Chapter 6
Nuclear and Extra-Nuclear Effects of Retinoid Acid Receptors: How They Are Interconnected

Aleksandr Piskunov, Ziad Al Tanoury and Cécile Rochette-Egly

Abstract The nuclear retinoic acid receptors (RAR α, β and γ) and their isoforms are ligand-dependent regulators of transcription, which mediate the effects of all-*trans* retinoic acid (RA), the active endogenous metabolite of Vitamin A. They heterodimerize with Retinoid X Receptors (RXRs α, β and γ), and regulate the expression of a battery of target genes involved in cell growth and differentiation. During the two last decades, the description of the crystallographic structures of RARs, the characterization of the polymorphic response elements of their target genes, and the identification of the multiprotein complexes involved in their transcriptional activity have provided a wealth of information on their pleiotropic effects. However, the regulatory scenario became even more complicated once it was discovered that RARs are phosphoproteins and that RA can activate kinase signaling cascades via a pool of RARs present in membrane lipid rafts. Now it is known that these RA-activated kinases translocate to the nucleus where they phosphorylate RARs and other retinoid signaling factors. The phosphorylation state of the RARs dictates whether the transcriptional programs which are known to be induced by RA are facilitated and/or switched on. Thus, kinase signaling pathways appear to be crucial for fine-tuning the appropriate physiological activity of RARs.

A. Piskunov · Z. Al Tanoury · C. Rochette-Egly (✉)
IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), INSERM, U964, CNRS, UMR7104, Université de Strasbourg, 1 rue Laurent Fries, BP 10142, 67404 Illkirch Cedex, France
e-mail: cegly@igbmc.fr

A. Piskunov
e-mail: piskunov@ibcgenerium.ru

Z. Al Tanoury
e-mail: tanouryz@igbmc.fr

M. A. Asson-Batres and C. Rochette-Egly (eds.), *The Biochemistry of Retinoic Acid Receptors I: Structure, Activation, and Function at the Molecular Level*, Subcellular Biochemistry 70,
DOI: 10.1007/978-94-017-9050-5_6,

Abbreviations

CAK	Cyclin-dependent kinase (CDK)-activating kinase
ChIP	Chromatin immunoprecipitation
ChIP-seq	ChIP coupled with deep sequencing
CRABP	Cellular retinoic acid binding protein
DBD	DNA binding domain
DR	Direct repeat
Erks	Extracellular-signal-regulated kinases
ES cells	Embryonic stem cells
FABP	Fatty acid binding protein
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HMT	Histone methyl transferase
iLBP	Intracellular lipid binding protein
IR	Inverted repeat
LBD	Ligand binding domain
LBP	Ligand binding pocket
MAPK	Mitogen activated protein kinase
MSK	Mitogen-and stress-activated protein kinase
N-CoR	Nuclear receptor corepressor
NMR	Nuclear magnetic resonance
NTD	N-terminal domain
PcG	Polycomb group proteins
PI3K	Phosphoinositide 3-kinase
Pin1	Protein interacting with NIMA (never in mitosis A)
PPAR	Peroxysome proliferator activated receptor
PRM	Proline rich motif
RA	Retinoic acid
RAR	Retinoic acid receptor
RARE	Retinoic acid response element
RNA-seq	high throughput qPCR sequencing
RXR	Retinoid X receptor
SAXS	Small angle X-ray
SH3	Src-homology-3
SRC	Steroid receptor coactivator
SMRT	Silencing mediator of retinoic acid receptor and thyroid hormone receptor
TBL1	Transducin beta like
TBLR1	TBL1-related protein 1
WW	Tryptophan-tryptophan

History: The Canonical Model for the Regulation of RAR-Target Gene Expression

The Functional Domains of RARs

Like most nuclear receptors (NRs) [49–51], RARs and RXRs exhibit a well-defined domain organization consisting of a central conserved DNA binding domain (DBD) linked to a variable N-terminal domain (NTD), and a C-terminal Ligand-Binding Domain (LBD) [10, 28, 73, 108] (Fig. 6.1a).

The structures of RAR and RXR LBDs are rather similar. This domain in each protein is composed of 12 conserved alpha helices and a beta-turn, separated by loops and folded into a three-layered, and parallel helical sandwich [20, 28, 103], with helices H4, H5, H8, H9 and H11 sandwiched between H1, H2 and H3 on one side, and H6, H7 and H10 on the other (Fig. 6.1a, b). In contrast, the C-terminal helix, H12, is more flexible and adopts conformations that differ from one RAR to the other. The conformation pf H12 also changes after RA binding.

The primary feature of the LBD is its functional complexity. It contains the Ligand-Binding Pocket (LBP) [19], the heterodimerization surface [21], and interaction surfaces involved in the binding of multiple coregulators (Fig. 6.1b). A well-described hydrophobic surface, generated by H3 and H4, is involved in the binding of corepressors/coactivators [57, 111] (see also Chap. 3 in this volume). The LBD also contains a recently described docking site for cyclin H, a subunit of the cyclin-dependent activating (CAK) sub complex of the general transcription factor, TFIIH, that is formed by loop L8-9 and the first amino acids of H9 [15] (Fig. 6.1b).

The DBD is composed of two zinc-nucleated modules and two alpha-helices [137, 138], which contribute to a second dimerization interface and define the contacts for specific DNA sequences, named RA response elements (RAREs). Classically, RAREs are composed of two direct repeats of a core hexameric motif (A/G) G (G/T) TCA separated by 1, 2 or 5 nucleotides and referred as DR1, DR2 and DR5 [8, 10, 48] (Fig. 6.1c). However, recent genome wide chromatin immunoprecipitation coupled with deep sequencing (ChIP-seq) technology has allowed identification of new RAR binding loci [85, 91] and revealed that RARs can occupy a larger repertoire of sites with an unexpected diversity in the spacing and the topology of the DNA binding elements, including DR0, DR8 and IR0 (inverted repeats) elements (Fig. 6.1c). Recent structural studies have also indicated that the architecture of DNA-bound heterodimers is dictated by the DNA sequence (Fig. 6.1d) [22, 104] (see also Chap. 2 in this volume).

In contrast to the DBD and the LBD, the NTD is not conserved between RARs and RXRs and even between the different subtypes and isoforms. As yet, high-resolution, three-dimensional structures of this region have not been produced [108]. Several biochemical and structural studies coupled to structure prediction algorithms suggest that the NTDs of RARs and RXRs, as well as of any member

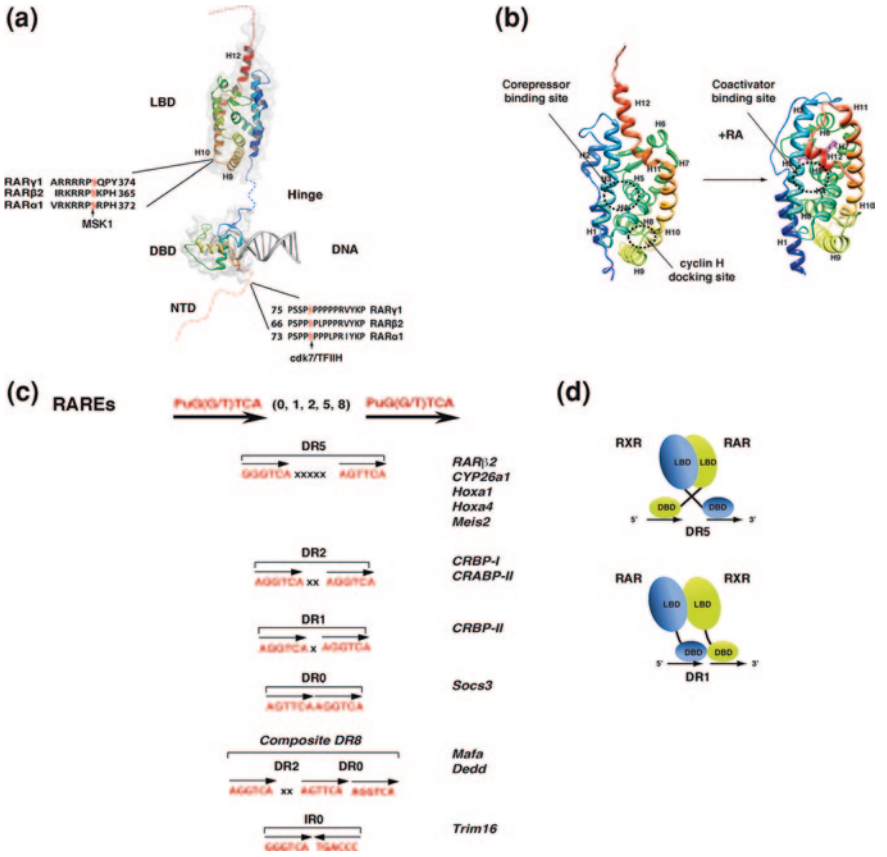

Fig. 6.1 Structure of RARs and of their DNA binding sites. **a** RARs depict a domain organization with an unstructured N-terminal domain (*NTD*), and two well structured domains: a central DNA binding domain (*DBD*) and a C-terminal ligand-binding domain (*LBD*). The phosphorylation sites located in the NTD and the LBD are shown. **b** Structural changes induced upon RA binding. The crystal structures of the unliganded RXRα and liganded RARγ LBDs are shown with the binding domains for corepressors, coactivators and cyclin H. Helices are represented as ribbons and labelled from H1 to H12. Adapted from Protein Data Bank 1lbd and 2lbd. **c** The retinoid response elements (*RAREs*) are composed of a direct repeat of the motif 5'- Pu G (G/T) TCA spaced by 0 (DR0), 1 (DR1), 2 (DR2), 5 (DR5) or 8 (DR8) base pairs. DR8 comprise three half sites with DR2 and DR0 spacing. Some RARE-associated genes are shown. **d** Binding of the RAR/RXR heterodimers to DR1 and DR5 RAREs (adapted from [104])

of the NR family, are naturally disordered [74, 131]. An interesting feature of these NTDs is that they contain phosphorylation sites [105], which are conserved between RARs (Fig. 6.1a) [112]. Moreover, they contain proline-rich motifs (PRMs) which are well known to bind proteins with Src-homology-3 (SH3) or tryptophan-tryptophan (WW) domains. Phosphorylation prevents or favors these interactions [7].

RAR-Mediated Gene Expression

RAR/RXR heterodimers control transcription *via* several distinct mechanisms, including both repression and activation. According to the canonical model, the transcriptional regulation of RA-target genes relies not only on the binding of RAR/RXR heterodimers to specific RAREs, but also on corepressors that dissociate and coactivators that associate with the LBD upon ligand-induced conformational changes [57, 81, 95, 133]. At the molecular level, the discrimination between corepressors and coactivators is governed by the ligand-induced, orientation of H12, which contributes in a critical manner to the generation or removal of cofactor interaction surfaces.

Repression of Transcription in the Absence of Ligand

In the absence of ligand and in a context of chromatin where the nucleosomes do not impede binding to RAREs, the RARα subtype is a strong repressor of target gene expression (Fig. 6.2a) [34]. In this unliganded state, H12 adopts an open conformation that unmasks a hydrophobic groove generated by H3 and H4 [76] (see also Chap. 3 in this volume). This interface specifically binds an LxxI/HIxxxI/L motif in the extended alpha helix box of the corepressors, NCoR or SMRT [98]. NCoR and SMRT are genetic paralogs with multiple protein variants, but SMRT is the favored corepressor for RARs [90]. According to recent studies, SMRT is recruited by the heterodimer only through the RAR partner [76] (see also Chap. 3 in this volume).

SMRT does not have intrinsic enzymatic activity, but serves as an adaptor to recruit other high molecular weight complexes that are endowed with histone deacetylase activity (HDACs) [95]. These complexes deacetylate lysine residues in the N-terminal tails of histones and maintain chromatin in a condensed and repressed state over the target promoter [34, 111] (Fig. 6.2a). The corepressor complexes also contain other components such as transducer β-like proteins (TBL1 and TBLR1), which serve as adaptors regulating corepressor assembly and function [94].

In contrast to RARα, the RARγ and RARβ subtypes poorly interact with corepressors [39, 60, 102], most probably due to the fact that, in these receptors, H12 interacts with H3 even in the absence of ligand, thus occluding the corepressor docking site.

Initiation of Transcription in Response to the Ligand: A Process Governed by the LBD via the Exchange of Coregulators

According to the canonical model, ligand binding to RARs must be understood in terms of structural features (Fig. 6.1b). When entering the cavity of the RARa LBP, the ligand induces a β-strand-to-α-helix secondary structure switch [76], which induces

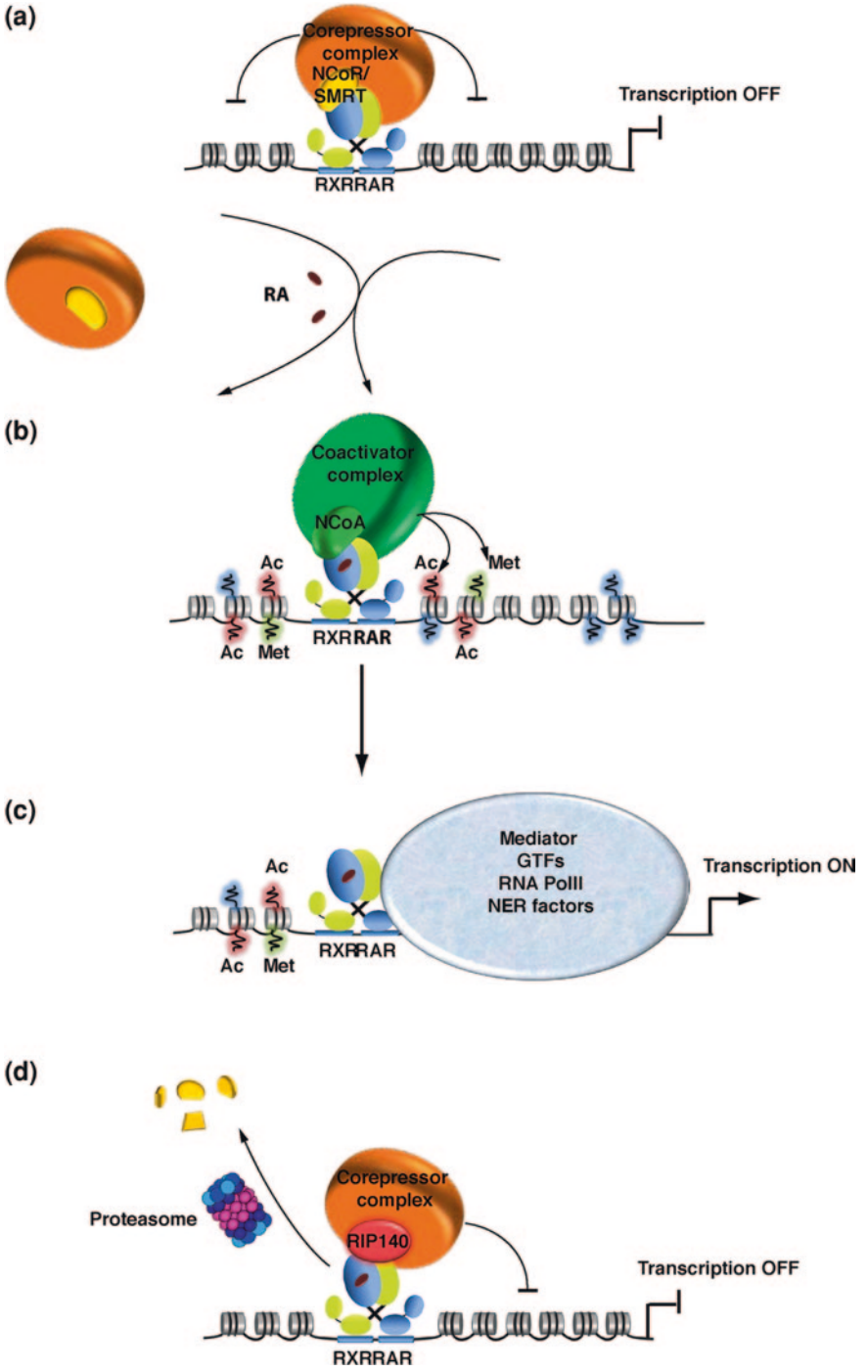
(a)
Corepressor complex NCoR/ SMRT
Transcription OFF
RXRRAR
RA
(b)
Coactivator complex
NCoA
Ac
Ac
Met
Ac Met
RXRRAR
Ac
(c)
Mediator
GTFs
RNA PolII
NER factors
Transcription ON
Ac
Ac Met
RXRRAR
(d)
Proteasome
Corepressor complex
RIP140
Transcription OFF
RXRRAR

Fig. 6.2 The classical model of activation of RA-target genes. **a** Repression in the absence of ligand. **b** Corepressors and coactivators exchange after ligand binding. **c** Recruitment of the transcription machinery and initiation of transcription. **d** End of the RA signal upon recruitment of non-conventional coativators such as RIP140, associated to large complexes with chromatin repressing activity. The end of the RA signal occurs also through the degradation of RARs by the ubiquitin proteasome system

repositioning of H11 relative to H10 and a concomitant swinging of H12 inward to pack against H3 and H4 in a mouse trap model that locks RA in the LBP. Consequently, corepressors are released and a new hydrophobic cleft is formed between H3, H4 and H12 [28], with a charge clamp between a conserved glutamate residue in H12 and a lysine in H3. This charge clamp specifically grips the ends of the helix specified by the LxxLL motif of the p160 subfamily of steroid receptor coactivators, SRC-1, SRC-2 and SRC-3 [81, 96]. The p160 coactivators have an intrinsic histone acetyl transferase (HAT) activity, and according to recent structural studies, only one coactivator molecule is recruited by the heterodimer through the RAR partner [22, 92].

When recruited to the LBD of the liganded RAR, the p160 coactivators initiate a dynamic, ordered and coordinated recruitment of other proteins with HAT activity [p300/CBP (CREB binding protein) and p/CAF (p300/CBP-associated factor)] or with histone methyl transferase (HMT) activities, such as Coactivator-associated arginine methyl-transferase 1 (CARM1) or Protein-arginine methyl transferase 1 (PRMT1) [57, 81, 111, 133] (Fig. 6.2b). Acetylation and methylation weaken histone DNA contacts and create marks forming an «*histone code*», which coordinates the recruitment of additional HATs or HMTs to histones for further chromatin decompaction. This code also orchestrates the recruitment of chromatin remodelers, which use the energy of ATP-hydrolysis to reposition nucleosomes by sliding them in *cis* or displacing them in *trans*, allowing the formation of nucleosome-free or nucleosome-spaced regions at the promoter.

The p160 coregulators also recruit large complexes which contain modules with other enzymatic activities, such as histone de-ubiquitinases [139] and histone lysine methyl transferases [42, 80]. Lysine methylation creates marks for the binding of other enzymes that erase repressive marks. According to a recent study, this step requires Poly (ADP-Ribose) glycohydrolase (PARG) which is corecruited at RAR-dependent promoters in response to RA [78]. Thus, due to their varied composition, the coactivator complexes provide an elegant mechanism to reorganize chromatin by writing activating histone marks, erasing repressive marks, and remodeling nucleosomes.

All of these steps pave the way for recruitment of the transcription machinery to the promoter region (Fig. 6.2c) [34, 111] including RNA Polymerase II, General Transcription Factors, and a specific subunit (DRIP205/TRAP220) of the multisubunit Mediator complex. According to recent studies, the transcription machinery assembles sequentially with nucleotide excision repair (NER) factors, in order to achieve optimal histone modifications, and thus, efficient RNA synthesis [77].

According to recent chromatin conformation capture technologies, RARs that bind to different enhancer elements of a gene can form loops [25]. An emerging view is that RARs, similar to other TFs, promote the formation of long range

chromatin loops, bridging genomic loci located even on different chromosomes, thus creating hot spots of transcription [115].

Turn "OFF" of Transcription

After the "ON" switch, transcription of the RA target genes has to be terminated. Several scenarios have been proposed, but it is still unclear whether termination activities are gene or cell-specific. One possibility is that liganded RARs recruit unconventional coregulators with LxxLL motifs which, in contrast to classical p160 SRCs, inhibit, rather than activate, the transcriptional activity of RARs (Fig. 6.2d). These coregulators include the receptor interacting protein of 140 kDa (RIP140/NRIP1) [62], the preferentially expressed antigen in melanoma (PRAME) [38], and the transcription Intermediary factor-1 alpha (TIF1α/Trim24) [75]. The mechanism of TIF1α-mediated repression has not been elucidated yet [67], but the repressive activities of RIP140 and PRAME have been attributed to the recruitment of HDACs and PcG proteins, respectively [38, 132].

According to another scenario, an efficient way to limit RAR function and/or to signal the end of the transcriptional process would be the degradation of RARs and RXRs by the ubiquitin proteasome system [126] (Fig. 6.2d). Supporting such a hypothesis, RARs have been shown to be ubiquitinated and degraded by the proteasome through the recruitment of TRIP1/SUG-1, which is a subunit of the 19S regulatory sub complex of the proteasome with an ATPase activity [41, 52].

Development of the Field: RARs also Have Non-canonical Extra-Nuclear Effects, Which Are Integrated in the Nucleus

RA Activates Kinase Signaling Pathways via a Pool of RARs Present in Membrane Lipid Rafts

It is now appreciated that RARs have additional extra-nuclear and non-transcriptional effects that activate kinase signaling pathways [4]. Studies from several laboratories have demonstrated that RA rapidly (within minutes after RA addition) and transiently activates several kinase cascades. RA activates p38 mitogen activated kinase (MAPK) in fibroblasts, mouse embryo carcinoma cells, mammary breast tumor cells, and leukemia cells [5, 25, 52, 100]. RA activates the p42/p44 MAPKs (also called Erks) in neurons, Sertoli cells, and embryonic stem cells [30, 33, 59, 87, 123].

Since the RA-induced activation of the MAPK pathways occurs after the activation of upstream cascades involving RhoGTPases [5, 33, 100], PI3 kinase and/or protein kinase B (PKB)/Akt [31, 87, 93] in the cytosol, it has been suggested that the RA-induced cytosolic activities must involve an atypical, non-genomic event similar to that described for steroid NRs [82, 99]. In line with this concept, RARα

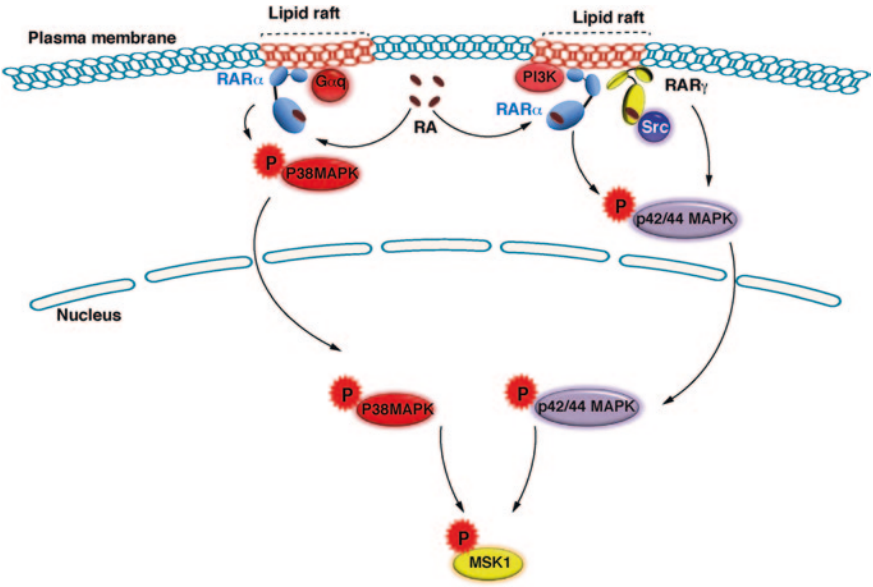

Fig. 6.3 Activation of kinase cascades by an extra nuclear pool of RARs. A subpopulation of RARα is present in membrane lipid rafts. Depending on the cell type, in response to RA, this pool of RARα can either interact with Gαq proteins or with PI3K to activate the p38 or p42/p44 MAPK pathways respectively. In other cell types, RARγ can also activate the p42/p44MAPK pathway via Src. Then the activated MAPKs translocate into the nucleus where they phosphorylate and activate MSK1

proteins have recently been found in lipid rafts isolated from the membranes of several cell types [87, 100] (Fig. 6.3). Moreover, the activation of p38MAPK has been shown to involve the interaction of RARα present in lipid rafts with Gαq proteins [100] (Fig. 6.3). However, the activation of Erks by RA did not involve Gαq proteins (Piskunov et al., unpublished results), but rather PI3K [31, 87, 93] or the *Src* kinase [33] (Fig. 6.3). Thus depending on the cell type, the extra nuclear effects of RA appear to involve different mechanisms and kinase cascades.

Once activated by RARα, p38MAPK and Erks translocate to the nucleus where they phosphorylate MSK1 (Fig. 6.3) [99]. Finally MAPKs and MSK1 phosphorylate several nuclear factors involved in the expression of RA-target genes, including RARs themselves and their coregulators.

In the Nucleus, RARs Are Rapidly Phosphorylated by a Cascade of Kinases

A few years after the cloning of RARs, it emerged that these receptors are also phosphoproteins [45, 106, 107, 109, 110]. However, at that time, the analysis of RAR phosphorylation was a challenging task because of its highly dynamic nature

and also because of the low ratio of phosphorylated *versus* non-phosphorylated RARs that are found in vivo [71]. The early studies of RAR phosphorylation required radioactive material and large amounts of recombinant NRs overexpressed in cultured cells or of bacterially expressed NRs purified and phosphorylated in vitro with different kinases. Though technically limited, these studies resulted in the identification of a number of phosphorylation sites in RARs and RXRs [1, 79, 105, 106, 110, 121]. Two main RAR phosphorylation sites were identified in intrinsically disordered regions; one in the loop between helices 9 and 10 of the LBD (S369 in RARα) and the other one in the NTD (S77 in RARα) [25] (Fig. 6.1a). The serine located in the LBD belongs to an Arginine-Lysine-rich motif and can be phosphorylated by several kinases such as the cyclic AMP dependent Protein Kinase (PKA) [110, 114] or MSK1 [25, 112]. In contrast, the serine located in the NTD belongs to a proline-rich motif and is phosphorylated by cdk7/cyclin H [106, 130], a kinase that belongs to the CAK subcomplex of the general transcription factor TFIIH. Most importantly, the correct positioning of the cdk7 kinase and thereby the efficiency of the NTD phosphorylation by cdk7 relies on the docking of cyclin H at a specific site in the LBD located in L8-9 and the N-terminal part of H9 [15] (Fig. 6.1b).

More recently, the emergence of new methods for enrichment of phosphopeptide samples, as well as the availability of phosphospecific antibodies, made it possible to analyze endogenous RAR phosphorylation. These studies revealed that the serines located in the LBD and in the NTD are both rapidly phosphorylated in response to RA via a cascade of kinases (Fig. 6.4). First, RA-activated MSK1 phosphorylates RARα at the serine located in the LBD [25]. Then, phosphorylation of this residue promotes phosphorylation of the serine located in the NTD through subtle conformational changes [25, 43]. Molecular dynamic simulations of the RARα LBD showed that phosphorylation of S369 (located in loop L9-10) leads to changes in the structural dynamics of the cyclin H binding site (composed of loop L8-9) situated at a 30 Å distance. This change in dynamics has been correlated with an increase in cyclin H binding and phosphorylation of the NTD at S77 by cdk7 [29, 112]. Thus, the coordinated phosphorylation of RARα results from a coordinated cascade that can be explained by changes in the structural features of the molecule.

This phosphorylation cascade has been described in cells that respond to RA via the activation of p38MAPK [25]. Whether it also occurs in cells that respond via the activation of Erks requires further investigations. Remarkably, the two phosphorylation sites are conserved between the mammalian RAR subtypes (α, β and γ) [112] (Fig. 6.1a) and the RARγ subtype is also phosphorylated at the same residues [9, 70, 110] through a similar cascade (our unpublished results). It is interesting to note that the serine residue located in the NTD has been conserved during evolution of chordates, indicating that the phosphorylation of this residue is likely important for RARs activity. In contrast, the serine residue located in the LBD is not present in non-mammalian RARs, suggesting that in other vertebrates, the phosphorylation cascade described above does not occur. Consequently, in other vertebrates, the phosphorylation of the NTD would be controlled by different regulatory circuits [112].

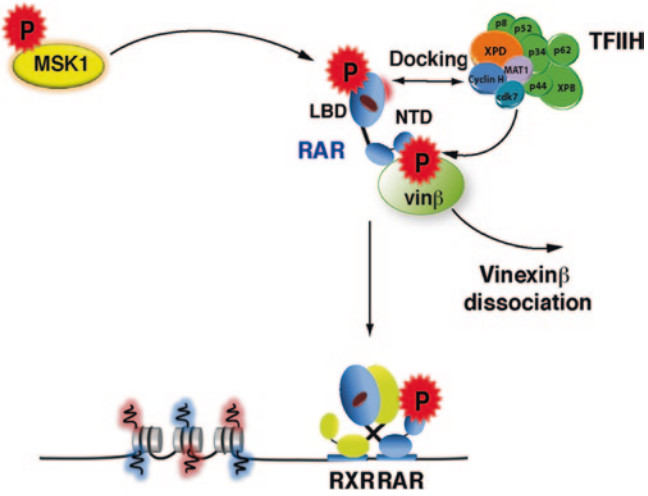

Fig. 6.4 RARs are phosphorylated by a cascade of cascades. Activated MSK1 phosphorylates RARs at a serine residue located in the LBD (loop L9-10). Phosphorylation of this residue induces conformational changes in loop L8-9, which promote the binding of the cyclin H subunit of the CAK subcomplex of TFIIH. Consequently the cdk7 kinase can phosphorylate the serine residue located in the NTD. In the case of RARγ, phosphorylation of the NTD induces the dissociation of vinexin β. Finally the phosphorylated RAR can be recruited to response elements located in the promoters of target genes

Consequences of RARs Phosphorylation at the NTD: Dissociation of Coregulators and Degradation by the Proteasome

The NTD is an intrinsically disordered region [74, 131], but the serine residue of this domain belongs to a PRM (Fig. 6.1a), which can form polyproline helices and bind proteins with SH3 or WW domains [65, 84, 136]. Recently, our laboratory identified vinexinβ as a new binding partner for the RARγ PRM [17]. Vinexinβ is an adaptor protein characterized by the presence of three SH3 domains, the third C-terminal one interacting with the PRM of RARγ. Recently, the combination of nuclear magnetic resonance (NMR), Circular dichroïsm, small angle X-ray scattering (SAXS) and molecular dynamics simulations revealed that phosphorylation of the serine residue located in the PRM of RARγ changes the global hydrodynamic behavior of the polyproline helix and decreases the propensity of the PRM to bind SH3 domains (Kieffer et al., unpublished results). Consequently, vinexinβ dissociates from RARγ [70] (Fig. 6.4).

In addition, at the end of the transcriptional process, phosphorylation of the N-terminal serine residue of RARγ and of an additional one located at position -2 has been shown to promote the ubiquitination of the receptor and its subsequent degradation by the proteasome [52, 68]. This is a typical example of interplay between different posttranslational modifications [118].

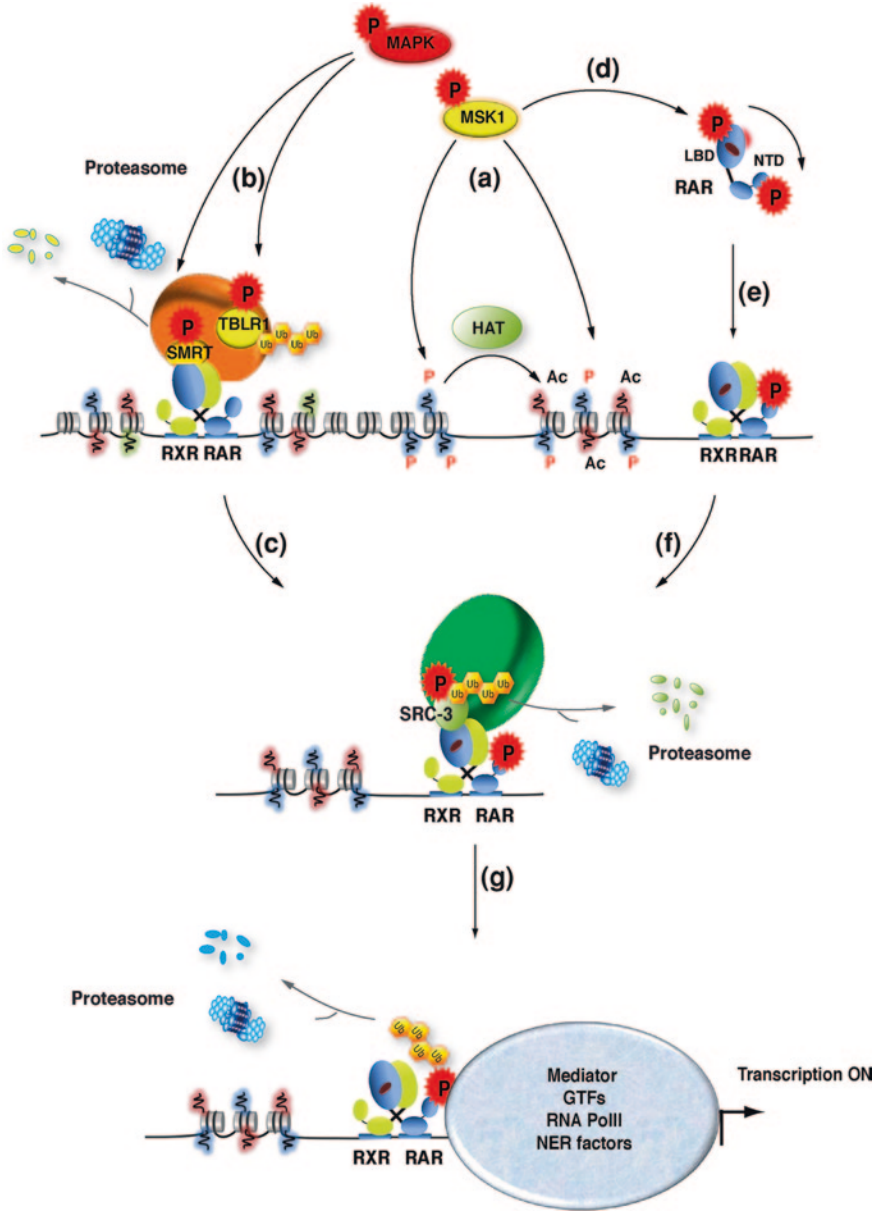
P
MAPK
P
MSK1
(d)
P
LBD
NTD
RAR
P
Proteasome
(b)
(a)
(e)
P
P
SMRT
TBLR1
Ub
HAT
Ac
P
Ac
RXR RAR
RXRRAR
(c)
(f)
P
Ub
SRC-3
Proteasome
P
RXR RAR
(g)
Proteasome
Ub
P
Mediator
GTFs
RNA PolII
NER factors
Transcription ON
RXR RAR

Fig. 6.5 Working models for the role of phosphorylations in the activation of RAR-target genes. When RARs already occupy RAREs in the absence of ligand, the RA-activated MAPKs phosphorylate components of the corepressor complexes such as SMRT and TBLR1. (**b**) Phosphorylation promotes their dissociation and their degradation by the proteasome, thus facilitating their exchange for coactivators. Then the coactivators such as SRC3 become also phosphorylated (**c**). Subsequently, they dissociate from RARs and are degraded, allowing the recruitment of other coregulators. Histones are also phosphorylated (**a**) and their phosphorylation induces the recruitment of HATs and remodeling complexes. Altogether these events cooperate to decompact chromatin at the promoters and pave the way for the recruitment of the transcription machinery (**g**). When RAREs are not occupied, phosphorylation of histones (**a**) and RARs (**d**) cooperate for the recruitment of RARs at their response elements (**e**). Next, liganded and DNA bound RARs recruit coactivator complexes, which decompact further chromatin. As above, coactivators phosphorylation (**f**) leads to their dissociation and degradation by the proteasome, thus facilitating the dynamics of coregulators exchange and the recruitment of the transcription machinery (**g**). Finally, RARs are degraded by the ubiquitin-proteasome system

The N-terminal PRM of RARα has been shown to bind the proline isomerase, Pin1, in a phospho-dependent manner [23, 53]. Pin1 is a WW domain-containing protein that is well known to induce *cis-trans* isomerization of proline residues that follow phosphorylated serines, and in so doing, to create new specific recognition sites for other interacting factors [135]. Pin1 interaction has been correlated with the degradation of RARα by the proteasome and the inhibition of RARα activity [53]. However the mechanism of the Pin1-mediated degradation of RARα remains to be defined.

Not only RARs but also Several Other Proteins Integrate MAPK Signaling and Become Phosphorylated in Response to RA

RA-activated MAPKs and MSK1 also phosphorylate other factors involved in RA target gene transcription, their phosphorylation favoring programs induced by the ligand. MSK1 is recruited at RAR-target promoters where it phosphorylates histone H3 tails at serines S10 and S28 (Fig. 6.5a). H3S10 phosphorylation has been correlated with the recruitment of HATs and the SWI/SNF ATP-dependent chromatin-remodeling complex [25, 99], while H3S28 phosphorylation induces the displacement of PcG complexes that maintain chromatin in a repressive state [47].

In addition, MSK1 and the upstream kinases, p38MAPK and Erks, phosphorylate several other actors in RA signaling, including RXRs, corepressors and coactivators. The important point is that phosphorylations alter protein structure, protein-protein interactions and protein activity, thus constituting an important cellular integration mechanism.

As an example, RA-activated p38MAPK rapidly phosphorylates rapidly RXRα at three residues located in the NTD [24, 128] by an as yet, unknown mechanism. MAPKs also phosphorylate components of corepressor complexes, such as SMRT and TBLR1 (Fig. 6.5b). Phosphorylation of SMRT induces its release from RARα [64] and disrupts its interaction with HDACs and other proteins in the corepressor complexes [129]. Consequently, the architecture, composition, and function of the

corepressor complexes are disrupted. A current model of TBLR1phosphorylation is that this adaptor mediates recruitment of the ubiquitin proteasome system to ubiquitinate and degrade NCoR, SMRT and HDACs [94, 97]. It has been proposed that the phosphorylation-dependent dissociation and degradation of components of the corepressor complexes mediates the exchange of corepressors with coactivators.

Coactivators are also phosphorylated in response to RA [54]. The p160 coactivator, SRC-3, is phosphorylated by p38MAPK at a serine residue located in the vicinity of the RAR binding domain. Phosphorylation of this residue results in dissociation of SRC3 from RARα. It also marks SRC-3 for ubiquitination and degradation by the proteasome [40] (Fig. 6.5c). This phosphorylation-ubiquitination-degradation process facilitates the dynamics of RARs-mediated transcription by allowing other coregulators to bind. The other components of the coactivator complexes, such as p300/CBP, can be also phosphorylated by several kinases in response to several signaling pathways [94, 96, 97], but whether they are phosphorylated in response to RA requires further investigation. Overall, coregulators respond to several signals that fine-tune their functional interactions with RARs and thus, their ability to modulate RAR transcriptional activity.

Current State of the Field

The RA-Induced Kinases and RAR Recruitment to DNA

Recent ChIP-seq profiles have confirmed that the occupancy of many RAREs is increased in response to RA [25, 70, 85, 89], and that there is a significant correlation between transcription activation and the binding of RAR/RXR heterodimers to DNA [89]. Though the mechanism of RAR/RXR recruitment to DNA in response to RA is still ill-defined, one cannot exclude a role for phosphorylation processes.

In the absence of RA, many RAREs are inaccessible due to a compact epigenetic landscape of chromatin. This implies that RAR binding requires an initial rapid modification of the chromatin environment in order to alleviate compaction and make the RAREs accessible. Among the candidates for chromatin reorganization, there are the RA-activated kinases. Indeed, RA-activated MSK1 is rapidly recruited to RAR-target genes promoters and phosphorylates histones H3 at serines S10 and S28 [25, 47]. Once phosphorylated, these serine residues are marks that induce the recruitment of remodeling complexes and the displacement of repressive complexes [47, 55, 56].

Another possibility is that the phosphorylation state of RARs themselves control their recruitment to DNA. To validate such a hypothesis, RNA-seq and ChIP experiments were performed with mutant mouse embryonic stem cell (mESC) lines expressing RAR phosphomutants in a RAR null background. Such experiments highlighted direct target genes whose expression is controlled by phosphorylation of the N-terminal serine residue of RARγ [2]. These studies also revealed that only RAREs with specific spacings recruit the phosphorylated form of RARγ in response to RA [2].

Then, the question was how phosphorylation of the N-terminal serine could promote recruitment of RARγ to specific RAREs in response to RA. It must be noted that this serine belongs to a PRM that is located in the vicinity of the DBD and which interacts with vinexin β, a repressor of RARγ-mediated transcription (see discussion, above). Remarkably, vinexin β bound to the nonphosphorylated form of RARγ prevents DNA binding, while vinexin β dissociation upon RARγ phosphorylation allows the binding of the receptor to DNA [70] (Fig. 6.4). Thus phosphorylation of the NTD that occurs in response to RA would promote DNA binding via the dissociation of proteins that occlude the DBD.

A Working Model for the Role of Phosphorylation in the Activation of RAR-Target Genes

RA-induced phosphorylation processes play an important role in the expression of RAR-target genes through modulating RAR recruitment to DNA, the sequential recruitment of the different classes of coregulators, and also the stability of the target proteins, thus constituting an important cellular integration mechanism. Two models can be proposed for the role of phosphorylation in the activation of RAR target genes. One for RARs already bound to DNA and another for RARs that are recruited to RAREs in response to RA (Fig. 6.5).

When RARs are constitutively bound to their DNA targets, ligand binding is the crucial molecular event that switches transcription from repression to activation via coregulator exchanges and chromatin reorganization. However it is now evident that RA-activated kinases provide additional layers of regulation for this switch through a phosphorylation code examplified by the phosphorylation of the corepressors and coactivators that promotes their dissociation from RARs and their degradation by the proteasome (Fig. 6.5b, c). Such a code facilitates the exchange of coregulator complexes and the reorganization of the epigenetic landscape. The kinases also phosphorylate histones, introducing additional marks for the recruitment of activating complexes and dissociation of repressive cofactors (Fig. 6.5a).

Not all RAREs are occupied in the absence of RA due to a compact genetic landscape. In this case, it is hypothesized that RA-activated kinases promote recruitment of RARs to DNA by first, phosphorylating histones, a process that alleviates chromatin compaction (Fig. 6.5a). The RA-activated kinases also phosphorylate RARs, but this process controls the recruitment of the receptors only to a subset of RAREs with a specific spacing (Fig. 6.5d, e). Additional approaches are required to investigate why phosphorylation controls RARs recruitment only to certain RAREs and not to the others. When RARs are recruited to DNA, it is evident that phosphorylation of the different coactivators also facilitates the dynamics of their association-dissociation for further chromatin decompaction and recruitment of transcription machinery (Fig. 6.5f, g). Finally, the phosphorylation of RARs signals their degradation by the ubiquitin proteasome system in order to stop the transcriptional process [16] (Fig. 6.5g).

Relevance: In vivo Relevance of the Cross Talk Between the Nuclear and Extra-Nuclear Effects of RARs

Embryonic Development and cell differentiation

Several genetic approaches performed in animals demonstrate that RARs and RXRs are the conductors of RA signaling during development [86, 113]. However, without further development of appropriate technology, current animal models cannot be used to study the role of RAR and RXR phosphorylation due to the complexity of the processes and signaling pathways. Instead, cell differentiation models have provided interesting tools to study the influence of RAR phosphorylation. These have included mouse embryo carcinoma cells (F9 cell line) which markedly resemble embryonic cells from the blastocyst and differentiate into primitive, parietal or visceral endoderm-like cells [18] after RA addition. They also included mouse embryonic stem cells that are pluripotent cells which self-renew indefinitely and have the propensity to differentiate in vitro into a larger variety of cell types [58, 134], such as neurons in response to RA [13].

Experiments from our laboratory revealed that differentiation of F9 cells into primitive endoderm [125] and ES cells into neurons [2] involves the RARγ2 subtype. Most interestingly, the generation of stable rescue cell lines expressing RARγ2 phosphomutants in a RARγ null background indicated that phosphorylation of the RARγ2 NTD is critically required for the RA-induced differentiation of these cells [2, 125]. Moreover, recent genome wide RNA-seq analysis experiments highlighted a subset of genes belonging to early phosphoRAR-regulated gene programs that are critical for triggering the effects of RA [2, 3]. These data suggest an important role for RAR phosphorylation in RA signaling, and pave the way for further investigations during embryonic and tissue development.

Cancer and Diseases

Available evidence suggests that the integrity of signaling pathways is required for the proper activity of RARs. Consequently, one can speculate that deregulation of the "*kinome*" would have deleterious downstream effects. Accordingly, in Xeroderma Pigmentosum patients, who are characterized by mutations affecting subunits of the general transcription factor, TFIIH, cdk7 does not efficiently phosphorylate RARα. This deficient phosphorylation has characteristic downstream consequences on the expression of RAR target genes [66] and has been correlated at least in part, to clinical abnormalities observed in patients.

In addition, in several cancers characterized by amplified or deregulated cytosolic kinase cascades [14], ending at Akt or MAPKs [119, 127] RARα has been shown to be aberrantly phosphorylated [121, 122]. Moreover, the RA-induced activation of the MAPK pathway is abrogated [100]. Subsequently, RARα is

degraded and/or its transcriptional activity is suppressed. Similarly, in hepatocellular carcinoma, RXRα is aberrantly phosphorylated in its LBD, with characteristic inhibition of its transcriptional activity [88]. Thus, one can postulate that aberrant kinome signaling and RAR/RXR phosphorylation and activity may correlate with tumoral growth and/or RA resistance [37].

There is an increasingly body of evidence indicating that RA signaling plays an important role in brain function, such as synaptic plasticity and learning and memory via transcriptional effects [72], as well as through non genomic effects involving a pool of RARα present in dendrites that becomes phosphorylated in response to RA [6, 30, 101]. An interesting observation is that RA signaling is also involved in the pathophysiology of Alzheimer's disease [72]. Alzheimer's disease is complex, but its striking and increasingly important characteristic is the aberrant expression and activity of several protein kinases [35, 61]. Therefore, one can suggest that in this disease, aberrant phosphorylation of RARα could have consequences on both its transcriptional activity and its extra nuclear effects on synaptic plasticity.

In conclusion, RA signaling and RAR phosphorylation represent potentially exploitable pathways for devising novel therapies in several diseases, including the Alzheimer disease [120].

Relevance in the Biology of Other Nuclear Receptors

According to the classical model, all-*trans* RA is channeled to RARs in the nucleus via the cellular RA-binding protein CRABPII, which is a small cytosolic protein belonging to the family of intracellular lipid binding proteins (iLBP) [26, 32, 36]. Such a process markedly facilitates formation of the liganded receptor. However, recent studies revealed that in certain CRABPII-deficient, cell types, such as brain, adipose tissue, skeletal muscle and skin, RA binds another fatty acid-binding protein of the iLBP family, FABP5. The interesting point is that upon RA binding, FABP5 does not deliver the ligand to RARs, but to the Peroxisome Proliferator-Activated Receptor β/δ (PPARβ/δ), another nuclear receptor, resulting in the regulation of genes that are not direct RAR targets [11, 116, 117]. Consequently, new functions of RA in the regulation of energy homeostasis and insulin responses were revealed [12]. PPARs are also known to be phosphoproteins [27] and to have nongenomic effects [83], but the β/δ subtype is the least studied in terms of phosphorylation. Whether it becomes phosphorylated in response to RA requires further investigation.

Some in vitro studies suggest that RA signaling could be mediated by other nuclear factors such as the Retinoic acid receptor related Orphan Receptor beta (RORβ) [124], the Chicken Ovalbumin Upstream Promoter Transcription Factor (COUP-TFII) [69] or the Testicular Receptors (TR2/4) [140], which are also able to integrate several signaling pathways [46, 63]. However, the in vivo relevance of such observations remains to be determined as well as whether these receptors can be also phosphorylated in response to RA.

Future Directions: What Is Still Left to do

The regulation of RAR-target gene activation by RA is controlled not only by simple on/off conformational switches of RARs, but also by kinase signaling pathways. Importantly, these signaling pathways target several actors in retinoid regulatory processes through phosphorylations that fine-tune the RA response via rapid changes in chromatin organization, RAR dynamics, coregulator interactions, and structural and functional shifts in protein-DNA interactions. The future challenges are to connect these data directly with new highly sensitive, real-time or large-scale technologies in order to get novel, critical information about the influence of phosphorylations on the regulation of RARs and RXRs activity. The last-generation dual linear ion trap mass spectrometers coupled with the Orbitrap technology should allow the identification of new phosphorylation sites in endogenous RARs, RXRs and their coregulators, and should provide information about their regulation by RA. Biophysical approaches, such as NMR, coupled to molecular dynamics simulations are other promising tools to investigate how phosphorylations fine-tune the structure of RARs and RXRs to control their recruitment to RAREs with specific spacings, but not to others with different spacings and/or sequences.

Finally, the recent TALEN (Transcription Activator-like Effector Nucleases) or CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-based technologies [44] should make possible the generation of point mutations at phosphorylation sites in vivo, providing a more powerful tool than the classical re-expression of a mutant in a null background. These tools coupled to RNA-seq, ChIP-seq and quantitative proteomics should provide interesting information about downstream gene expression and changes in protein complexes induced by RAR phosphorylation. Large-scale and quantitative phosphorylation screens of RARs, combined with other large-scale data sets, should pave the way to breakthroughs in disease-related research. In conclusion, further insights into the effects of RA will likely continue to reveal new targets and mechanisms that will help explain their pleiotropic effects and how these features might be manipulated in the treatment of metabolic disorders.

Acknowledgments Funds from the Agence Nationale pour la Recherche (ANR-05-BLAN-0390-02 and ANR-09-BLAN-0297-01), the Foundation pour la Recherche Medicale (FRM, DEQ20090515423), the Institut National du Cancer (INCa-PLO7-96099 and PL09-194) and the Association pour la Recherche sur le Cancer (ARC 3169, ARC SL220110603474) supported this work. This study was also supported by the grant ANR-10-LABX-0030-INRT, a French State fund managed by the ANR under the frame programme Investissements d'Avenir labelled ANR-10-IDEX-0002-02. INCA supported ZA while FRM and the lady TATA Memorial Trust supported AP.

References

1. Adam-Stitah S, Penna L, Chambon P, Rochette-Egly C (1999) Hyperphosphorylation of the retinoid X receptor alpha (RXRa) by activated c-Jun N-terminal Kinases (JNKs). J Biol Chem 274:18932–18941
2. Al Tanoury Z, Gaouar S, Piskunov A, Ye T, Urban S, Jost B, Keime C, Davidson I, Dierich A, Rochette-Egly C (2014) Phosphorylation of the retinoic acid receptor RARγ2 is crucial for the neuronal differentiation of mouse embryonic stem cells. J Cell Sci 127:2095–2105

3. Al Tanoury Z, Piskunov A, Andriamoratsiresy D, Gaouar S, Lutzing R, Ye T, Jost B, Keime C, Rochette-Egly C (2013a) Genes involved in cell adhesion and signaling: a new repertoire of retinoic acid receptors target genes in mouse embryonic fibroblasts. J Cell Sci 127:521–533
4. Al Tanoury Z, Piskunov A, Rochette-Egly C (2013b) Vitamin A and retinoid signaling: genomic and nongenomic effects: thematic review series: fat-soluble vitamins: vitamin A. J Lipid Res 54:1761–1775
5. Alsayed Y, Uddin S, Mahmud N, Lekmine F, Kalvakolanu DV, Minucci S, Bokoch G, Platanias LC (2001) Activation of Rac1 and the p38 mitogen-activated protein kinase pathway in response to all-trans-retinoic acid. J Biol Chem 276:4012–4019
6. Aoto J, Nam CI, Poon MM, Ting P, Chen L (2008) Synaptic signaling by all-trans retinoic acid in homeostatic synaptic plasticity. Neuron 60:308–320
7. Ball LJ, Kuhne R, Schneider-Mergener J, Oschkinat H (2005) Recognition of proline-rich motifs by protein-protein-interaction domains. Angew Chem Int Ed Engl 44:2852–2869
8. Balmer JE, Blomhoff R (2005) A robust characterization of retinoic acid response elements based on a comparison of sites in three species. J Steroid Biochem Mol Biol 96:347–354
9. Bastien J, Adam-Stitah S, Riedl T, Egly JM, Chambon P, Rochette-Egly C (2000) TFIIH interacts with the retinoic acid receptor gamma and phosphorylates its AF-1-activating domain through cdk7. J Biol Chem 275:21896–21904
10. Bastien J, Rochette-Egly C (2004) Nuclear retinoid receptors and the transcription of retinoid-target genes. Gene 328:1–16
11. Berry DC, Noy N (2007) Is PPARbeta/delta a retinoid receptor? PPAR Res 2007:73256
12. Berry DC, Noy N (2009) All-trans-retinoic acid represses obesity and insulin resistance by activating both peroxisome proliferation-activated receptor beta/delta and retinoic acid receptor. Mol Cell Biol 29:3286–3296
13. Bibel M, Richter J, Lacroix E, Barde YA (2007) Generation of a defined and uniform population of CNS progenitors and neurons from mouse embryonic stem cells. Nat Protoc 2:1034–1043
14. Blume-Jensen P, Hunter T (2001) Oncogenic kinase signalling. Nature 411:355–365
15. Bour G, Gaillard E, Bruck N, Lalevee S, Plassat JL, Busso D, Samama JP, Rochette-Egly C (2005) Cyclin H binding to the RARalpha activation function (AF)-2 domain directs phosphorylation of the AF-1 domain by cyclin-dependent kinase 7. Proc Natl Acad Sci USA 102:16608–16613
16. Bour G, Lalevee S, Rochette-Egly C (2007) Protein kinases and the proteasome join in the combinatorial control of transcription by nuclear retinoic acid receptors. Trends Cell Biol 17:302–309
17. Bour G, Plassat JL, Bauer A, Lalevee S, Rochette-Egly C (2005) Vinexin beta Interacts with the non-phosphorylated AF-1 Domain of retinoid receptor gamma (RARg) and represses RARg-mediated transcription. J Biol Chem 280:17027–17037
18. Bour G, Taneja R, Rochette-Egly C (2006) Mouse embryocarcinoma F9 cells and retinoic acid. A model to study the molecular mechanisms of endodermal differentiation. In: Taneja R (ed) Nuclear Receptors in development, vol 16. Elsevier Press Inc, Netherlands, pp 211–253
19. Bourguet W, Germain P, Gronemeyer H (2000) Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications. Trends Pharmacol Sci 21:381–388
20. Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D (1995) Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha. Nature 375:377–382
21. Bourguet W, Vivat V, Wurtz JM, Chambon P, Gronemeyer H, Moras D (2000) Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. Mol Cell 5:289–298
22. Brelivet Y, Rochel N, Moras D (2012) Structural analysis of nuclear receptors: from isolated domains to integral proteins. Mol Cell Endocrinol 348:466–473
23. Brondani V, Schefer Q, Hamy F, Klimkait T (2005) The peptidyl-prolyl isomerase Pin1 regulates phospho-Ser77 retinoic acid receptor alpha stability. Biochem Biophys Res Commun 328:6–13

24. Bruck N, Bastien J, Bour G, Tarrade A, Plassat JL, Bauer A, Adam-Stitah S, Rochette-Egly C (2005) Phosphorylation of the retinoid x receptor at the omega loop, modulates the expression of retinoic-acid-target genes with a promoter context specificity. Cell Signal 17:1229–1239
25. Bruck N, Vitoux D, Ferry C, Duong V, Bauer A, de The H, Rochette-Egly C (2009) A coordinated phosphorylation cascade initiated by p38MAPK/MSK1 directs RARalpha to target promoters. EMBO J 28:34–47
26. Budhu AS, Noy N (2002) 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 22:2632–2641
27. Burns KA, Vanden Heuvel JP (2007) Modulation of PPAR activity via phosphorylation. Biochim Biophys Acta 1771:952–960
28. Chambon P (1996) A decade of molecular biology of retinoic acid receptors. FASEB J 10:940–954
29. Chebaro Y, Amal I, Rochel N, Rochette-Egly C, Stote R, Dejaegere A (2013) Phosphorylation of the retinoic acid receptor alpha induces a mechanical allosteric regulation and changes in internal dynamics. Plos Comp Biol 9:e1003012
30. Chen N, Napoli JL (2008) All-trans-retinoic acid stimulates translation and induces spine formation in hippocampal neurons through a membrane-associated RARalpha. FASEB J 22:236–245
31. Cheung YT, Lau WK, Yu MS, Lai CS, Yeung SC, So KF, Chang RC (2009) Effects of all-trans-retinoic acid on human SH-SY5Y neuroblastoma as in vitro model in neurotoxicity research. Neurotoxicology 30:127–135
32. Delva L, Bastie JN, Rochette-Egly C, Kraiba R, Balitrand N, Despouy G, Chambon P, Chomienne C (1999) Physical and functional interactions between cellular retinoic acid binding protein II and the retinoic acid-dependent nuclear complex. Mol Cell Biol 19:7158–7167
33. Dey N, De PK, Wang M, Zhang H, Dobrota EA, Robertson KA, Durden DL (2007) CSK controls retinoic acid receptor (RAR) signaling: a RAR-c-SRC signaling axis is required for neuritogenic differentiation. Mol Cell Biol 27:4179–4197
34. Dilworth FJ, Chambon P (2001) Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription. Oncogene 20:3047–3054
35. Ding Y, Qiao A, Wang Z, Goodwin JS, Lee ES, Block ML, Allsbrook M, McDonald MP, Fan GH (2008) Retinoic acid attenuates beta-amyloid deposition and rescues memory deficits in an Alzheimer's disease transgenic mouse model. J Neurosci 28:11622–11634
36. Dong D, Ruuska SE, Levinthal DJ, Noy N (1999) Distinct roles for cellular retinoic acid-binding proteins I and II in regulating signaling by retinoic acid. J Biol Chem 274:23695–23698
37. Duong V, Rochette-Egly C (2011) The molecular physiology of nuclear retinoic acid receptors. From health to disease. Biochim Biophys Acta 1812:1023–1031
38. Epping MT, Wang L, Edel MJ, Carlee L, Hernandez M, Bernards R (2005) The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. Cell 122:835–847
39. Farboud B, Hauksdottir H, Wu Y, Privalsky ML (2003) Isotype-restricted corepressor recruitment: a constitutively closed helix 12 conformation in retinoic acid receptors beta and gamma interferes with corepressor recruitment and prevents transcriptional repression. Mol Cell Biol 23:2844–2858
40. Ferry C, Gaouar S, Fischer B, Boeglin M, Paul N, Samarut E, Piskunov A, Pankotai-Bodo G, Brino L, Rochette-Egly C (2011) Cullin 3 mediates SRC-3 ubiquitination and degradation to control the retinoic acid response. Proc Natl Acad Sci USA 108:20603–20608
41. Ferry C, Gianni M, Lalevee S, Bruck N, Plassat JL, Raska I Jr, Garattini E, Rochette-Egly C (2009) SUG-1 plays proteolytic and non-proteolytic roles in the control of retinoic acid target genes via its interaction with SRC-3. J Biol Chem 284:8127–8135
42. Fujiki R, Chikanishi T, Hashiba W, Ito H, Takada I, Roeder RG, Kitagawa H, Kato S (2009) GlcNAcylation of a histone methyltransferase in retinoic-acid-induced granulopoiesis. Nature 459:455–459

43. Gaillard E, Bruck N, Brelivet Y, Bour G, Lalevee S, Bauer A, Poch O, Moras D, Rochette-Egly C (2006) Phosphorylation by PKA potentiates retinoic acid receptor alpha activity by means of increasing interaction with and phosphorylation by cyclin H/cdk7. Proc Natl Acad Sci USA 103:9548–9553
44. Gaj T, Gersbach CA, Barbas CF 3rd (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31:397–405
45. Gaub MP, Rochette-Egly C, Lutz Y, Ali S, Matthes H, Scheuer I, Chambon P (1992) Immunodetection of multiple species of retinoic acid receptor alpha: evidence for phosphorylation. Exp Cell Res 201:335–346
46. Gay F, Barath P, Desbois-Le Peron C, Metivier R, Le Guevel R, Birse D, Salbert G (2002) Multiple phosphorylation events control chicken ovalbumin upstream promoter transcription factor I orphan nuclear receptor activity. Mol Endocrinol 16:1332–1351
47. Gehani SS, Agrawal-Singh S, Dietrich N, Christophersen NS, Helin K, Hansen K (2010) Polycomb group protein displacement and gene activation through MSK-dependent H3K27me3S28 phosphorylation. Mol Cell 39:886–900
48. Germain P, Altucci L, Bourguet W, Rochette-Egly C, Gronemeyer H (2003) Nuclear receptor superfamily: principles of signaling. Pure Appl Chem IUPAC 75:1619–1664
49. Germain P, Chambon P, Eichele G, Evans RM, Lazar MA, Leid M, De Lera AR, Lotan R, Mangelsdorf DJ, Gronemeyer H (2006a) International union of pharmacology. LX. Retinoic acid receptors. Pharmacol Rev 58:712–725
50. Germain P, Chambon P, Eichele G, Evans RM, Lazar MA, Leid M, De Lera AR, Lotan R, Mangelsdorf DJ, Gronemeyer H (2006b) International union of pharmacology. LXIII. Retinoid X receptors. Pharmacol Rev 58:760–772
51. Germain P, Staels B, Dacquet C, Spedding M, Laudet V (2006) Overview of nomenclature of nuclear receptors. Pharmacol Rev 58:685–704
52. Gianni M, Bauer A, Garattini E, Chambon P, Rochette-Egly C (2002) Phosphorylation by p38MAPK and recruitment of SUG-1 are required for RA-induced RARγ degradation and transactivation. EMBO J 21:3760–3769
53. Gianni M, Boldetti A, Guarnaccia V, Rambaldi A, Parrella E, Raska I Jr, Rochette-Egly C, Del Sal G, Rustighi A, Terao M, Garattini E (2009) Inhibition of the peptidyl-prolyl-isomerase Pin1 enhances the responses of acute myeloid leukemia cells to retinoic acid via stabilization of RARalpha and PML-RARalpha. Cancer Res 69:1016–1026
54. Gianni M, Parrella E, Raska I, Gaillard E, Nigro EA, Gaudon C, Garattini E, Rochette-Egly C (2006) P38MAPK-dependent phosphorylation and degradation of SRC-3/AIB1 and RARalpha-mediated transcription. EMBO J 25:739–751
55. Gillespie RF, Gudas LJ (2007) Retinoic acid receptor isotype specificity in F9 teratocarcinoma stem cells results from the differential recruitment of coregulators to retinoic response elements. J Biol Chem 282:33421–33434
56. Gillespie RF, Gudas LJ (2007) Retinoid regulated association of transcriptional co-regulators and the polycomb group protein SUZ12 with the retinoic acid response elements of Hoxa1, RARbeta(2), and Cyp26A1 in F9 embryonal carcinoma cells. J Mol Biol 372:298–316
57. Glass CK, Rosenfeld MG (2000) The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 14:121–141
58. Gudas LJ, Wagner JA (2011) Retinoids regulate stem cell differentiation. J Cell Physiol 226:322–330
59. Gupta P, Ho PC, Huq MM, Ha SG, Park SW, Khan AA, Tsai NP, Wei LN (2008) Retinoic acid-stimulated sequential phosphorylation, PML recruitment, and SUMOylation of nuclear receptor TR2 to suppress Oct4 expression. Proc Natl Acad Sci USA 105:11424–11429
60. Hauksdottir H, Farboud B, Privalsky ML (2003) Retinoic acid receptors beta and gamma do not repress, but instead activate target gene transcription in both the absence and presence of hormone ligand. Mol Endocrinol 17:373–385
61. Hilgeroth A, Tell V, Kramer S, Totzke F, Schachtele C (2013) Approaches to a multitargeting drug development: first profiled 3-Ethoxycarbonyl-1-aza-9-oxafluorenes representing a perspective compound class targeting alzheimer disease relevant kinases CDK1, CDK5 and GSK-3beta. Med Chem

62. Hu X, Chen Y, Farooqui M, Thomas MC, Chiang CM, Wei LN (2004) Suppressive effect of receptor-interacting protein 140 on coregulator binding to retinoic acid receptor complexes, histone-modifying enzyme activity, and gene activation. J Biol Chem 279:319–325
63. Jetten AM, Kurebayashi S, Ueda E (2001) The ROR nuclear orphan receptor subfamily: critical regulators of multiple biological processes. Prog Nucleic Acid Res Mol Biol 69:205–247
64. Jonas BA, Varlakhanova N, Hayakawa F, Goodson M, Privalsky ML (2007) Response of SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) and N-CoR (nuclear receptor corepressor) corepressors to mitogen-activated protein kinase kinase kinase cascades is determined by alternative mRNA splicing. Mol Endocrinol 21:1924–1939
65. Kay BK, Williamson MP, Sudol M (2000) The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains. FASEB J 14:231–241
66. Keriel A, Stary A, Sarasin A, Rochette-Egly C, Egly JM (2002) XPD mutations prevent TFIIH-dependent transactivation by nuclear receptors and phosphorylation of RARalpha. Cell 109:125–135
67. Khetchoumian K, Teletin M, Tisserand J, Mark M, Herquel B, Ignat M, Zucman-Rossi J, Cammas F, Lerouge T, Thibault C, Metzger D, Chambon P, Losson R (2007) Loss of Trim24 (Tif1alpha) gene function confers oncogenic activity to retinoic acid receptor alpha. Nat Genet 39:1500–1506
68. Kopf E, Plassat JL, Vivat V, de The H, Chambon P, Rochette-Egly C (2000) 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 275:33280–33288
69. Kruse SW, Suino-Powell K, Zhou XE, Kretschman JE, Reynolds R, Vonrhein C, Xu Y, Wang L, Tsai SY, Tsai MJ, Xu HE (2008) Identification of COUP-TFII orphan nuclear receptor as a retinoic acid-activated receptor. PLoS Biol 6:e227
70. Lalevee S, Bour G, Quinternet M, Samarut E, Kessler P, Vitorino M, Bruck N, Delsuc MA, Vonesch JL, Kieffer B, Rochette-Egly C (2010) Vinexinss, an atypical "sensor" of retinoic acid receptor gamma signaling: union and sequestration, separation, and phosphorylation. FASEB J 24:4523–4534
71. Lalevee S, Ferry C, Rochette-Egly C (2010) Phosphorylation control of nuclear receptors. Methods Mol Biol 647:251–266
72. Lane MA, Bailey SJ (2005) Role of retinoid signalling in the adult brain. Prog Neurobiol 75:275–293
73. Laudet V, Gronemeyer H (2001) Nuclear receptor factsbook. Academic Press, London
74. Lavery DN, McEwan IJ (2005) Structure and function of steroid receptor AF1 transactivation domains: induction of active conformations. Biochem J 391:449–464
75. Le Douarin B, Zechel C, Garnier JM, Lutz Y, Tora L, Pierrat P, Heery D, Gronemeyer H, Chambon P, Losson R (1995) The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18. EMBO J 14:2020–2033
76. le Maire A, Teyssier C, Erb C, Grimaldi M, Alvarez S, de Lera AR, Balaguer P, Gronemeyer H, Royer CA, Germain P, Bourguet W (2010) A unique secondary-structure switch controls constitutive gene repression by retinoic acid receptor. Nat Struct Mol Biol 17:801–807
77. Le May N, Mota-Fernandes D, Velez-Cruz R, Iltis I, Biard D, Egly JM (2010) NER factors are recruited to active promoters and facilitate chromatin modification for transcription in the absence of exogenous genotoxic attack. Mol Cell 38:54–66
78. Le May N, Iltis I, Amé J, Zhovner A, Biard D, Egly JM, Schreiber V, Coin F (2012) Poly (ADP-ribose) glycohydrolase regulates retinoic acid receptor-mediated gene expression. Mol Cell 48:785–798
79. Lee HY, Suh YA, Robinson MJ, Clifford JL, Hong WK, Woodgett JR, Cobb MH, Mangelsdorf DJ, Kurie JM (2000) Stress pathway activation induces phosphorylation of retinoid X receptor. J Biol Chem 275:32193–32199
80. Lee MG, Villa R, Trojer P, Norman J, Yan KP, Reinberg D, Di Croce L, Shiekhattar R (2007) Demethylation of H3K27 regulates polycomb recruitment and H2A ubiquitination. Science 318:447–450

81. Lefebvre P, Martin PJ, Flajollet S, Dedieu S, Billaut X, Lefebvre B (2005) Transcriptional activities of retinoic acid receptors. Vitam Horm 70:199–264
82. Losel R, Wehling M (2003) Nongenomic actions of steroid hormones. Nat Rev Mol Cell Biol 4:46–56
83. Luconi M, Cantini G, Serio M (2010) Peroxisome proliferator-activated receptor gamma (PPARgamma): is the genomic activity the only answer? Steroids 75:585–594
84. Macias MJ, Wiesner S, Sudol M (2002) WW and SH3 domains, two different scaffolds to recognize proline-rich ligands. FEBS Lett 513:30–37
85. Mahony S, Mazzoni EO, McCuine S, Young RA, Wichterle H, Gifford DK (2011) Ligand-dependent dynamics of retinoic acid receptor binding during early neurogenesis. Genome Biol 12:R2
86. Mark M, Ghyselinck NB, Chambon P (2009) Function of retinoic acid receptors during embryonic development. Nucl Recept Signal 7:e002
87. Masia S, Alvarez S, de Lera AR, Barettino D (2007) Rapid, nongenomic actions of retinoic acid on phosphatidylinositol-3-kinase signaling pathway mediated by the retinoic acid receptor. Mol Endocrinol 21:2391–2402
88. Matsushima-Nishiwaki R, Okuno M, Adachi S, Sano T, Akita K, Moriwaki H, Friedman SL, Kojima S (2001) Phosphorylation of retinoid X receptor alpha at serine 260 impairs its metabolism and function in human hepatocellular carcinoma. Cancer Res 61:7675–7682
89. Mendoza-Parra MA, Walia M, Sankar M, Gronemeyer H (2011) Dissecting the retinoid-induced differentiation of F9 embryonal stem cells by integrative genomics. Mol Syst Biol 7:538
90. Mengeling BJ, Goodson ML, Bourguet W, Privalsky ML (2012) SMRTepsilon, a corepressor variant, interacts with a restricted subset of nuclear receptors, including the retinoic acid receptors alpha and beta. Mol Cell Endocrinol 351:306–316
91. Moutier E, Ye T, Choukrallah MA, Urban S, Osz J, Chatagnon A, Delacroix L, Langer D, Rochel N, Moras D, Benoit G, Davidson I (2012) Retinoic acid receptors recognise the mouse genome through binding elements with diverse spacing and topology. J Biol Chem 287:26328–26341
92. Osz J, Brelivet Y, Peluso-Iltis C, Cura V, Eiler S, Ruff M, Bourguet W, Rochel N, Moras D (2012) Structural basis for a molecular allosteric control mechanism of cofactor binding to nuclear receptors. Proc Natl Acad Sci USA 109:E588–E594
93. Pan J, Kao YL, Joshi S, Jeetendran S, Dipette D, Singh US (2005) Activation of Rac1 by phosphatidylinositol 3-kinase in vivo: role in activation of mitogen-activated protein kinase (MAPK) pathways and retinoic acid-induced neuronal differentiation of SH-SY5Y cells. J Neurochem 93:571–583
94. Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG (2004) A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. Cell 116:511–526
95. Perissi V, Jepsen K, Glass CK, Rosenfeld MG (2010) Deconstructing repression: evolving models of co-repressor action. Nat Rev Genet 11:109–123
96. Perissi V, Rosenfeld MG (2005) Controlling nuclear receptors: the circular logic of cofactor cycles. Nat Rev Mol Cell Biol 6:542–554
97. Perissi V, Scafoglio C, Zhang J, Ohgi KA, Rose DW, Glass CK, Rosenfeld MG (2008) TBL1 and TBLR1 phosphorylation on regulated gene promoters overcomes dual CtBP and NCoR/SMRT transcriptional repression checkpoints. Mol Cell 29:755–766
98. Perissi V, Staszewski LM, McInerney EM, Kurokawa R, Krones A, Rose DW, Lambert MH, Milburn MV, Glass CK, Rosenfeld MG (1999) Molecular determinants of nuclear receptor-corepressor interaction. Genes Dev 13:3198–3208
99. Piskunov A, Rochette-Egly C (2011) MSK1 and nuclear receptor signaling. In: MSKs, Arthur S, Vermeulen L (eds) Landes Biosciences, Austin
100. Piskunov A, Rochette-Egly C (2012) A retinoic acid receptor RARalpha pool present in membrane lipid rafts forms complexes with G protein alphaQ to activate p38MAPK. Oncogene 31:3333–3345
101. Poon MM, Chen L (2008) Retinoic acid-gated sequence-specific translational control by RARalpha. Proc Natl Acad Sci USA 105:20303–20308

102. Privalsky ML (2004) The role of corepressors in transcriptional regulation by nuclear hormone receptors. Annu Rev Physiol 66:315–360
103. Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, Moras D (1995) Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. Nature 378:681–689
104. Rochel N, Ciesielski F, Godet J, Moman E, Roessle M, Peluso-Iltis C, Moulin M, Haertlein M, Callow P, Mely Y, Svergun DI, Moras D (2011) Common architecture of nuclear receptor heterodimers on DNA direct repeat elements with different spacings. Nat Struct Mol Biol 18:564–570
105. Rochette-Egly C (2003) Nuclear receptors: integration of multiple signalling pathways through phosphorylation. Cell Signal 15:355–366
106. Rochette-Egly C, Adam S, Rossignol M, Egly JM, Chambon P (1997) Stimulation of RAR alpha activation function AF-1 through binding to the general transcription factor TFIIH and phosphorylation by CDK7. Cell 90:97–107
107. Rochette-Egly C, Gaub MP, Lutz Y, Ali S, Scheuer I, Chambon P (1992) Retinoic acid receptor-beta: immunodetection and phosphorylation on tyrosine residues. Mol Endocrinol 6:2197–2209
108. Rochette-Egly C, Germain P (2009) Dynamic and combinatorial control of gene expression by nuclear retinoic acid receptors. Nucl Recept Signal 7:e005
109. Rochette-Egly C, Lutz Y, Saunders M, Scheuer I, Gaub MP, Chambon P (1991) Retinoic acid receptor gamma: specific immunodetection and phosphorylation. J Cell Biol 115:535–545
110. Rochette-Egly C, Oulad-Abdelghani M, Staub A, Pfister V, Scheuer I, Chambon P, Gaub MP (1995) Phosphorylation of the retinoic acid receptor-alpha by protein kinase A. Mol Endocrinol 9:860–871
111. Rosenfeld MG, Lunyak VV, Glass CK (2006) Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. Genes Dev 20:1405–1428
112. Samarut E, Amal I, Markov G, Stote R, Dejaegere A, Laudet V, Rochette-Egly C (2011) Evolution of nuclear retinoic acid receptors alpha (RARa) phosphorylation sites. Serine gain provides fine-tuned regulation. Mol Biol Evol 28:2125–2137
113. Samarut E, Rochette-Egly C (2012) Nuclear retinoic acid receptors: conductors of the retinoic acid symphony during development. Mol Cell Endocrinol 348:348–360
114. Santos NC, Kim KH (2010) Activity of retinoic acid receptor-alpha is directly regulated at its protein kinase A sites in response to follicle-stimulating hormone signaling. Endocrinology 151:2361–2372
115. Schoenfelder S, Sexton T, Chakalova L, Cope NF, Horton A, Andrews S, Kurukuti S, Mitchell JA, Umlauf D, Dimitrova DS, Eskiw CH, Luo Y, Wei CL, Ruan Y, Bieker JJ, Fraser P (2010) Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. Nat Genet 42:53–61
116. Schug TT, Berry DC, Shaw NS, Travis SN, Noy N (2007) Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. Cell 129:723–733
117. Schug TT, Berry DC, Toshkov IA, Cheng L, Nikitin AY, Noy N (2008) Overcoming retinoic acid-resistance of mammary carcinomas by diverting retinoic acid from PPARbeta/delta to RAR. Proc Natl Acad Sci USA 105:7546–7551
118. Sims RJ 3rd, Reinberg D (2008) Is there a code embedded in proteins that is based on post-translational modifications? Nat Rev Mol Cell Biol 9:815–820
119. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177–182
120. Sodhi RK, Singh N (2013) All-trans retinoic acid rescues memory deficits and neuropathological changes in mouse model of streptozotocin-induced dementia of Alzheimer's type. Prog Neuropsychopharmacol Biol Psychiatry 40:38–46

121. Srinivas H, Juroske DM, Kalyankrishna S, Cody DD, Price RE, Xu XC, Narayanan R, Weigel NL, Kurie JM (2005) c-Jun N-terminal kinase contributes to aberrant retinoid signaling in lung cancer cells by phosphorylating and inducing proteasomal degradation of retinoic acid receptor alpha. Mol Cell Biol 25:1054–1069
122. Srinivas H, Xia D, Moore NL, Uray IP, Kim H, Ma L, Weigel NL, Brown PH, Kurie JM (2006) Akt phosphorylates and suppresses the transactivation of retinoic acid receptor alpha. Biochem J 395:653–662
123. Stavridis MP, Collins BJ, Storey KG (2010) Retinoic acid orchestrates fibroblast growth factor signalling to drive embryonic stem cell differentiation. Development 137:881–890
124. Stehlin-Gaon C, Willmann D, Zeyer D, Sanglier S, Van Dorsselaer A, Renaud JP, Moras D, Schule R (2003) All-trans retinoic acid is a ligand for the orphan nuclear receptor ROR beta. Nat Struct Biol 10:820–825
125. Taneja R, Rochette-Egly C, Plassat JL, Penna L, Gaub MP, Chambon P (1997) Phosphorylation of activation functions AF-1 and AF-2 of RAR alpha and RAR gamma is indispensable for differentiation of F9 cells upon retinoic acid and cAMP treatment. EMBO J 16:6452–6465
126. Tansey WP (2001) Transcriptional activation: risky business. Genes Dev 15:1045–1050
127. Tari AM, Lim SJ, Hung MC, Esteva FJ, Lopez-Berestein G (2002) Her2/neu induces all-trans retinoic acid (ATRA) resistance in breast cancer cells. Oncogene 21:5224–5232
128. Tarrade A, Bastien J, Bruck N, Bauer A, Gianni M, Rochette-Egly C (2005) Retinoic acid and arsenic trioxide cooperate for apoptosis through phosphorylated RXR alpha. Oncogene 24:2277–2288
129. Varlakhanova N, Hahm JB, Privalsky ML (2011) Regulation of SMRT corepressor dimerization and composition by MAP kinase phosphorylation. Mol Cell Endocrinol 332:180–188
130. Wang A, Alimova IN, Luo P, Jong A, Triche TJ, Wu L (2010) Loss of CAK phosphorylation of RAR{alpha} mediates transcriptional control of retinoid-induced cancer cell differentiation. FASEB J: Official Publ Fed Am Soc Exp Biol 24:833–843
131. Warnmark A, Treuter E, Wright AP, Gustafsson JA (2003) Activation functions 1 and 2 of nuclear receptors: molecular strategies for transcriptional activation. Mol Endocrinol 17:1901–1909
132. Wei LN, Hu X, Chandra D, Seto E, Farooqui M (2000) Receptor-interacting protein 140 directly recruits histone deacetylases for gene silencing. J Biol Chem 275:40782–40787
133. Westin S, Rosenfeld MG, Glass CK (2000) Nuclear receptor coactivators. Adv Pharmacol 47:89–112
134. Wilson V, Olivera-Martinez I, Storey KG (2009) Stem cells, signals and vertebrate body axis extension. Development 136:1591–1604
135. Wulf G, Finn G, Suizu F, Lu KP (2005) Phosphorylation-specific prolyl isomerization: is there an underlying theme? Nat Cell Biol 7:435–441
136. Zarrinpar A, Lim WA (2000) Converging on proline: the mechanism of WW domain peptide recognition. Nat Struct Biol 7:611–613
137. Zechel C, Shen XQ, Chambon P, Gronemeyer H (1994) Dimerization interfaces formed between the DNA binding domains determine the cooperative binding of RXR/RAR and RXR/TR heterodimers to DR5 and DR4 elements. EMBO J 13:1414–1424
138. Zechel C, Shen XQ, Chen JY, Chen ZP, Chambon P, Gronemeyer H (1994) The dimerization interfaces formed between the DNA binding domains of RXR, RAR and TR determine the binding specificity and polarity of the full-length receptors to direct repeats. EMBO J 13:1425–1433
139. Zhao Y, Lang G, Ito S, Bonnet J, Metzger E, Sawatsubashi S, Suzuki E, Le Guezennec X, Stunnenberg HG, Krasnov A, Georgieva SG, Schule R, Takeyama K, Kato S, Tora L, Devys D (2008) A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Mol Cell 29:92–101
140. Zhou XE, Suino-Powell KM, Xu Y, Chan CW, Tanabe O, Kruse SW, Reynolds R, Engel JD, Xu HE (2011) The orphan nuclear receptor TR4 is a vitamin A-activated nuclear receptor. J Biol Chem 286:2877–2885