repression of transcription, respectively. Most of these coregulators are involved in modifying chromatin and nucleosome structure such that chromatin is relaxed or condensed, and in bridging between the upstream enhancer domains and the transcription preinitiation complex. Retinoid regulation of the rate of transcription of target genes and the duration of the retinoid response is further complicated by covalent modification of the retinoid receptors by phosphorylation involved in coactivator association and ubiquitinylation involved in the degradation of retinoid receptors. This review presents a prototype retinoid responsive gene that encodes the phosphoenolpyruate carboxykinase (PEPCK) gene as an example of a specific mechanism of retinoid regulation of a metabolic gene. The retinoid response elements and overall mechanism of retinoid regulation of the PEPCK gene have been well documented by both in vitro and in vivo methods. We provide detailed information on the specific nuclear receptors, coactivators and chromatin modification events that occur when vitamin A is deficient and, therefore, retinoids are not available to activate the nuclear retinoid-signaling cascade.
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## 1. Introduction

The research link between molecular biology and nutrition is producing new insights into how nutrients directly regulate gene expression. Advances in the area of vitamin A, or retinoid, regulation of gene expression provide one of the best examples of the productivity of this combined bionutrition approach. Vitamin A and its bioactive metabolites have pleiotropic effects in all tissues of vertebrate organisms involving cellular development, proliferation, differentiation, metabolism and apoptosis. The widespread nature of the bioactivities of retinoids is due, in large part, to the ability of retinoids to regulate the expression of target genes. Historically, our understanding of the molecular mechanism of retinoid action was rapidly advanced when the nuclear retinoid receptors were cloned and characterized, and the consensus DNA retinoid response elements in target genes identified [1–3]. This has led to a proliferation of information on the mechanism of action of the downstream metabolites of retinol, all-*trans* and 9-*cis* retinoic acid (RA), which are the active retinoids involved in regulating gene expression in the nucleus of the cell. This review will cover the general state of knowledge regarding

*Abbreviations:* CBP, (cAMP response element binding protein)-binding protein; ChIP, chromatin immunoprecipitation; COUPTF, chicken ovalbumin upstream promoter transcription factor; HAT, histone acetyltransferase; HNF, hepatic nuclear factor; K, lysine; PEPCK, phosphoenolpyruvate carboxykinase; PGC, peroxisome proliferator-activated receptor-γ coactivator-1; PIC, preinitiation complex; PPAR, peroxisome proliferator-activated receptor; RA, retinoic acid; RAR, retinoic acid receptor; RARE, retinoic acid response element; RNA Pol II, RNA polymerase II; RXR, retinoid X receptor; SRC-1, steroid receptor coactivator-1; VAD, vitamin A deficient; VAS, vitamin A sufficient.

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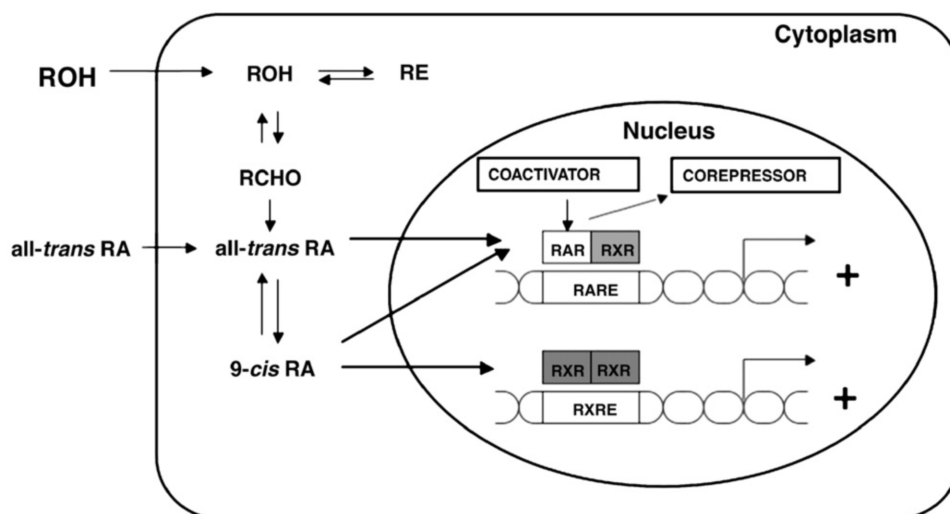


Fig. 1. Intracellular retinoid metabolism, transport, and nuclear action.

retinoid regulation of gene expression and describe a model gene to provide, in detail, a molecular mechanism of retinoid regulation of a specific gene. The prototype gene is the well-characterized retinoid-responsive gene encoding the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK). The PEPCK gene is presented as a prototype gene and may represent the molecular mechanism by which retinoids regulate the expression of numerous other metabolic genes that have been shown to be responsive to retinoids.

## 2. Nuclear retinoid receptor pathways

The bioactive retinoids all-*trans* and 9-*cis* RA are ligands that bind and activate cognate retinoid receptors, and these receptors, in turn, function as transcription factors that regulate the expression of target genes. The general course of retinoid metabolism, transport and nuclear action is diagramed in Fig. 1. In the nucleus there are two distinct classes of retinoid receptors, the retinoic acid receptors (RAR) and retinoid X receptors (RXR). Both the RAR and RXR bind the naturally occurring metabolites of vitamin A with a similar order of affinity (all-*trans* RA>retinal>retinyl acetate>retinol), but RXR requires from 10- to 40-fold more ligand for comparable activation [4]. A downstream metabolite of all-*trans* RA, 9-*cis* RA, has been shown to be a naturally occurring, high-affinity ligand for the RXR [5]. There are multiple RAR and RXR subtypes — $\alpha$, $\beta$ and $\gamma$ — that are encoded by separate genes at distinct chromosomal loci, and each receptor subtype has different isoforms generated by alternate splicing and differential promoter usage of the individual gene [6]. In addition to the multiplicity of RAR and RXR genes and their respective mRNA isoforms, their biological activity is further extended by interaction of the retinoid receptors with other nuclear receptors (NRs). The RXRs serve as heterodimeric partners for RARs, thyroid hormone receptors (TRs), vitamin D receptors (VDRs), the peroxisome proliferator-activated receptor (PPAR), the chicken ovalbumin upstream promoter-transcription factor (COUPTF), farnesoid X-activated receptor, liver-X receptor and other characterized NRs [7–11]. The RXR can also function as a homodimer dependent on the 9-*cis* RA ligand [12]. Many of the ligands for the NRs that heterodimerize with RXR are products of lipid metabolism, such as fatty acids, leukotrienes, prostaglandins and cholesterol derivatives [13]. RARs and RXRs have conserved modular structures, particularly in the DNA binding and ligand binding domains, which define, in part, their membership in the nonsteroid, thyroid/retinoid/vitamin D superfamily of NRs. These are Class II NRs. Class II receptors are defined as NRs that heterodimerize with RXR and bind to direct repeat (DR) elements in target genes [4]. Recently, Desvergne et al. [14] subdivided RXRs into a subcategory defined as “metabolic sensors” referring to NRs that have been shown to respond to, and transmit the signal of, alterations in metabolite concentrations. Class II NRs are characterized by a variable NH2-terminal region (A/B) which has a ligand-independent activation function (AF1); a conserved DNA binding domain (C) consisting of two zinc fingers required for DNA recognition and binding [15–17]; a hinge region (D); and a multi-functional ligand-binding domain (LBD) (E) consisting of a conserved carboxy terminal motif required for ligand-dependent transactivation (AF2), homo- and heterodimerization, and the association of coactivator and corepressor proteins [18–20].

The classic retinoid response element of a target gene is a direct repeat of the motif 5′-PuG(G/T)TCA-3′ spaced by 1, 2 or 5 base pairs (DR1, DR2 and DR5, respectively) [1,21,22]. The DR2 and DR5 elements preferentially bind RXR-RAR heterodimers with the RXR monomer binding the 5′ motif. Whereas the DR1 binds RXR-RAR heterodimers with the reverse polarity, i.e., RXR binding the 3′

motif, and this can be inhibitory [2,3,23]. DR1 elements also bind RXR as a homodimer and, therefore, provide an RXRE [24]. The DNA binding domains of each NR of a dimer pair determine the specificity of DNA response element recognition and binding affinity to the respective DR elements.

## 3. Coactivators and corepressors associated with retinoid receptors

It has been established that RARs are ligand-dependent transcription factors, having the ability to repress transcription in the absence of ligand. Ligand binding induces conformational changes in helix 12 of the AF2 domain of RARs [25] providing a hydrophilic surface for association with a diverse group of nuclear proteins referred to as coactivators [26]. To date, numerous functional groups of coactivators have been characterized. The p160 family is a large coactivator family with three distinct members, each with splice variants, all of which interact with retinoid receptors. For simplicity, the three p160 members can be identified as steroid receptor coactivator 1 (SRC-1) (includes SRC-1/NCoA-1), SRC-2 (includes TIF-2/GRIP-1/NCoA-2) and SRC-3 (includes pCIP/ACTR/AIB1/TRAM1/RAC3) [13,27,28,29]. The SRC coactivators act in a ligand and AF2-dependent manner and bind to both nonsteroid and steroid NRs at the AF2 domain of each monomer of an NR dimer. The SRC proteins have a series of conserved motifs, LxxLL, referred to as NR boxes in the receptor interaction domain that are involved in binding of the coactivator to retinoid receptors [30]. Ligand, or agonist, binding to either RAR or RXR of a RAR-RXR heterodimer initiates the recruitment of an SRC coactivator to the hydrophobic LBD of either receptor. Indeed, a single SRC coactivator can bind to the hydrophobic LBD of each of both RAR and RXR in a RAR-RXR heterodimer via two NR boxes, in the presence of ligand for both RAR and RXR [31]. The SRC coactivators have been shown to have histone acetyl transferase (HAT) activity. SRC coactivators have the ability to acetylate core histones in nucleosomes, thereby relaxing the chromatin structure and providing a more accessible DNA for transcription initiation [32,33].

Another category of coactivators are referred to as cointegrators, of which the E1A-associated 300 kDa protein (p300), a homologue of CREB binding protein (CBP), designated CBP/p300 ([34,35], is an example. CBP/p300 is a large nuclear protein that can interact with both upstream enhancers and the basal transcription machinery at the start site of transcription [36]. CBP/p300 also has been shown to have HAT activity [32,37]. A central role has been proposed for CBP/p300 as an “integrator” of multiple signaling pathways because CBP/p300 is required for *trans*-activation of target genes by CREB, AP-1 and the NRs [38]. Also in this category is the murine mediator multiprotein complex, related to the yeast mediator that reversibly recruits the RNA polymerase II holoenzyme [39].

Another functional group of coactivators is represented by SWI/SNF (first identified in yeast as a mating-type switching gene) [40]. SWI/SNF is a 2-MDa, 12 subunit multi-protein complex that functions as an ATP-dependent chromatin remodeling complex [41–44]. Chromatin remodeling allows for the subsequent binding of coactivators with HAT activity, as well as the association of the general transcription machinery [45,46], as described in more detail in the following section.

Countering the action of coactivators are corepressors, several of which have been identified related to retinoid signaling [47–51]. The nuclear receptor corepressor (NCoR) and the silencing mediator for retinoid and thyroid hormone receptor (SMRT) were initially identified as highly homologous corepressors of unliganded thyroid hormone receptor (TR) [48,52]. Both proteins have four autonomous repression domains and inhibit transcription of target genes by binding to the ligand binding domain of NRs, such as RAR and RXR [53]. The binding of corepressors alters the AF2 domain of NRs and recruits a large protein complex containing histone deacetylases (HDAC) to a target gene [54–56]. Current studies indicate that NCoR associates directly with HDAC3, HDAC4 and HDAC5, whereas SMRT associates with HDAC5 and HDAC7 or with HDAC3 [57]. The HDAC activity causes local histone deacetylation and the resultant hypoacetylated chromatin is more condensed and restricts access of transcription factors and the general transcription machinery to the DNA template [58,59].

In addition to the above coactivators and corepressors, another type of coactivator has been identified that is unique to the retinoid receptor nuclear complex, the cellular retinoic acid binding protein II (CRABPII). CRABPII has been shown to function as a nuclear coactivator by Delva et al. [60], thereby extending the known function of CRABPII. CRABPII binds to RAR and RXR in the nucleus and enhances RA-mediated *trans*-activation of target genes. It should be noted, however, that CRABPII is unlike other coactivators because it is a ligand-binding protein, it interacts with retinoid receptors only and it does not have structural homology with other coactivators. The novel role of CRABPII may be that it regulates the nuclear availability of ligand to the retinoid receptor, rather than functioning as a classic coactivator. Indeed, Noy et al. [61,62] have shown that the CRABPII–RAR complex is a short-lived intermediate that provides for “channeling” of small lipophilic ligands such as all-*trans* RA to RARs.

## 4. Chromatin structure and transcriptional activation

In order to understand vitamin A regulation of gene expression, it is important to note that any target gene is present within the complex structure of chromatin. Chromatin can be tightly packaged, or condensed, or it can take on a relaxed configuration at sites in the DNA that are being actively transcribed. The individual unit of chromatin is the

nucleosome that is made up of the DNA helix making 1.65 turns around a histone octomer [63–65]. The histone octomer is made up of pairs of histones: H2A, H2B, H3 and H4. H3 and H4 make up the core tetramer with H2a and H2B dimers flanking the H3–H4 tetramer [44]. A well-characterized mode of covalent modification of core histones is that of acetylation, via the action of coactivator HAT activity, as described above. The N-terminal tails of histones in the core octamer of nucleosomes are acetylated [66–68]. Acetylation neutralizes the charge of the histones, thus loosening the binding of histone proteins to negatively charged DNA and the nucleosome to nucleosome association, relaxing the chromatin structure at the site of acetylation.

In the case of retinoids, this series of molecular events is induced when RA ligand binds to cognate retinoid receptors; this causes the corepressors to dissociate and coactivators to be recruited. Most coactivators have either intrinsic HAT activity or are associated with HATs (or, in some cases, methyltransferases). Coactivators also include cointegrators, such as the murine mediator complex, and ATP-dependent chromatin remodeling complexes, such as SWI/SNF, that are recruited to the promoter site of a target gene [69]. The sequence of binding events may vary depending on the signaling mechanism and/or the target gene; however, in combination, coactivators with HAT activity, cointegrators and chromatin remodeling complexes decompress chromatin structure and activate transcription initiation. Chromatin remodeling involves the repositioning of nucleosomes in the chromatin by sliding them further along the DNA (*cis*-displacement) or displacing the nucleosome to another DNA strand (*trans* displacement). Eventually, the early-stage coactivators themselves dissociate and chromatin remodeling allows retinoic acid receptors to recruit a murine mediator complex, the SRB- and mediator-containing complex (SMCC) [70]. The mediator complex was first identified in yeast and subsequently shown to have a mammalian counterpart, the SMCC complex. This mediator is a 20-subunit complex that facilitates the entry of RNA polymerase II (Pol II) to the promoter transcription start site at the TATTA box, thus facilitating the formation of the transcription preinitiation complex (PIC) that determines the rate of transcription of a eukaryotic gene.

## 5. Sequential molecular events

It has been determined that the above processes are interdependent and occur in a temporally specified sequence for any given target gene, although the sequence is not the same for every gene. For retinoid signaling, they have shown that unliganded RAR-RXR heterodimers are bound in weak association with a cognate RARE, and with the addition of ligand a tighter binding is achieved through the recruitment of the ATP-dependent SWI/SNF complex that directs nucleasome remodeling and relaxation of the chromatin structure, as indicated above. This is followed by recruitment of coactivators with HAT activity/association. In subsequent steps, the liganded RAR-RXR with associated coactivators recruits general transcription factors, the TFIID complexes and RNA Pol II to form the transcription PIC. The last step of RNA Pol II association is dependent upon the large multisubunit SMCC complex [39,43,71]. A subunit of the SMCC complex, TRAP220 (or DRIP205), binds to the AF-2 domain of ligand-bound retinoid receptors [39,72]. It is postulated that the SMCC complex may either recruit RNA Pol II directly via binding the carboxy terminal domain (CTD) of RNA Pol II or associate at an RARE as a preformed SMCC–RNA Pol II (holoenzyme) complex [43,71]. The recruitment of RNA Pol II and the completion of the transcription preinitiation complex initiate transcription of the retinoid-target gene, and the rate of this complex formation determines the overall rate of transcription of the gene.

## 6. Phosphorylation of retinoid receptors

The molecular mechanism of retinoid action is further complicated by covalent modification of retinoid receptors by phosphorylation/dephosphorylation in response to numerous signaling pathways [73–75]. For example, signaling pathways initiated by mitogen-activated protein (MAP) kinases, phosphatidyl inositol 3-kinase (PI3K) and cyclic AMP-dependent protein kinase (PKA) are involved in the control of retinoid-regulated transcription. RARs are phosphorylated in the ligand binding domain (LBD) (or E domain) at serine residues by PKA [76], and RXRs are phosphorylated in the LBD by MAP kinases at both tyrosines and serines [77,78]. Phosphorylation sites are also found in the A/B domains, predominantly at serine residues, and also, to a lesser extent, in the F domain, although the F domain is not present in RXRs and is of unknown function in RARs. Coregulators such as the Silencing Mediator of Retinoic acid and Thyroid hormone receptor (SMRT) corepressor are also phosphorylated [79]. Phosphorylation may modulate the ability of retinoid receptors to recruit coactivators and/or affect corepressor association, as well as general transcription factor association [80]. Phosphorylation, in addition to assisting in *trans*-activation of target genes, may also be involved in subsequent degradation or nuclear export of retinoid receptors [75].

## 7. Retinoid receptor degradation: the ubiquitin–proteasome system

A final critical step in the molecular mechanism of retinoid action is the degradation of the retinoid receptors, which effectively “turns off” the retinoid signal. Targeted degradation of RARs and RXRs is accomplished through the ubiquitin–proteasome system [81–85]. Retinoid receptors are ubiquitinylated at multiple sites and then are targets for destruction by the 26S proteosome [86–89]. Overall, degradation determines the duration of the retinoid receptor

response. The above-mentioned phosphorylation of retinoid receptors is part of the ubiquitin-mediated degradation process. For example, the AF-1 domain of the RARγ gene is phosphorylated at two serine residues in response to different kinases. Phosphorylation of these serine residues serves as a checkpoint controlling both RARγ *trans*-activation capacity and RARγ degradation by the 26S proteasome. It is postulated that phosphorylation assists in the association/disassociation of coregulators and also allows for the recruitment of a ubiquitin ligases. Therefore, phosphorylation may regulate both *trans*-activation by and the degradation of RARγ through first increasing the association of coactivators and, subsequently, increasing RARγ ubiquitylation [87]. This has only been shown for RARγ, however, and is one model of many possible mechanisms that may relate phosphorylation and ubiquitinylation. Another model is that phosphorylation induces the dissociation of inhibitors bound to retinoid receptors, thereby making the nearby lysine residue available for ubiquitin ligases recruited at the AF-2 domain [75]. Recently, Zimmerman et al. [90] have shown that RXR export from the nucleus can also be regulated. Upon inflammation-mediated signaling, RXRα is targeted for nuclear export and degradation [90]. This mechanism may explain the decrease in expression of RXR-dependent genes in the inflamed liver. Furthermore, ligand or agonist binding to retinoid receptors leads to retinoid receptor degradation via the ubiquitin/proteosome pathway. Osburn et al. [91] have shown that the half-life of ligand-bound RXR is half of that in the absence of ligand. In addition, the binding of ligand or agonist to RAR, TR or RXR leads to increased degradation of both NRs of the dimer pair.

## 8. Retinoid receptor cross-talk with other NRs: the role PPARs

Closely related members of the nuclear receptor super family are the PPARs that form heterodimers with RXRs. Three isoforms of PPAR, designated α, β and γ, have been cloned and characterized [92–94]. The classical peroxisome proliferator-inducible pathway, that of peroxisomal β-oxidation [95–97], utilizes very long fatty acids as substrates, and these very long chain fatty acids, in turn, are the naturally occurring ligands of PPARα, the predominant receptor isoform in liver [92]. Like other NRs, PPARs possess a highly conserved DNA binding domain, with two zinc fingers, that recognizes peroxisome proliferator response elements (PPREs) in the promoter regions of target genes. After ligand binding, PPARs heterodimerize with RXR, and the PPAR-RXR heterodimers bind to DNA sequences containing direct repeats of the hexanucleotide sequence AGGTCA separated by one nucleotide, i.e., DR-1 response elements. PPARs are expressed in all tissues and are activated by a variety of synthetic ligands, such as synthetic peroxisome proliferators, thiazolidinedione antidiabetic drugs, fibrate hypolipidemic drugs and endogenous ligands such as polyunsaturated fatty acids (PUFAs) and eicosanoids [98,99]. PPARα is a major target of PUFA-regulated gene expression and PUFAs induce the expression of the genes of hepatic fatty acid oxidation, mediated by PPAR activation. Interestingly, recent evidence shows that fatty acids are also RXR ligands and that PUFAs may serve as endogenous RXR ligands, as well as 9-*cis* RA [100,101]. Indeed, it has been shown that very long chain PUFAs, in particular DHA, can activate RXR as a heterodimer with RAR. Therefore, there is a high degree of cross talk between RARs, RXRs and PPARs, and the potential for shared ligand activation and reciprocal signaling effects.

## 9. PPAR and RXR signaling

It is well documented that n-3 and n-6 PUFAs are activators of PPARα in liver and stimulate fatty acid oxidation [102]. PPARα was the first transcription factor identified as a nuclear receptor activated by endogenous PUFAs. Additionally, 9-*cis* RA can act as a single ligand to induce the PPAR-RXR heterodimer [1]. In fact, Yang et al. [103] have shown that the coactivator SRC-1 is recruited to PPAR-RXR only in the presence of 9-*cis* RA, not PPAR ligand. Additionally, RXR-RXR homodimers are able to bind PPREs within PPAR-responsive genes and induce expression in response to 9-*cis* RA rather than PPAR ligands, as demonstrated for the malic enzyme (ME) gene PPRE [104]. As well, the RXR-RXR homodimer can substitute for PPAR-RXR at PPREs in target genes such as ME and regulate downstream metabolic pathways [104]. Therefore, rexinoids, specifically 9-*cis* RA, can drive the PPAR signal independent of PPAR ligands, or 9-*cis* RA can drive the RXR-RXR homodimer to activate a PPRE, independent of PPAR itself.

## 10. A prototype retinoid-responsive gene: the phosphoenolpyruvate carboxykinase (PEPCK) gene

A listing and categorization of retinoid-responsive genes cited 532 RA-target genes that were categorized based on evidence of direct, classic retinoid signaling versus indirect RA regulation [105]. Of the 532 genes, the gene encoding phosphoenolpyruvate carboxykinase (PEPCK) was listed in the category of direct, classic RA-targeted genes, where the evidence of RA regulation was indisputable. Therefore, we will present the mechanism of RA regulation of the PEPCK gene as a model, or prototype, of genes that are directly regulated by RA. The PEPCK gene provides a useful model as it is exemplary of the pleiotropic effects of retinoids. In this case, RA regulates a gene that encodes a protein that is an enzyme of carbohydrate metabolism and not involved in retinoid absorption, transport, metabolism or degradation.

The gene for PEPCK encodes a rate-determining enzyme in the gluconeogenic pathway in liver, and, as such, it is a differentiation marker for hepatic parenchymal cells. The PEPCK gene is expressed in a tissue-specific manner, it is

restricted to a specific developmental time course and it is subject to complex hormonal and nutrient regulation [106–113]. All-*trans* RA and 9-*cis* RA, at equal concentrations, induce *trans*-activation of the PEPCK promoter by approximately sevenfold in H4IIE hepatoma cells [114–116]. The first region of the PEPCK promoter to be defined as a minimal RARE (−451/−433) (RARE1) has sequence homology to the RAREs of both laminin B1 and the RARβ genes [114]. A second direct repeat RARE (RARE2) has been identified between −337 and −321 and shown to bind the RXRα-RARα heterodimer in vitro [117]. Since RARE2 competes for the binding of RXRα-RARα more efficiently than the RARE1, Scott et al. [117] suggest that the response of the PEPCK gene to RA could be mediated primarily by RARE2. There is a third upstream RARE from −1003 to −987 (RARE3) that binds RXRα-PPARγ from adipose tissue [118].

In vivo, it has been demonstrated that vitamin A deficiency and all-*trans* RA supplementation decrease and increase respectively the expression of the endogenous PEPCK gene in mouse liver [119]. These studies showed the physiologic relevance of retinoid regulation of the PEPCK gene. Under conditions that induce the hepatic PEPCK gene, i.e., food deprivation or cAMP treatment, the PEPCK gene is not induced in the vitamin A-deficient (VAD) mouse. Inhibition of the PEPCK gene by vitamin A deficiency is reversed by all-*trans* or 9-*cis* RA treatment to a similar extent. By comparison, PEPCK transgenes, driven by either a −460 or a −355 bp PEPCK promoter sequence, are inhibited by vitamin A deficiency, but are differentially induced by retinoids, indicating that RARE1 is required for the 9-*cis* RA response and RARE2 is sufficient for the all-*trans* RA response of the PEPCK gene in mouse liver [120].

## 11. Effect of vitamin A status on NR and coactivator association with the PEPCK RAREs

Examination of the direct interaction of NRs with RAREs within the PEPCK 5′ flanking sequence indicates that both retinoid receptors and other NRs bind the PEPCK DNA elements; HNF4α and RXRα are the major nuclear receptors that bind RARE1, and RXRα, RARα and COUPTFII are the major nuclear receptors that bind RARE2 [120]. Taken together with the differences in retinoid responsiveness of the PEPCK(460) and PEPCK(355) transgenes, all-*trans* RA signals are transmitted via RARα-RXRα activation and binding to PEPCK RARE2, while 9-*cis* RA signals are transmitted via RXRα-RXRα activation and binding to RARE1, with both retinoid isomers maintaining activation of the PEPCK gene. Binding of orphan receptors HNF-4α and COUPTFII at RARE1 and RARE2 sites, respectively, is a potential competitor of retinoid signaling.

In addition to the above NRs that bind PEPCK RAREs and are involved in retinoid regulation of the PEPCK gene, PPARα also binds to the PEPCK RAREs. There is a reduction in PPARα binding in VAD liver, especially at RARE3 [121]. PPAR-RXR heterodimers are capable of activating target genes, including PEPCK, in response to PPARα-specific ligands and to both 9-*cis* and all-*trans* RA [98,122]. Of particular interest related to PEPCK gene expression is the fact that PPARα is required for the cellular response to fasting in liver, both in terms of fatty acid oxidation and gluconeogenesis [123,124]. In PPARα$^{-/-}$ mice, the normal increase in hepatic fatty acid oxidation due to fasting does not occur and genes encoding enzymes of mitochondrial fatty acid oxidation are not induced [125]. Scribner et al. [121] have shown that, in the absence of retinoids, PPARα binding at PEPCK RARE3 is significantly diminished under the same conditions that PEPCK gene expression is decreased. This should decrease gluconeogenesis, due to decreased PPARα binding and activation of PEPCK gene transcription. Therefore, both metabolic responses to fasting, increased fatty acid oxidation and increased gluconeogenesis, are impaired, in part, by a defect in the retinoid-PPARα signaling mechanism.

The decrease in PPARα binding to PEPCK RAREs in VAD liver is amplified by, and correlated with, a decreased PPARγ coactivator (PGC)-1α association, most notably at RARE3 of the PEPCK promoter/regulatory domain [126]. A decrease in both PPARα and PGC-1α binding to the PEPCK RARE3 is consistent with the spring-trap model proposed by Spiegelman et al. [127,128] in which PGC-1α "docking" at PPARγ is required for PGC-1α's ability to coactivate genes and for its subsequent binding to coactivators CBP and SRC-1 in complex formation. Therefore, a major component of the mechanism of the VAD-induced decrease in PEPCK gene expression may be the diminution in PPARα association at the PEPCK RAREs and, subsequently, decreased docking of PGC-1α, leading to decreased complex formation including association of CBP and/or SRC-1. The decrease in complex formation would result in decreased localized histone acetylation and impaired bridging between RARE3 and the PIC at the start site of transcription. It has been suggested that at the PEPCK promoter, PGC-1α functions through both PPARα and HNF-4α [129]. In vivo experiments show that PGC-1α association with PPARα occurs at RARE3 and that this is decreased in VAD liver [126]. Furthermore, PGC-1α association with HNF-4α occurs at RARE1 and this binding is also potentially decreased in VAD liver [126]. Hypothetically, the decrease in PGC-1α association at both RARE1 and RARE3 contributes to a decrease in formation of the complex with CBP and SRC-1 and, therefore, decreased histone acetylation and bridging between RARE1 and RARE3 and the PIC, with a subsequent decrease in transcription rate of the PEPCK gene.

## 12. Effect of vitamin A status on histone modification in the PEPK promoter/regulatory domain

Covalent modification of nucleosomal histones in the PEPCK promoter/regulatory domain is also affected by RA

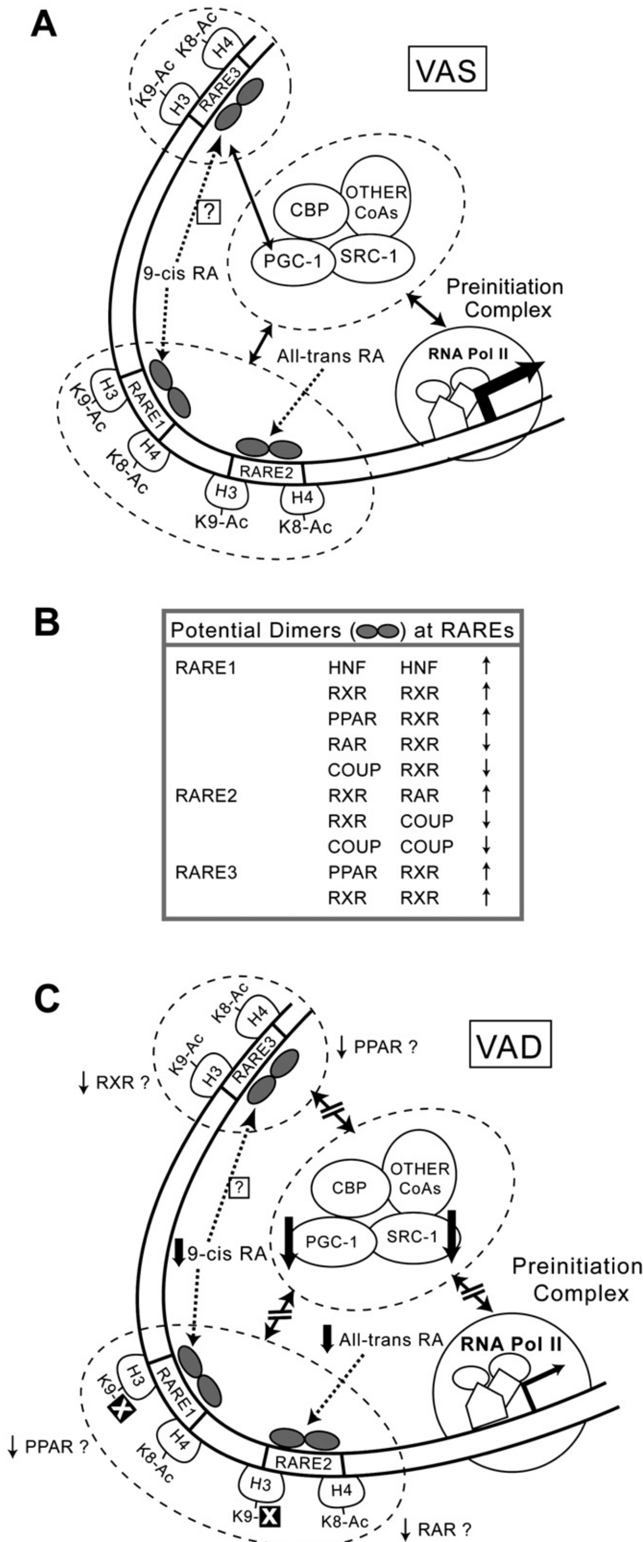


Fig. 2. Model for retinoid regulation of the PEPCK gene.

status in liver. Coactivator association and the acetylation of specific lysines on histones H3 within the core octamer of the nucleosome are changed with vitamin A deficiency in mouse liver. Histones H3 and H4 are acetylated at RARE1, RARE2 and RARE3 of the mouse PEPCK promoter in vivo, with measurable differences in H3 acetylation in VAD mice. Of the lysine residues of the core histones, H3 lysine 9 and H4 lysine 8 are acetylated, while H3 lysine 14 and H4 lysines 5, 12 and 16 are not [126]. Selective acetylation of lysine residues of core histones, rather than global acetylation of all histones in an activated gene, is consistent with the concept of an inherent “histone code” in chromatin that is both tissue- and gene-specific [59,130,131]. This program is thought to be reliant upon native chromatin structure, dictated by association with highly specialized coregulator proteins and dependent upon other post-translational histone modifications [132–135]. Thus, the acetylation of H3 lysine 9 and H4 lysine 8 is part of the histone code for PEPCK gene expression, and acetylation/deacetylation of H3 lysine 9 is dependent on vitamin A status in liver.

Therefore, decreased PEPCK gene expression in vitamin A deficiency is also partly explained by the decrease in H3 lysine 9 acetylation localized to RARE1/RARE2, which contributes to decreased chromatin relaxation. The specific mechanism that causes decreased H3 lysine 9 acetylation in VAD mouse liver has not been fully elucidated. In vitro studies have identified many coactivators with HAT activity that have the ability to acetylate lysine residues on multiple histones, but it is still not clear if these in vitro results correspond to what occurs in native chromatin in vivo related to individual histone acetylation patterns of specific genes [32,136–138]. However, studies using free histones and isolated mononucleosomes have shown that SRC-1 preferentially acetylates H3 lysines 9 and 14 [136,139]. Assessed by the in vivo chromatin immunoprecipitation (ChIP) assay, it has been shown that there is a decrease in SRC-1 binding to the PEPCK RARE1/RARE2 domain. This, in turn, may be the cause of the decrease in H3 lysine acetylation at PEPCK RARE1RARE2 [126].

CBP also has HAT activity and exhibits a substrate preference for specific lysines in histones. H4 lysine 8 serves as a substrate for acetylation by CBP in vitro [32,137,138]. As indicated above, H4 lysine 8 is acetylated at the PEPCK promoter in vivo, with no alteration in lysine 8 acetylation in VAD mouse liver. This is consistent with CBP being constitutively associated with the PEPCK promoter, regardless of changes in vitamin A status. Potentially, acetylation of H4 lysine 8 has a broader purpose for maintaining basal transcription of the PEPCK gene, whereas H3 lysine 9 acetylation is linked to changes in acute transcriptional regulation. Because CBP can also acetylate HNF-4α [140], it is possible that CBP participation in PEPCK gene regulation includes functionally relevant acetylation of nonhistone proteins that bind the PEPCK RAREs.

## 13. Molecular model of vitamin A (or retinoid) regulation of the PEPCK gene

A model of vitamin A regulation of the PEPCK gene in mouse liver is shown in Fig. 2. Highlighted are the PEPCK

RAREs and the NR hetero- and homodimers that have been shown, by both in vitro and in vivo methods, to bind the respective RAREs (Fig. 2A and 2B). Also indicated are the coactivators that have been shown by in vivo ChIP assay to bind to specific PEPCK RAREs in mouse liver. Potential complex formation amongst the coactivators and bridging between the RARE domains and the PIC, as well as RNA Pol II association, is also shown. The sites of histone H3 and H4 acetylation within the RARE1/RARE2 proximal domain and the RARE3 distal domain are indicated. In VAD liver, decreased hepatic PEPCK gene expression occurs under the same conditions where there is a measurable decrease in association of RNA Pol II at PEPCK RARE1/RARE2 in vivo [141]. Subsequent reassociation of RNA Pol II at the PEPCK promoter occurs with the administration of physiologic doses of both all-*trans* and 9-*cis* RA and mirrors the increase measured in PEPCK mRNA under the same conditions. Duong et al. [142] have also shown in vivo association of RNA Pol II with the PEPCK promoter associated with coactivator binding. Therefore, all-*trans* and 9-*cis* RA, in combination, activate PEPCK gene transcription, mediated through differential regulation of RAREs 1–3 [141], although not necessarily via retinoid receptors alone, resulting in increased association of RNA Pol II and PIC formation. Vitamin A deficiency decreases PEPCK gene expression by decreasing both SRC-1 association at the RARE1/RARE2 domain and PPARα and PGC-1α association at RARE3 and potentially RARE1/RARE2 (Fig. 2C), therefore disrupting components of the coactivator complex that are involved in histone acetylation and bridging the RAREs with the transcription start site. The decrease in SRC-1 binding at PEPCK RARE1/RARE2 constitutes a decrease in localized HAT activity, consistent with a decrease in histone H3 lysine 9 acetylation at RARE/RARE2. The decrease in SRC-1 association could also potentially weaken the bridge between the proximal RAREs and the PIC. Decreased PPARα association, leading to decreased PGC-1α recruitment, also contributes to weakening the multiprotein complex by PGC-1α's absence and decreasing recruitment of other accessory proteins that bridge the RAREs with the PIC. Overall, the combined effect in VAD liver is decreased coactivator association and, therefore, decreased localized histone acetylation, resulting in decreased association of RNA Pol II, decreased PIC formation and a decreased rate of transcription of the PEPCK gene.

## 14. Conclusions

Taken together, the PEPCK gene provides a useful prototype for examining the detailed molecular mechanism of vitamin A, or RA, regulation of eukaryotic genes. In particular, the PEPCK gene may most accurately represent metabolic genes that share a similar mechanism. As shown here, both in vitro and in vivo results are consistent and describe a complex interplay of both all-*trans* and 9-*cis* RA binding to, and activation of, RAR, RXRs and, potentially, PPARs, that interact with specific RAREs in the promoter domains of the PEPCK gene. The RAREs have been identified in specific domains of the PEPCK gene and have different DR configurations and, potentially, tissue- and RA isomer specificity.

In vivo, vitamin A deficiency induces a series of changes in the binding of NRs, primarily PPARα, to the PEPCK promoter, with more significant changes in coactivator binding and core histone acetylation with correlated changes in RNA Pol II association. To date, there is no information on the mechanism of vitamin A regulation of the PEPCK gene related to the roles of large multisubunit proteins such as the murine mediator SMCC or the SWI/SNF complex. Nor is there current information on the role of phosphorylation, ubiquitinylation and degradation of retinoid receptors related to retinoid regulation of the PEPCK gene. Although significant strides have been made in the full characterization of retinoid regulation of PEPCK gene expression in liver, much remains to be done to fully characterize the complex molecular events of vitamin A regulation of this prototype metabolic gene.

## References

[1] Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. Cell 1995;83:841–50.
[2] Chambon P. A decade of molecular biology to retinoic acid receptors. FASEB J 1996;10:940–54.
[3] Laudet V, Gronemeyer H. Nuclear receptor factsbook. London: Academic Press; 2001.
[4] Mangelsdorf DJ, Thummel C, Reato M, Herrlich P, Schutz G, Umesono K, et al. The nuclear receptor superfamily: the second decade. Cell 1995;83:835–9.
[5] Heyman RA, Mandelsdorf DJ, Dyck JA, Stein RB, Eichele G, Evans RM, et al. 9-Cis retinoic acid is a high affinity ligand for the retinoid X receptor. Cell 1992;68:397–406.
[6] Chambon P, Zelent A, Petkovich M, Mendelsohn C, Leroy P, Drust A, et al. The family of retinoic acid nuclear receptors. In: Saurat JH, editor. Retinoids: 10 years on. Basel: Karger; 1991. p. 10–27.
[7] Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM. Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. Nature 1992;358:771–4.
[8] Forman BM, Umesono K, Chen J, Evans RM. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. Cell 1995;81:541–50.
[9] Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA, Mangelsdorf DJ. LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev 1995;9:1033–45.
[10] Perlman T, Rangarajan PN, Umesono K, Evans RM. Determinants for selective RAR and TR recognition of direct repeat HREs. Genes Dev 1993;7:1411–22.
[11] Perlmann T, Jansson L. A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1. Genes Dev 1995;9:769–82.
[12] Mangelsdorf DJ, Borgmeyer U, Heyman RA, Zhou JY, Ong ES, Oro AE, et al. Characterization of three RXR genes that mediate the action of 9-cis retinoic acid. Genes Dev 1992;6:329–44.
[13] Aranda A, Pascual A. Nuclear hormone receptors and gene expression. Physiol Rev 2001;81:1269–304.

[14] Desvergne B, Michalik L, Wahli W. Transcriptional regulation of metabolism. Physiol Rev 2006;86:465–514.
[15] Luisi BF, Xu WX, Otwinowski A, Freedman LP, Yamamoto KR, Sigler PB. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. Nature 1991;352:497–505.
[16] Lee MS, Kliewer SA, Provencal J, Wright PE, Evans RM. Structure of the retinoid X receptor alpha DNA binding domain: a helix required for homodimeric DNA binding. Science 1993;260:1117–21.
[17] Schwabe JW, Chapman L, Finch JT, Rhodes D. The crystal structure of the estrogen receptor DNA-binding domain bound to DNA: how receptors discriminate between their response elements. Cell 1993; 75:567–78.
[18] Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schulman IG, Juguilon H, et al. Role of CBP/p300 in nuclear receptor signalling. Nature 1996;383:99–103.
[19] Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 2000;14:121–41.
[20] Wurtz JM, Bourguet W, Renaud JP, Vivat V, Chambon P, Moras D, et al. A canonical structure for the ligand-binding domain of nuclear receptors. Nat Struct Biol 1996;3:87–94.
[21] de The H, Vivanco-Ruiz MM, Tiollais P, Stunnenberg H, Dejean A. Identification of a retinoic acid responsive element in the retinoic acid receptor beta gene. Nature 1990;343:177–80.
[22] Leid M, Kastner P, Chambon P. Multiplicity generates diversity in the retinoic acid signalling pathways. Trends Biochem Sci 1992;17: 427–33.
[23] Rastinejad F, Wagner T, Zhao Q, Khorasanizadeh S. Structure of the RXR-RAR DNA-binding complex on the retinoic acid response element DR1. EMBO J 2000;19:1045–54.
[24] Mangelsdorf DJ, Umesono K, Kliewer SA, Borgmeyer U, Ong ES, Evans RM. A direct repeat in the cellular retinol-binding protein type II gene confers differential regulation by RXR and RAR. Cell 1991; 66:555–61.
[25] Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, et al. Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. Nature 1995;378:681–9.
[26] Moras D, Gronemeyer H. The nuclear receptor ligand-binding domain: structure and function. Curr Opin Cell Biol 1998; 10:384–91.
[27] Onate SA, Boonyaratanakornkit V, Spencer TE, Tsai SY, Tsai MJ, Edwards DP, et al. The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors. J Biol Chem 1998;273:12101–8.
[28] Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, et al. Ligand binding and coactivator assembly of the peroxisome proliferator-activated receptor gamma. Nature 1998;395:137–43.
[29] Darimont BD, Wagner RL, Apriletti JW, Stallcup MR, Kushner PJ, Baxter JD, et al. Structure and specificity of nuclear receptor–coactivator interactions. Genes Dev 1998;12:3343–56.
[30] Heery DM, Kalkhoven E, Hoare S, Parker MG. A signature motif in transcriptional coactivators mediates binding to nuclear receptors. Nature 1997;387:733–6.
[31] Germain P, Iyer J, Zechel C, Gronemeyer H. Co-regulator recruitment and the mechanism of retinoic acid receptor synergy. Nature 2002;415:187–92.
[32] Bannister AJ, Kouzarides T. The CBP-coactivator is a histone acetyltransferase. Nature 1996;384:641–3.
[33] Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 1996;87:953–9.
[34] Voegel JJ, Heine MJ, Tini M, Vivat V, Chambon P, Gronemeyer H. The coactivator TIF2 contains three nuclear receptor binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways. EMBO J 1998;17:507–19.
[35] McInerney EM, Rose DW, Flynn SE, Westin S, Mullen TM, Krones A, et al. Determinants of coactivator LXXLL motif specificity in nuclear receptor transcriptional activation. Genes Dev 1998;12: 3357–68.
[36] Janknecht R, Hunter T. A growing coactivator network. Nature 1996; 383:22–3.
[37] Chen JD. Steroid/nuclear receptor coactivators. Vitam Horm 2000; 58:391–448.
[38] Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, et al. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 1996;85:403–14.
[39] Malik S, Roeder RG. Transcriptional regulation through mediator-like coactivators in yeast and metazoan cells. Trends Biochem Sci 2000;25:277–83.
[40] Cosma MP. Ordered recruitment: gene-specific mechanism of transcription activation. Mol Cell 2002;10:227–36.
[41] Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. Cell 2000;103:263–71.
[42] Orphanides G, Reinberg D. RNA polymerase II elongation through chromatin. Nature 2000;407:471–5.
[43] Dilworth FJ, Chambon P. Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription. Oncogene 2001;20:3047–54.
[44] Adelman K, Lis JT. How does Pol II overcome the nucleosome barrier? Mol Cell 2002;9:451–2.
[45] Fry CJ, Peterson CL. Chromatin remodeling enzymes: who's on first? Curr Biol 2001;11:R185–97.
[46] Aalfs JD, Kingston RE. What does chromatin remodeling mean? Trends Biochem Sci 2000;25:548–55.
[47] Chen H, Evans R. A transcriptional corepressor that interacts with nuclear hormone receptors. Nature 1995;377:454–7.
[48] Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, et al. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor corepressor. Nature 1995; 377:397–404.
[49] Dressel U, Thormeyer D, Altincicek B, Paulutat A, Eggert M, Schneider S, et al. Alien, a highly conserved protein with characteristics of a corepressor for members of the nuclear hormone receptor superfamily. Mol Cell Biol 1999;19:3383–94.
[50] Perissi V, Staszewski LM, McInerney EM, Kurokawa R, Krones R, Rose DW, et al. Molecular determinants of nuclear receptor–corepressor interaction. Genes Dev 1999;13:3198–208.
[51] Ordentlich P, Downes M, Evans RM. Corepressors and nuclear hormone receptor function. Curr Top Microbiol Immunol 2001;254: 101–16.
[52] Lavigne AC, Mengus G, Gangloff YG, Wurtz JM, Davidson I. Human TAF(II)55 interacts with the vitamin D(3) and thyroid hormone receptors and with derivatives of the retinoid X receptor that have altered transactivation properties. Mol Cell Biol 1999;19:5486–94.
[53] Horwitz KB, Jackson TA, Bainm DL, Richer JK, Takimoto GS, Tung L. Nuclear receptor coactivators and corepressors. Mol Endocrinol 1996;10:1167–77.
[54] Nagy L, Kao HY, Love JD, Li C, Banayo E, Gooch JT, et al. Mechanism of corepressor binding and release from nuclear hormone receptors. Genes Dev 1999;13:3209–16.
[55] Heinzel T, Tavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, et al. A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature 1997;387: 43–8.
[56] Alland L, Muhle R, Hou H, Potes J, Chin L, Schreiber-Agus N, et al. Role for NCoR and histone deacetylase in Sin3-mediatedd transcriptional repression. Nature 1997;387:49–55.
[57] Jones PL, Sachs LM, Rouse N, Wade PA, Shi Y-B. Multiple NCoR complexes contain distinct histone deacetylases. J Biol Chem 2001; 276:8807–11.
[58] Kouzarides T. Histone methylation in transcriptional control. Curr Opin Genet Dev 2002;12:198–209.
[59] Jenuwin T, Allis CD. Translating the histone code. Science 2001; 293:1074–80.

[60] Delva L, Bastie JN, Rochette-Egly C, Kraiba R, Balitrand N, Despouy G, et al. Physical and functional interactions between cellular retinoic acid binding protein II and the retinoic acid-dependent nuclear complex. Mol Cell Biol 1999;19:7158–67.
[61] Dong D, Noy N. Heterodimer formation by retinoid X receptor: regulation by ligands and by the receptor's self-association properties. Biochemistry 1998;37:10691–700.
[62] Budhu AS, Noy N. Direct channeling of retinoic acid between cellular retinoic acid-binding protein II and retinoic acid receptor sensitizes mammary carcinoma cells to retinoic acid-induced growth arrest. Mol Cell Biol 2002;22:2632–41.
[63] Wang JC. The path of DNA in the nucleosome. Cell 1982;29:724–6.
[64] Richmond TJ, Finch JT, Rushton B, Rhodes D, Klug A. Structure of the nucleosome core particle at 7 A resolution. Nature 1984;311:532–7.
[65] Felsenfeld G, McGhee JD. Structure of the 30 nm chromatin fiber. Cell 1986;44:375–7.
[66] Chen H, Lin RJ, Xie W, Wilpitz D, Evans RM. Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. Cell 1999;98:675–86.
[67] Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000;403:41–5.
[68] Roth SY, Denu JM, Allis CD. Histone acetyltransferases. Ann Rev Biochem 2001;70:81–120.
[69] Narlikar GJ, Fan HY, Kingston RE. Cooperation between complexes that regulate chromatin structure and transcription. Cell 2002;108:475–87.
[70] Ito M, Yuabn CX, Mallik S, Gu W, Fondell JD, Yamamura S, et al. Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. Mol Cell 1999;3:361–70.
[71] Woychik NA, Hampsey M. The RNA polymerase II machinery: structure illuminates function. Cell 2002;108:453–63.
[72] Kingston RE, Narlikar GJ. ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. Genes Dev 1999;13:2339–52.
[73] Delmotte MH, Tahayato A, Formstecher P, Lefebvre P. Serine 157, A retinoic acid receptor alpha residue phosphorylated by protein kinase C in vitro, is involved in RXR.RARalpha heterodimerization and transcriptional activity. J Biol Chem 1999;274:38225–31.
[74] Rochette-Egly C. Nuclear receptors: integration of multiple signalling pathways through phosphorylation. Cell Signal 2003;15:355–66.
[75] Bastien J, Rochette-Egly C. Nuclear retinoid receptors and the transcription of retinoid-target genes. Gene 2004;328:1–16.
[76] Rochette-Egly C, Oulad-Abdelghani M, Staub A, Pfister V, Scheuer I, Chambon P, et al. Phosphorylation of the retinoic acid receptor-alpha by protein kinase A. Mol Endocrinol 1995;9:860–71.
[77] Lee HY, Suh YA, Robinson MJ, Clifford JL, Hong WK, Woodgett JR, et al. Stress pathway activation induces phosphorylation of retinoid X receptor. J Biol Chem 2000;275:32193–9.
[78] Matsushima-Nishiwaki R, Okuno M, Adachi S, Sano T, Akita K, Moriwaki H, et al. Phosphorylation of retinoid X receptor alpha at serine 260 impairs its metabolism and function in human hepatocellular carcinoma. Cancer Res 2001;61:7675–82.
[79] Hong SH, Privalsky ML. The SMRT corepressor is regulated by a MEK-1 kinase pathway: inhibition of corepressor function is associated with SMRT phosphorylation and nuclear export. Mol Cell Biol 2000;20:6612–25.
[80] Keriel A, Stary A, Sarasin A, Rochette-Egly C, Egly JM. XPD mutations prevent TFIIH-dependent transactivation by nuclear receptors and phosphorylation of RARalpha. Cell 2002;109:125–35.
[81] Zhu J, Gianni M, Kopf E, Honore N, Chelbi-Alix M, Koken M, et al. Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor a (RARa) and oncogenic RARa fusion proteins. Proc Natl Acad Sci U S A 1999;96:14807–12.
[82] Kopf E, Plassat JL, Vivat V, de The H, Chambon P, Rochette-Egly C. Dimerization with retinoid X receptors and phosphorylation modulate the retinoic acid-induced degradation of retinoic acid receptors alpha and gamma through the ubiquitin–proteasome pathway. J Biol Chem 2000;275:33280–8.
[83] Tanaka T, Rodriguez de la Concepcion ML, De Luca LM. Involvement of all-trans-retinoic acid in the breakdown of retinoic acid receptors alpha and gamma through proteasomes in MCF-7 human breast cancer cells. Biochem Pharmacol 2001;61:1347–55.
[84] Conaway RC, Brower CS, Conaway JW. Emerging roles of ubiquitin in transcription regulation. Science 2002;296:1254–8.
[85] Gianni M, Kopf E, Bastien J, Oulad-Abdelghani M, Garattini E, Chambon P, et al. Down-regulation of the phosphatidylinositol 3-kinase/Akt pathway is involved in retinoic acid-induced phosphorylation, degradation, and transcriptional activity of retinoic acid receptor gamma 2. J Biol Chem 2002;277:24859–62.
[86] DeMartino GN, Slaughter CA. The proteasome, a novel protease regulated by multiple mechanisms. J Biol Chem 1999;274:22123–6.
[87] Gianni M, Bauer A, Garattini E, Chambon P, Rochette-Egly C. Phosphorylation by p38MAPK and recruitment of SUG-1 are required for RA-induced RARg degradation and transactivation. EMBO J 2002;21:3760–9.
[88] Gonzalez F, Delahodde A, Kodadek T, Johnston SA. Recruitment of a 19S proteasome subcomplex to an activated promoter. Science 2002;296:548–50.
[89] Muratani M, Tansey WP. How the ubiquitin–proteasome system controls transcription. Nat Rev Mol Cell Biol 2003;4:192–201.
[90] Zimmerman TL, Thevananther S, Ghose R, Burns AR, Karpen SJ. Nuclear export of retinoid X receptor alpha in response to interleukin-1beta-mediated cell signaling: roles for JNK and SER260. J Biol Chem 2006;281:15434–40.
[91] Osburn DL, Shao G, Seidel HM, Schulman IG. Ligand-dependent degradation of retinoid X receptors does not require transcriptional activity or coactivator interactions. Mol Cell Biol 2001;21:4909–18.
[92] Isseman I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 1990;347:645–50.
[93] Dreyer C, Keller H, Mahfoudi A, Loudet V, Krey G, Wahli W. Positive regulation of peroxisomal β-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors. Biol Cell 1993;77:67–77.
[94] Tonotoz P, Hu E, Speigelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma2, a lipid activated transcription factor. Cell 1994;79L:1147–56.
[95] Osumi T, Wen JK, Hashimoto T. Two cis-acting elements in the peroxisome proliferators-responsive element enhancer region of rat acyl-CoA oxidase gene. Biochem Biophys Res Commun 1991;175:866–71.
[96] Tugwood JD, Isseman I, Anderson RG, Bundell KR, McPheat WL, Green S. The mouse peroxisome proliferators activated receptor recognizes a response element in the 5′-flanking sequence of rat acyl coA oxidase. Embo J 1992;11:433–9.
[97] Zhang X-K, Lehmann J, Hoffman B, Dawson M, Camerson J, Graupner G, et al. Homodimer formation of retinoid X receptor induced by 9-cis retinoic acid. Nature 1992;358:587–91.
[98] Desvergne B, Wahli W. Peroxisome proliferators-activated receptors: nuclear control of metabolism. Endocr Rev 1999;20:649–88.
[99] Reddy JK. Peroxisome proliferators and peroxisome proliferator-activated receptor alpha. Am J Pathol 2004;164:2305–21.
[100] Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D. Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha. Nature 1995;375:377–82.
[101] Egea PF, Rochel N, Birck C, Vachette P, Timmins PA, Moras D. Effects of ligand binding on the association properties and conformation in solution of retinoic acid receptors RXR and RAR. J Mol Biol 2001;307:557–76.
[102] Jump DB, Botolin D, Wang Y, Xu J, Christian B, Demeure O. Fatty acid regulation of hepatic gene transcription. J Nutr 2005;135:2503–6.

[103] Yang W, Rachez C, Freedman LP. Discrete roles for peroxisome proliferators-activated receptor gamma and retinoid X receptor in recruiting nuclear receptor coactivators. Mol Cell Biol 2000;20: 8008–17.
[104] Ijpenberg A, Tan NS, Gelman L, Kersten S, Seydoux J, Xu J, et al. Polarity and specific sequence requirements of peroxisome proliferators-activated receptor/retinoid X receptor heterodimer binding to DNA. A functional analysis of the malic enzyme gene PPAR response element. Embo J 2004;23:2083–91.
[105] Balmer JE, Blomhoff R. Gene expression regulation by retinoic acid. J Lipid Res 2002;43:1773–808.
[106] Granner DK, Andreone T, Sasaki K, Beale E. Inhibition of transcription of the phosphoenolpyruvate carboxykinase gene by insulin. Nature 1983;305:549–51.
[107] Imai E, Stromstedt PE, Quinn PD, Carlstedt-Duke J, Gustafsson J, Granner DK. Characterization of a complex glucocorticoid response unit in the phosphoneolpyruvate carboxyukinase gene. Mol Cell Biol 1990;10:4712–9.
[108] Lamers WH, Hanson RW, Meisner HM. cAMP stimulates transcription of the gene for cytosolic phosphoenolpuriuvate carboxykinase in rat liver nuclei. Proc Natl Acad Sci U S A 1982;79:5137–41.
[109] McGrane MM, Yun JS, Moorman AF, Lamers WH, Hendrick GK, Arafah BM, et al. Metabolic effects of developmental, tissue- and cell-specific expression of a chimeric phosphoenolpyruvate carboxykinase (GTP)/bovine growth hormone gene in transgenic mice. J Biol Chem 1990;265:22371–9.
[110] McGrane MM, Hanson RW. From diet to DNA: dietary patterning of gene expression. Nutr Clin Pract 1992;7:16–21.
[111] O'Brien RM, Lucas PC, Forest CD, Magnuson MA, Granner DK. Identification of a sequence in the PEPCK gene that mediates a negative effect of insulin on transcription. Science 1990; 249:533–7.
[112] Sasaki K, Cripe TP, Kocks SR, Adreone TL, Petersen DD, Bealse EG, et al. Multihormonal regulation of phosphoenolpyruvate carboxykinasse gene transcription. J Biol Chem 1984;259: 8288–92.
[113] Short JM, Wynshaw-Boris A, Short HP, Hanson RW. Characterization of phosphoenolpyruvate carboxykinase (GTP) promoter-regulatory region. J Biol Chem 1986;261:9721–6.
[114] Hall RK, Scott DK, Noisin EL, Lucas PC, Granner DK. Activation of the phosphoenolpyruvate carboxykinase gene retinoic acid response element is dependent on a retinoic acid receptor/coregulator complex. Mol Cell Biol 1992;12:5527–35.
[115] Lucas PC, O'Brien RM, Mitchell JA, Davis CM, Imai E, Forman BM, Samuels HH, Granner DK. A retinoic acid response element is part of a pleiotropic domain in the phosphoenolpyruvate carboxykinase gene. Proc Natl Acad Sci U S A 1991;88:2184.
[116] Lucas PC, Forman BM, Samuels HH, Granner DK. Specificity of a retinoic acid response element in the phosphoenolpyruvate carboxykinase gene promoter: consequences of both retinoic acid and thyroid hormone receptor binding. Mol Cell Biol 1991;11: 5164–70.
[117] Scott DK, Mitchell JA, Granner KD. Identification and characterization of a second retinoic acid response element in the phosphoenolpyruvate carboxykinase gene promoter. J Biol Chem 1996;271: 6260–4.
[118] Devine JH, Eubank DW, Clouthier DE, Tontonoz P, Spiegelman RE, Hammer RE, et al. Adipose expression of the phosphoenolpyruvate carboxykinase promoter requires peroxisome proliferators-activated receptor gamma and 9-cis retinoic acid receptor binding to an adipocyte-specific enhancer in vivo. J Biol Chem 1999;274: 13604–12.
[119] Shin D-J, McGrane MM. Vitamin A regulates genes involved in hepatic gluconeogenesis in mice: phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. J Nutr 1997;127:1274–8.
[120] Shin D-J, Odom DP, Scribner KB, Ghoshal S, McGrane MM. Retinoid regulation of the phosphoenolpyruvate carboxykinase gene in liver. Mol Cell Endocrinol 2002;195:39–54.
[121] Scribner KB, Odom DP, McGrane MM. Nuclear receptor binding to the retinoic acid response elements of the phosphoenolpyruvate carboxykinase gene in vivo: effects of vitamin A deficiency. J Nutr Biochem 2006.
[122] Eubank DW, Duplus E, Williams SC, Forest C, Beale EG. Peroxisome proliferator-activated receptor gamma and chicken ovalbumin upstream promoter transcription factor II negatively regulate the phosphoenolpyruvate carboxykinase promoter via a common element. J Biol Chem 2001;276:30561–9.
[123] Leone TC, Weinheimer CJ, Kelly DP. A critical role for PPAR in the cellular fasting response: the PPAR alpha null mouse as a model of fatty acid oxidation disorders. Proc Natl Acad Sci U S A 1999;96: 7473–7.
[124] Xu J, Xiao G, Trujillo C, Chang V, Blanco L, Joseph SB, et al. PPARalppha influences substrate utilization for hepatic glucose production. J Biol Chem 2002;277:50237–44.
[125] Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Roles of PPARs in health and disease. J Clin Invest 1999;103: 1489–98.
[126] Scribner KB, Odom DP, McGrane MM. Vitamin A status in mice affects the histone code of the phosphoenolpyruvate carboxykinase gene in liver. J Nutr 2005;135:2774–9.
[127] Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley B, et al. Activation of PPARgamma coactivator 1 through transcription factor socking. Science 1999;286:1368–71.
[128] Monsalve M, Wu Z, Adelmant G, Puigserver P, Fan M, Spiegelman BM. Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1. Mol Cell 2002;6(2):307–16.
[129] Rhee J, Inoue Y, Yoon JC, Puigserver P, Fan M, Gonzales FJ, et al. Regulation of hepatic fasting response by PPARgamma coactivator 1 alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. Proc Natl Acad Sci U S A 2003;100:4012–7.
[130] Hebbes TR, Thorne AW, Crane-Robinson C. A direct link between core histone acetylation and transcriptionally active chromatin. Embo J 1988;7:1395–402.
[131] Edmondson DG, Davie JK, Zhou J, Mirnikjoo B, Tatchell K, Dent SYR. Site specific loss of acetylation upon phosphorylation of histone H3. J Biol Chem 2002;277:29496–502.
[132] Kuo M-H, Brownell JE, Sobel RE, Ranalli TA, Cook RG, Edmondson DG, et al. Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. Nature 1996;383:269–72.
[133] Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, et al. Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell 1997;90:569–80.
[134] Zhang W, Bieker JJ. Acetylation and modulation of erythroid druppel-like factor activity by interaction with histone acetyltransferases. Proc Natl Acad Sci U S A 1998;95:9855–60.
[135] Grant PA, Eberharter A, John S, Cook RG, Turner BM, Workman JL. Expanded lysine acetylation specificity of Gcn5 in native complexes. J Biol Chem 1999;274:5895–900.
[136] Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen C, et al. Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 1997;389:9498.
[137] Schiltz RL, Mizzen CA, Vassilev A, Cook RG, Allis CD, Nakatani Y. Overlapping but distinct patterns of histone acetylation by the human coactivators p300 and PCAF within nucleosomal substrates. J Biol Chem 1999;274:1189–92.
[138] Ito K, Barnes PJ, Adcock IM. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. Mol Cell Biol 2000; 20:6891–903.
[139] Xu J, Li Q. Review of the in vivo functions of the p160 steroid receptor coactivator family. Mol Endocrinol 2003;17:1681–92.

[140] Soutoglou E, Katrakili N, Talianidis I. Acetylation regulates transcription factor activity at multiple levels. Mol Cell 2000;5: 745–51.
[141] Scribner KB, McGrane MM. RNA polymerase II association with the phosphoenolpyruvate carboxykinase (PEPCK) promoter is reduced in vitamin A deficient liver. J Nutr 2003;133:4112–7.
[142] Duong DT, Waltner-Law ME, Sears R, Sealy L, Granner DK. Insulin inhibits hepatocellular glucose production by utilizing liver-enriched transcriptional inhibitory protein to disrupt the association of CREB-binding protein and RNA polymerase II with the phosphoenolpyruvate carboxykinase gene promoter. J Biol Chem 2002;277:32234–42.