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**Abstract**—Retinoid is a term for compounds that bind to and activate retinoic acid receptors (RAR $\alpha$ , RAR $\beta$ , and RAR $\gamma$ ), members of the nuclear hormone receptor superfamily. The most important endogenous retinoid is all-*trans*-retinoic acid. Retinoids regulate a wide variety of essential biological processes,

such as vertebrate embryonic morphogenesis and organogenesis, cell growth arrest, differentiation and apoptosis, and homeostasis, as well as their disorders. This review summarizes the considerable amount of knowledge generated on these receptors.

## Introduction

The retinoic acid receptors (RARs<sup>1</sup>) mediate both organismal and cellular effects of retinoids. “Retinoids” is a generic term that covers compounds including both naturally dietary vitamin A (retinol) metabolites and active synthetic analogs (Sporn et al., 1976; Chambon, 2005). Both experimental and clinical studies have revealed that retinoids regulate a wide variety of essential biological processes, such as vertebrate embryonic morphogenesis and organogenesis, cell growth arrest, differentiation and apoptosis, and homeostasis, as well as their disorders (Sporn et al., 1976; Blomhoff, 1994; Sporn et al., 1994; Kastner et al., 1995; Chambon, 2005). All-*trans*-retinoic acid (ATRA), the most potent biologically active metabolite of vitamin A, can both prevent and rescue the main defects caused by vitamin A defi-

ciency (VAD) in adult animals (Kastner et al., 1995). As early as 1925 preclinical studies demonstrated that VAD correlated with the development of squamous metaplasia in rodents (Wolbach and Howe, 1925). This and subsequent studies anticipated a strong rationale for the use of retinoids in the treatment and prevention of cancer (Hong and Sporn, 1997). The most impressive example of retinoid anticancer activity is the treatment of patients with acute promyelocytic leukemia (APL), a subtype of acute myelogenous leukemia, since upon addition of ATRA to the therapy approximately 72% of patients with APL can be cured (de Thé et al., 1990a; Degos and Wang, 2001; Lin et al., 1999).

## RARs

Retinoids exert their pleiotropic effects through the three RAR subtypes [RAR $\alpha$  (NR1B1), first identified in 1987 independently by Pierre Chambon’s and Ron Evans’s groups, RAR $\beta$  (NR1B2), and RAR $\gamma$  (NR1B3)] that originate from three distinct genes (Giguere et al., 1987; Petkovich et al., 1987; Chambon, 1996). For each RAR subtype, several isoforms exist that differ from one another in their N-terminal region A. These isoforms arise from the differential usage of two promoters and alternative splicing. The downstream promoters, referred to as P2, are induced by retinoids owing to the presence of a retinoic acid response element (RARE, see below). There are two major isoforms for RAR $\alpha$  ( $\alpha$ 1 and  $\alpha$ 2) and for RAR $\gamma$  ( $\gamma$ 1 and  $\gamma$ 2) and four major isoforms for RAR $\beta$  ( $\beta$ 1 and  $\beta$ 3 initiated at the P1 promoter and  $\beta$ 2 and  $\beta$ 4 initiated at the P2 promoter). RARs function as

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<sup>1</sup> Abbreviations: RAR, retinoic acid receptor; ATRA, all-*trans*-retinoic acid; VAD, vitamin A deficiency; APL, acute promyelocytic leukemia; RARE, retinoic acid response element; RXR, retinoid X receptor; DR, direct repeat; NCoR, nuclear receptor corepressor; SMRT, silencing mediator for retinoid and thyroid hormone receptors; CRABP, cellular retinoic acid-binding protein; HCAC, histone deacetylase; AF, activation function; AP-1, activator protein-1; 9CRA, 9-*cis* retinoic acid; RA, retinoic acid; PML, promyelocyte leukemia protein; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; PLZF, promyelocytic leukemia zinc finger.

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heterodimers with the three retinoid X receptors [RXR $\alpha$  (NR2B1), RXR $\beta$  (NR2B2), and RXR $\gamma$  (NR2B3)] (Mangelsdorf and Evans, 1995; Kastner et al., 1997; Mark et al., 1999). In vitro studies demonstrated that RXR-RAR heterodimers act as ligand-dependent transcriptional regulators by binding to the specific RARE DNA sequences found in the promoter region of retinoid target genes. RAREs correspond to direct repeats of polymorphic arrangements of the canonical motif 5'-PuG(G/T)CA separated by five (generally referred to as DR5) or one (DR1) or two (DR2) nucleotides (Leid et al., 1992; Mangelsdorf and Evans, 1995). In DR5 and DR2 elements, RXRs occupy the 5' element, whereas RARs occupy the 3' element (5'-RXR-RAR-3'). In contrast, the polarity of heterodimers is reversed in DR1 elements (5'-RAR-RXR-3') (Kurokawa et al., 1994; Rastinejad et al., 2000; Rastinejad, 2001). Strikingly, and contrary to DR2 or DR5 context (see below), specific RAR agonists do not induce the dissociation of corepressors from the RAR-RXR heterodimer bound to a DR1 leading to repressive activity (Kurokawa et al., 1995). DR5 elements were identified in the promoters of genes such as the *RAR $\beta$ 2* gene (de The et al., 1990b), several *Hox* genes that are key players in the specification of the antero-posterior axis during development (Boncinelli et al., 1991; Tabin, 1995; Dupe et al., 1997), and the cytochrome P450RAI (*CYP26*) gene whose product is implicated in the catabolism of ATRA (Loudig et al., 2000). DR2 elements were found in the promoters of the cellular retinol binding protein I (Smith et al., 1991) and *CRABPII* (Durand et al., 1992) genes, CRABPs functioning in retinoid storage and intracellular transport (for other retinoid target genes, see McCaffery and Drager, 2000; Laudet and Gronemeyer, 2002).

A molecular mechanism by which RXR-RAR heterodimers regulate transcription of target genes has been proposed (Glass and Rosenfeld, 2000). In the absence of RAR agonist, the RXR-RAR heterodimer recruits the corepressor proteins NCoR or SMRT and associated factors such as histone deacetylases (HDACs) or DNA-methyl transferases that may lead to an inactive condensed chromatin structure, preventing transcription. Upon RAR agonist binding, corepressors are released, and coactivator complexes such as histone acetyltransferases or histone arginine methyltransferases are recruited to activate transcription (Nagy et al., 1997; Hu and Lazar, 2000; Aranda and Pascual, 2001; Privalsky, 2001; McKenna and O'Malley, 2002; Perissi and Rosenfeld, 2005). Recently, poly(ADP-ribose) polymerase 1, which can interact directly with RAR $\alpha$ , has been shown to be indispensable to RAR-mediated transcription from the *RAR $\beta$ 2* promoter (Pavri et al., 2005).

Whereas RAR agonists can autonomously activate transcription through such heterodimers, RXRs are unable to respond to RXR-selective agonists (rexinoids) in the absence of RAR ligand. The molecular basis of this

phenomenon, referred to as RXR subordination or silencing, has been dissected. Agonist binding to RXR is unable to induce the dissociation of corepressor from the RXR-RAR heterodimers, preventing coactivator recruitment (Westin et al., 1998; Germain et al., 2002). A synergistic transcriptional activation is observed when RAR and RXR partners are simultaneously bound to agonists, indicating that RXRs are not transcriptionally silent partners in RXR-RAR heterodimers (Lotan et al., 1995b).

The essential role of gene silencing by RARs has been demonstrated for two developmental processes, namely the skeletal development in the mouse and head formation in *Xenopus* (for a review, see Weston et al., 2003), and is underscored by the pathogenesis of APL in which an inappropriate repression by oncogenic RAR $\alpha$  fusion proteins blocks myeloid differentiation leading to APL. The repressive model of unliganded heterodimers is based mainly on studies involving RAR $\alpha$ , which can strongly interact with corepressors. However, recent findings suggest differences in cofactor stoichiometry and patterns of interactions among the distinct RAR subtypes, as unliganded RAR $\beta$  was shown to poorly associate corepressors and to be a significant transcriptional activator, contrasting with the strong repressing activity of unliganded RAR $\alpha$  (Germain et al., 2002; Farboud et al., 2003; Hauksdottir et al., 2003).

RARs also integrate a variety of signaling pathways, notably through posttranslational modifications (Rochette-Egly and Chambon, 2001; Laudet and Gronemeyer, 2002; Rochette-Egly, 2003). Among these modifications, phosphorylation of RARs has been shown to play a critical role in the retinoid response. Both AF-1 domains and LBDs of RARs are substrates for various kinases activated by a variety of signals (Bastien and Rochette-Egly, 2004). Another particularly interesting feature of RARs has been revealed by the studies of several genes such as osteocalcin or collagenase showing the inhibition of the transcription factor complex activator protein-1 (AP-1)-driven transactivation by liganded RARs (Lafyatis et al., 1990; Nicholson et al., 1990; Schule et al., 1990; Chen et al., 1995; Resche-Rigon and Gronemeyer, 1998). However, the mechanism of this cross-talk remains elusive.

### Expression and Function of Retinoid Acid Receptors

In situ hybridization revealed the expression of all three RARs during mouse embryonic development. Whereas RAR $\alpha$  is present in most tissues, both RAR $\beta$  and RAR $\gamma$  expressions are more selective (Dolle et al., 1990). These differences in tissue distribution suggest that RARs have distinct physiological functions.

The specific role of each RAR has been studied in great detail in the RA-responsive F9 murine embryonal carcinoma cell line. Interestingly, F9 cells represent a simple

cell-autonomous model system for analyzing RAR signaling under in vitro conditions that mimics, at least to some extent, physiological processes occurring during early embryogenesis (for a review, see Rochette-Egly and Chambon, 2001). Both synthetic RAR isotype-selective ligands and knockouts of the individual RARs through homologous recombination followed by re-expression of wild-type or mutant RARs have been used. Overall these experiments revealed important insights regarding the complexity and the selectivity of retinoid signaling. In F9 cells RXR-RAR heterodimers are the functional units that selectively mediate the target gene expression and the differentiation and the growth arrest controlled by retinoids, and the AF-2 ligand-dependent transcriptional activity of RXRs is subordinated to their RAR heterodimeric partner. More specifically RXR $\alpha$ -RAR $\gamma$  heterodimers are necessary for growth arrest, visceral endodermal differentiation, and primitive endodermal differentiation, whereas RXR $\alpha$ -RAR $\alpha$  is required for parietal endodermal differentiation in the presence of cAMP. In addition the different roles of RAR phosphorylations have been revealed in the context of the differentiation induction in F9 cells. For instance, phosphorylation within the RAR $\gamma$  AF-1 activation domain is required for primitive endodermal differentiation and for induction of retinoid target genes, but in a differential promoter-dependent manner, and for degradation of RXR $\alpha$ -RAR $\gamma$  heterodimers by the ubiquitin-proteasome system (Taneja et al., 1997) (for reviews, see Rochette-Egly, 2003; Bastien and Rochette-Egly, 2004). Furthermore, the RAR $\beta$ 2-null F9 cell line exhibits no growth arrest in response to retinoids in contrast to wild-type, RAR $\alpha$ <sup>-/-</sup>, and RAR $\gamma$ <sup>-/-</sup> F9 cell lines (Faria et al., 1999). However, RAR knockouts may generate artificial functional redundancies between individual RARs that do not exist under wild-type conditions (Taneja et al., 1996). Overall these investigations with the F9 cells and previous gene transfection studies demonstrated that the individual RAR subtypes can have distinct activities even within the same cell line. In the same line, in vitro studies have shown that, even though other RAR subtypes are also expressed, RAR $\alpha$  agonists induce the inhibition of proliferation of some breast cancer cell lines and the differentiation of leukemic cells (Dawson et al., 1995; Chen et al., 1996).

The above studies on F9 were complemented by genetic strategies in the mouse to determine the function of RARs under physiological conditions. This was mainly performed by Pierre Chambon's laboratory by knockout of the three RAR subtypes as well as the eight RAR isoforms (see above) through homologous recombination in embryonic stem cells. In combination with pharmacological approaches using RAR antagonists to block the retinoid signaling pathway, the generation of such germline mutations has provided many valuable insights on the developmental functions of RARs (for comprehensive reviews, see Mark et al., 2004, 2005). However, because

of the functional redundancies observed between RARs artifactually generated by knockouts, the number of organs that need retinoids for their development might be underestimated, and these studies have failed to reveal many of the physiological functions of RARs, notably in adult animals. Despite this fact, they provided the genetic evidence that RARs transduced retinoid signals in vivo and revealed that the various RAR subtypes have distinct functionalities during embryogenesis. Briefly, all RAR single-null mutant mice are viable and altogether display some aspects of the postnatal and fetal VAD syndromes. Specifically, RAR $\alpha$ -null mutant males are sterile as a result of a degeneration of the seminiferous epithelium that inhibits spermatogenesis (Li et al., 1993; Lufkin et al., 1993). RAR $\beta$ -null mice display abnormalities in the vitreous body in eyes (Grondona et al., 1996) and impaired abilities in locomotion and motor coordination (Krezel et al., 1998). RAR $\gamma$  inactivation causes both skeletal and epithelial defects (Lohnes et al., 1993; Ghyselinck et al., 1997; Chapellier et al., 2002). In contrast to RAR single-null knockout mice, mutants lacking a pair of RAR subtypes (double-null mutants) or two or more isoforms belonging to distinct subtypes exhibit a number of defects leading to a dramatically reduced viability and all the known manifestations of the VAD syndrome. Also such genetic studies have revealed that retinoid signals are transduced by specific RXR $\alpha$ -RAR( $\alpha$ ,  $\beta$ , or  $\gamma$ ) heterodimers during development.

### Natural Retinoids and Synthetic Analogs

Natural retinoids are produced in vivo from the oxidation of vitamin A. Synthesis of retinoic acid from retinol is a two-step process in which alcohol dehydrogenases perform the oxidation of vitamin A to all-*trans*-retinaldehyde, followed by oxidation of the latter to ATRA by retinaldehyde dehydrogenases (of which four have been characterized, RALDH1–4), which is the rate-limiting step in its production. ATRA is in turn metabolized by CYP26 to hydroxylated metabolites that can also activate all three RARs (Fujii et al., 1997; White et al., 1997). However, genetic approaches using the RALDH1a2 null mutation and the CYP26 null mutation demonstrated that the main function of CYP26 is to degrade endogenous ATRA and to protect cells from excess ATRA rather than to synthesize active hydroxylated retinoids (Niederreither et al., 2002). RARs bind with high affinity not only ATRA but also 9-*cis* retinoic acid (9CRA), an isomerization product of ATRA. Whereas ATRA can bind only to RARs, 9CRA can bind to both RAR and RXR. However, because 9CRA has not been consistently detected in mammalian cells unless the medium contained ATRA, the consideration of 9CRA as a natural bioactive retinoid remains controversial (see "LXIII. Retinoid X Receptors" on page 760 of this issue).

Given the importance of the retinoid signaling pathway, a major research effort has been directed to the identification of potent synthetic retinoids leading to the generation of a panel of modulators with activities ranging from agonists to inverse agonists (Klein et al., 1996; Thacher et al., 2000; Kagechika and Shudo, 2005). Such configurationally and/or conformationally restricted analogs of ATRA are valuable tools for dissecting the role of each RAR in several processes. Retinoids were also used as therapeutic agents for the treatment and prevention of cancer and hyperproliferative diseases (see below) (Thacher et al., 2000; Altucci and Gronemeyer, 2001; Clarke et al., 2004a; Dawson, 2004; Vivat-Hannah and Zusi, 2005). The crystal structures of the LBDs of all three RARs bound to various ligands have been solved, providing molecular details of the determinants of both subtype selectivity and the agonist/antagonist-induced structural changes (Renaud et al., 1995; Bourguet et al., 2000; Germain et al., 2004). These 3D structure determinations together with comparison of RAR sequences revealed only three divergent residues into the ligand-binding pockets of all three RARs that are critical for the recognition of subtype-specific ligands. This finding has been confirmed by swapping of these residues (Gehin et al., 1999). Accordingly, it has been possible to generate entirely subtype-selective ligands but also molecules that have complex activities such as ligands that are RAR $\alpha$  and RAR $\gamma$  antagonists and RAR $\beta$  agonists (Chen et al., 1995; Germain et al., 2004). Interestingly, selective retinoids that dissociate the inhibition of AP-1 activity from the classic RARE-dependent activation of transcription have been identified (Fanjul et al., 1994; Chen et al., 1995). Such compounds are promising therapeutic agents and provide valuable tools to address the mechanism of the RAR/AP-1 cross-talk, the importance of which for growth control and cancer is now established.

### Diseases, Treatments, and Chemoprevention

The RARs have been associated with several diseases such as cancer or skin disorders on the basis of epidemiological, clinical, and experimental investigations in human and animals. Then retinoids are used in a variety of chemopreventive and chemotherapeutic settings. The recognized potential of the retinoids in skin disorders is demonstrated by the clinical use of ATRA, 9CRA, and 13-*cis*-retinoic acid for dermatological indications including acne, psoriasis, or photoaging (for reviews, see Thacher et al., 2000; Zouboulis, 2001; Dawson, 2004). In addition to these RA isomers, two synthetic retinoids are available for the treatment of stable plaque psoriasis [the RAR $\beta/\gamma$ -selective agonist tazarotene (AGN190168)] (Marks, 1997; McClelland, 1998) and for acne [adapalene (CD271)] (Galvin et al., 1998; Zhu et al., 2001).

Aberrant retinoid signaling mechanisms have been linked to cancer. The most direct implication of RAR in

human disease is given by APL, which is caused by a reciprocal chromosomal translocation between RAR $\alpha$  and promyelocyte leukemia protein (PML) human genes, leading to the alteration of the signaling of both RAR $\alpha$  and PML (de Thé et al., 1990a). The resulting fusion protein PML-RAR $\alpha$  displays increased binding efficiency to the transcriptional corepressors NCoR and SMRT compared with RAR $\alpha$ , inducing the recruitment of HDAC complexes and the silencing of RAR target genes. This process, in turn, arrests myelopoiesis at the promyelocyte stage and prevents the differentiation of APL cells, which might normally occur in the presence of endogenous ATRA. Importantly, the use of supraphysiological doses of ATRA has led to remission in patients with APL, revealing the potential of retinoids for chemotherapeutic applications. This successful therapy is supposed to overcome the negative effects of PML-RAR $\alpha$  by inducing the dissociation of silencing complexes from PML-RAR $\alpha$  and then the activation of differentiation processes. In addition, high concentrations of ATRA can induce postmaturation apoptosis through the induction of the tumor-selective death ligand tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, also called Apo2L), a most promising molecule in cancer research (Altucci and Gronemeyer, 2001). However, with this therapy some patients with APL have a relapse and become resistant to ATRA. Interestingly, the RAR $\alpha$ -selective agonist Am80 can induce complete remission in patients previously treated by ATRA who have had relapses, highlighting interest on the generation of even more selective retinoids (Kagechika et al., 1988; Tobita et al., 1997; Takeuchi et al., 1998).

The RAR $\alpha$  gene can translocate with other genes, such as the promyelocytic leukemia zinc finger (PLZF) gene product, that are insensitive to ATRA. In the case of the PLZF-RAR $\alpha$  fusion protein, the PLZF moiety is constitutively associated to corepressor complexes independently of ATRA, which is supposed to lead to the ATRA insensitivity.

Strong evidence supports the idea that retinoids pharmacologically prevent carcinogenesis in a variety of tissues. Retinoids are used as chemopreventive agents for the treatment of preneoplastic diseases such as oral leukoplakia, cervical dysplasia, and xeroderma pigmentosum (Lotan, 1996; Lippman and Lotan, 2000; Sun and Lotan, 2002). However, the promises of preclinical studies demonstrating the efficacy of retinoids did not consistently translate into clinical response for the treatment of other solid tumors. Interestingly, both experimental investigations and analyses of the natural course of solid human tumor development suggest that RAR $\beta$  may act as a potential tumor suppressor. Indeed, its expression is selectively lost in many neoplastic tissues, including non-small cell lung cancer, squamous cell carcinomas of the head and neck, and breast cancer (Castillo et al., 1997; Widschwendter et al., 1997; Xu et al., 1997a,b; Picard et al., 1999). The restoration of

RAR $\beta$  expression with concomitant retinoic treatment was associated with a clinical response of oral leukoplakia (Lotan et al., 1995a). Furthermore, a recently identified novel RAR $\beta$  isoform, referred to as RAR $\beta$ 1', which apparently arises from an alternative splicing of RAR $\beta$ 1, may function as a tumor suppressor gene in the lung with biological functions distinct from those of previously known RAR $\beta$  isoforms (Petty et al., 2005).

### Ongoing Research

Despite their promising therapeutic value for various indications, the administration of retinoids is strongly limited by severe associated toxic side effects due to the pleiotropic functions of these agents. These effects include teratogenicity, increases in serum triglycerides, mucocutaneous cytotoxicity, headache, and bone toxicity. Therefore, research in progress on retinoid therapy is focused on overcoming both the unwanted side effects of currently used retinoids in the clinic and intrinsic or acquired ATRA resistance in patients and their consequences (Freemantle et al., 2003). First, more work is required to understand better the molecular pathways induced by RARs, notably those underlying the antiproliferative and anticancer activities of retinoids, even though multiple mechanisms that modulate the complex retinoid signaling pathways and their cross-reactions are gradually being elucidated. For instance, the increased understanding of the regulation of RAR activities through phosphorylation should provide new insights in the developmental processes and in cancer (Bastien and Rochette-Egly, 2004).

Second, combinations with other chemopreventive agents that may also enhance the clinical efficacy of retinoids are increasingly sought. Indeed, increased understanding of epigenetic dysregulations that occur during the development of carcinogenesis suggest that ATRA resistance might be combatted by the use of epigenetic modifying agents such as HCAC inhibitors or DNA methyl transferase inhibitors in combination with retinoids, some of which are in clinical trials (Bachman et al., 2003; Egger et al., 2004; Feinberg and Tycko, 2004; Altucci et al., 2005). Several studies revealed other candidates for combinations treatment such as tumor necrosis factor or TRAIL (Altucci and Gronemeyer, 2001; Zusi et al., 2002). Interestingly the recent demonstration of retinoid-induced tumor suppression activities through a network involving the tumor suppressor interferon-regulator factor 1 and TRAIL provides new avenues for the therapeutic combination of retinoids and interferons that is already being tested clinically (Lippman et al., 1997; Clarke et al., 2004b, 2005).

Lastly, the improved use of retinoids in therapy will require the generation of novel synthetic RAR ligands harboring increased selective properties both to decrease the adverse effects associated with retinoid treatments and to overcome resistance to retinoids. Among

the novel compounds, atypical retinoids, such as *N*-(4-hydroxyphenyl) retinamide or CD437, have emerged as potential anticancer agents because of their antiproliferative and apoptotic actions with little toxicity compared with classic retinoids (Ortiz et al., 2002; Dawson, 2004). Despite these compounds being classified as retinoids because of their binding to RARs, their antitumoral effects, at least in part, seem to be independent of the RXR-RAR heterodimer function (Holmes et al., 2000). Furthermore, the development of RAR $\beta$ -selective ligands will be of prime importance because of the tumor-suppression potential of RAR $\beta$ .

Tables 1 through 3 summarize the major molecular, physiological, and pharmacological properties of RAR subtypes.

### REFERENCES

- Altucci L, Clarke N, Nebbioso A, Scognamiglio A, and Gronemeyer H (2005) Acute myeloid leukemia: therapeutic impact of epigenetic drugs. *Int J Biochem Cell Biol* **37**:1752–1762.
- Altucci L and Gronemeyer H (2001) The promise of retinoids to fight against cancer. *Nat Rev Cancer* **1**:181–193.
- Aranda A and Pascual A (2001) Nuclear hormone receptors and gene expression. *Physiol Rev* **81**:1269–1304.
- Bachman KE, Park BH, Rhee I, Rajagopalan H, Herman JG, Baylin SB, Kinzler KW, and Vogelstein B (2003) Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene. *Cancer Cell* **3**:89–95.
- Bastien J and Rochette-Egly C (2004) Nuclear retinoid receptors and the transcription of retinoid-target genes. *Gene* **328**:1–16.
- Blomhoff R (1994) Transport and metabolism of vitamin A. *Nutr Rev* **52**:S13–23.
- Boncinelli E, Simeone A, Acampora D, and Mavilio F (1991) HOX gene activation by retinoic acid. *Trends Genet* **7**:329–334.
- Bourguet W, Vivat V, Wurtz JM, Chambon P, Gronemeyer H, and Moras D (2000) Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. *Mol Cell* **5**:289–298.
- Castillo L, Milano G, Santini J, Demard F, and Pierrefite V (1997) Analysis of retinoic acid receptor  $\beta$  expression in normal and malignant laryngeal mucosa by a sensitive and routine applicable reverse transcription-polymerase chain reaction enzyme-linked immunosorbent assay method. *Clin Cancer Res* **3**:2137–2142.
- Chambon P (1996) A decade of molecular biology of retinoic acid receptors. *FASEB J* **10**:940–954.
- Chambon P (2005) The nuclear receptor superfamily: a personal retrospect on the first two decades. *Mol Endocrinol* **19**:1418–1428.
- Chapellier B, Mark M, Messaddeq N, Calleja C, Warot X, Brocard J, Gerard C, Li M, Metzger D, Ghyselinck NB, et al. (2002) Physiological and retinoid-induced proliferations of epidermis basal keratinocytes are differently controlled. *EMBO (Eur Mol Biol Organ) J* **21**:3402–3413.
- Chen JY, Clifford J, Zusi C, Starrett J, Tortolani D, Ostrowski J, Reczek PR, Chambon P, and Gronemeyer H (1996) Two distinct actions of retinoid-receptor ligands. *Nature (Lond)* **382**:819–822.
- Chen JY, Penco S, Ostrowski J, Balaguer P, Pons M, Starrett JE, Reczek P, Chambon P, and Gronemeyer H (1995) RAR-specific agonist/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation. *EMBO (Eur Mol Biol Organ) J* **14**:1187–1197.
- Clarke N, Germain P, Altucci L, and Gronemeyer H (2004a) Retinoids: potential in cancer prevention and therapy. *Expert Rev Mol Med* **6**:1–23.
- Clarke N, Jimenez-Lara AM, Voltz E, and Gronemeyer H (2004b) Tumor suppressor IRF-1 mediates retinoid and interferon anticancer signaling to death ligand TRAIL. *EMBO (Eur Mol Biol Organ) J* **23**:3051–3060.
- Clarke N, Nebbioso A, Altucci L, and Gronemeyer H (2005) TRAIL: at the center of drugable anti-tumor pathways. *Cell Cycle* **4**:914–918.
- Dawson MI (2004) Synthetic retinoids and their nuclear receptors. *Curr Med Chem Anti-Cancer Agents* **4**:199–230.
- Dawson MI, Chao WR, Pine P, Jong L, Hobbs PD, Rudd CK, Quick TC, Niles RM, Zhang XK, Lombardo A, et al. (1995) Correlation of retinoid binding affinity to retinoic acid receptor  $\alpha$  with retinoid inhibition of growth of estrogen receptor-positive MCF-7 mammary carcinoma cells. *Cancer Res* **55**:4446–4451.
- de The H, Chomienne C, Lanotte M, Degos L, and Dejean A (1990a) The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor  $\alpha$  gene to a novel transcribed locus. *Nature (Lond)* **347**:558–561.
- de The H, Vivanco-Ruiz MM, Tiollais P, Stunnenberg H, and Dejean A (1990b) Identification of a retinoic acid responsive element in the retinoic acid receptor  $\beta$  gene. *Nature (Lond)* **343**:177–180.
- Degos L and Wang ZY (2001) All *trans* retinoic acid in acute promyelocytic leukemia. *Oncogene* **20**:7140–7145.
- Dolle P, Ruberte E, Leroy P, Morriss-Kay G, and Chambon P (1990) Retinoic acid receptors and cellular retinoid binding proteins. I. A systematic study of their differential pattern of transcription during mouse organogenesis. *Development* **110**:1133–1151.
- Dupe V, Davenne M, Brocard J, Dolle P, Mark M, Dierich A, Chambon P, and Rijli

- FM (1997) In vivo functional analysis of the Hoxa-1 3' retinoic acid response element (3'RARE). *Development* **124**:399–410.
- Durand B, Saunders M, Leroy P, Leid M, and Chambon P (1992) All-*trans* and 9-*cis* retinoic acid induction of CRABP II transcription is mediated by RAR-RXR heterodimers bound to DR1 and DR2 repeated motifs. *Cell* **71**:73–85.
- Egger G, Liang G, Aparicio A, and Jones PA (2004) Epigenetics in human disease and prospects for epigenetic therapy. *Nature (Lond)* **429**:457–463.
- Fanjul A, Dawson MI, Hobbs PD, Jong L, Cameron JF, Harlev E, Graupner G, Lu XP, and Pfahl M (1994) A new class of retinoids with selective inhibition of AP-1 inhibits proliferation. *Nature (Lond)* **372**:107–111.
- Farboud B, Hauksdottir H, Wu Y, and 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.
- Faria TN, Mendelsohn C, Chambon P, and Gudas LJ (1999) The targeted disruption of both alleles of RAR $\beta$  in F9 cells results in the loss of retinoic acid-associated growth arrest. *J Biol Chem* **274**:26783–26788.
- Feinberg AP and Tycko B (2004) The history of cancer epigenetics. *Nat Rev Cancer* **4**:143–153.
- Freemantle SJ, Spinella MJ, and Dmitrovsky E (2003) Retinoids in cancer therapy and chemoprevention: promise meets resistance. *Oncogene* **22**:7305–7315.
- Fujii H, Sato T, Kaneko S, Gotoh O, Fujii-Kuriyama Y, Osawa K, Kato S, and Hamada H (1997) Metabolic inactivation of retinoic acid by a novel P450 differentially expressed in developing mouse embryos. *EMBO (Eur Mol Biol Organ) J* **16**:4163–4173.
- Galvin SA, Gilbert R, Baker M, Guibal F, and Tuley MR (1998) Comparative tolerance of adapalene 0.1% gel and six different tretinoin formulations. *Br J Dermatol* **139** (Suppl 52):34–40.
- Gehin M, Vivat V, Wurtz JM, Losson R, Chambon P, Moras D, and Gronemeyer H (1999) Structural basis for engineering of retinoic acid receptor isotype—selective agonists and antagonists. *Chem Biol* **6**:519–529.
- Germain P, Iyer J, Zechel C, and Gronemeyer H (2002) Coregulation and the mechanism of retinoic acid receptor synergy. *Nature (Lond)* **415**:187–192.
- Germain P, Kammerer S, Perez E, Peluso-Ilitis C, Tortolani D, Zusi FC, Starrett J, Lapointe P, Daris JP, Marinier A, et al. (2004) Rational design of RAR-selective ligands revealed by RAR $\beta$  crystal structure. *EMBO (Eur Mol Biol Organ) Rep* **5**:877–882.
- Ghyselinck NB, Dupe V, Dierich A, Messaddeq N, Garnier JM, Rochette-Egly C, Chambon P, and Mark M (1997) Role of the retinoic acid receptor  $\beta$  (RAR $\beta$ ) during mouse development. *Int J Dev Biol* **41**:425–447.
- Giguere V, Ong ES, Segui P, and Evans RM (1987) Identification of a receptor for the morphogen retinoic acid. *Nature (Lond)* **330**:624–629.
- Glass CK and Rosenfeld MG (2000) The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev* **14**:121–141.
- Grondona JM, Kastner P, Gansmuller A, Decimo D, Chambon P, and Mark M (1996) Retinal dysplasia and degeneration in RAR $\beta$ 2/RAR $\gamma$ 2 compound mutant mice. *Development* **122**:2173–2188.
- Hauksdottir H, Farboud B, and 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.
- Holmes WF, Dawson MI, Soprano RD, and Soprano KJ (2000) Induction of apoptosis in ovarian carcinoma cells by AHPN/CD437 is mediated by retinoic acid receptors. *J Cell Physiol* **185**:61–67.
- Hong WK and Sporn MB (1997) Recent advances in chemoprevention of cancer. *Science (Wash DC)* **278**:1073–1077.
- Hu X and Lazar MA (2000) Transcriptional repression by nuclear hormone receptors. *Trends Endocrinol Metab* **11**:6–10.
- Kagechika H, Kawachi E, Hashimoto Y, Himi T, and Shudo K (1988) Retinobenzoid acids. I. Structure-activity relationships of aromatic amides with retinoid activity. *J Med Chem* **31**:2182–2192.
- Kagechika H and Shudo K (2005) Synthetic retinoids: recent developments concerning structure and clinical utility. *J Med Chem* **48**:5875–5883.
- Kastner P, Mark M, and Chambon P (1995) Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? *Cell* **83**:859–869.
- Kastner P, Mark M, Ghyselinck N, Krezel W, Dupe V, Grondona JM, and Chambon P (1997) Genetic evidence that the retinoid signal is transduced by heterodimeric RXR/RAR functional units during mouse development. *Development* **124**:313–326.
- Klein ES, Pino ME, Johnson AT, Davies PJ, Nagpal S, Thacher SM, Krasinski G, and Chandraratna RA (1996) Identification and functional separation of retinoic acid receptor neutral antagonists and inverse agonists. *J Biol Chem* **271**:22692–22696.
- Krezel W, Ghyselinck N, Samad TA, Dupe V, Kastner P, Borrelli E, and Chambon P (1998) Impaired locomotion and dopamine signaling in retinoid receptor mutant mice. *Science (Wash DC)* **279**:863–867.
- Kurokawa R, DiRenzo J, Boehm M, Sugarman J, Gloss B, Rosenfeld MG, Heyman RA, and Glass CK (1994) Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding. *Nature (Lond)* **371**:528–531.
- Kurokawa R, Soderstrom M, Horlein A, Halachmi S, Brown M, Rosenfeld MG, and Glass CK (1995) Polarity-specific activities of retinoic acid receptors determined by a co-repressor. *Nature (Lond)* **377**:451–454.
- Lafayette R, Kim SJ, Angel P, Roberts AB, Sporn MB, Karin M, and Wilder RL (1990) Interleukin-1 stimulates and all-*trans*-retinoic acid inhibits collagenase gene expression through its 5' activator protein-1-binding site. *Mol Endocrinol* **4**:973–980.
- Laudet V and Gronemeyer H (2002) *The Nuclear Receptor Facts Book*, Academic Press, San Diego.
- Leid M, Kastner P, and Chambon P (1992) Multiplicity generates diversity in the retinoic acid signalling pathways. *Trends Biochem Sci* **17**:427–433.
- Li E, Sucov HM, Lee KF, Evans RM, and Jaenisch R (1993) Normal development and growth of mice carrying a targeted disruption of the  $\alpha$ 1 retinoic acid receptor gene. *Proc Natl Acad Sci USA* **90**:1590–1594.
- Lin RC, Egan DA, and Evans RM (1999) Molecular genetics of acute promyelocytic leukemia. *Trends Genet* **15**:179–184.
- Lippman SM and Lotan R (2000) Advances in the development of retinoids as chemopreventive agents. *J Nutr* **130**:479S–482S.
- Lippman SM, Lotan R, and Schleuniger U (1997) Retinoid-interferon therapy of solid tumors. *Int J Cancer* **70**:481–483.
- Lohnes D, Kastner P, Dierich A, Mark M, LeMeur M, and Chambon P (1993) Function of retinoic acid receptor  $\gamma$  in the mouse. *Cell* **73**:643–658.
- Lotan R (1996) Retinoids in cancer chemoprevention. *FASEB J* **10**:1031–1039.
- Lotan R, Dawson MI, Zou CC, Jong L, Lotan D, and Zou CP (1995a) Enhanced efficacy of combinations of retinoic acid- and retinoid X receptor-selective retinoids and alpha-interferon in inhibition of cervical carcinoma cell proliferation. *Cancer Res* **55**:232–236.
- Lotan R, Xu XC, Lippman SM, Ro JY, Lee JS, Lee JJ, and Hong WK (1995b) Suppression of retinoic acid receptor- $\beta$  in premalignant oral lesions and its up-regulation by isotretinoin. *N Engl J Med* **332**:1405–1410.
- Loudig O, Babichuk C, White J, Abu-Abed S, Mueller C, and Petkovich M (2000) Cytochrome P450RAI(CYP26) promoter: a distinct composite retinoic acid response element underlies the complex regulation of retinoic acid metabolism. *Mol Endocrinol* **14**:1483–1497.
- Lufkin T, Lohnes D, Mark M, Dierich A, Gorry P, Gaub MP, LeMeur M, and Chambon P (1993) High postnatal lethality and testis degeneration in retinoic acid receptor  $\alpha$  mutant mice. *Proc Natl Acad Sci USA* **90**:7225–7229.
- Mangelsdorf DJ and Evans RM (1995) The RXR heterodimers and orphan receptors. *Cell* **83**:841–850.
- Mark M, Ghyselinck NB, and Chambon P (2004) Retinoic acid signalling in the development of branchial arches. *Curr Opin Genet Dev* **14**:591–598.
- Mark M, Ghyselinck NB, and Chambon P (2006) Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signalling pathway during mouse embryogenesis. *Annu Rev Pharmacol Toxicol* **46**:451–480.
- Mark M, Ghyselinck NB, Wendling O, Dupe V, Mascrez B, Kastner P, and Chambon P (1999) A genetic dissection of the retinoid signalling pathway in the mouse. *Proc Natl Acad Sci USA* **96**:609–613.
- Marks R (1997) Clinical safety of tazarotene in the treatment of plaque psoriasis. *J Am Acad Dermatol* **37**:S25–S32.
- McCaffery P and Drager UC (2000) Regulation of retinoic acid signaling in the embryonic nervous system: a master differentiation factor. *Cytokine Growth Factor Rev* **11**:233–249.
- McClelland PB (1998) Obtaining the optimal treatment outcome with tazarotene. *Dermatol Nurs* **10**:343–348.
- McKenna NJ and O'Malley BW (2002) Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell* **108**:465–474.
- Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, Schreiber SL, and Evans RM (1997) Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell* **89**:373–380.
- Nicholson RC, Mader S, Nagpal S, Leid M, Rochette-Egly C, and Chambon P (1990) Negative regulation of the rat stromelysin gene promoter by retinoic acid is mediated by an AP1 binding site. *EMBO (Eur Mol Biol Organ) J* **9**:4443–4454.
- Niederreither K, Abu-Abed S, Schuhbaur B, Petkovich M, Chambon P, and Dolle P (2002) Genetic evidence that oxidative derivatives of retinoic acid are not involved in retinoid signaling during mouse development. *Nat Genet* **31**:84–88.
- Ortiz MA, Bayon Y, Lopez-Hernandez FJ, and Piedrafitra FJ (2002) Retinoids in combination therapies for the treatment of cancer: mechanisms and perspectives. *Drug Resist Updat* **5**:162–175.
- Pavri R, Lewis B, Kim TK, Dilworth FJ, Erdjument-Bromage H, Tempst P, de Murcia G, Evans R, Chambon P, and Reinberg D (2005) PARP-1 determines specificity in a retinoid signaling pathway via direct modulation of mediator. *Mol Cell* **18**:83–96.
- Perissi V and Rosenfeld MG (2005) Controlling nuclear receptors: the circular logic of cofactor cycles. *Nat Rev Mol Cell Biol* **6**:542–554.
- Petkovich M, Brand NJ, Krust A, and Chambon P (1987) A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature (Lond)* **330**:444–450.
- Petty WJ, Li N, Biddle A, Bounds R, Nitkin C, Ma Y, Dragnev KH, Freemantle SJ, and Dmitrovsky E (2005) A novel retinoic acid receptor  $\beta$  isoform and retinoid resistance in lung carcinogenesis. *J Natl Cancer Inst* **97**:1645–1651.
- Picard E, Seguin C, Monhoven N, Rochette-Egly C, Siat J, Borrelly J, Martinet Y, Martinet N, and Vignaud JM (1999) Expression of retinoid receptor genes and proteins in non-small-cell lung cancer. *J Natl Cancer Inst* **91**:1059–1066.
- Privalsky ML (2001) Regulation of SMRT and N-CoR corepressor function. *Curr Top Microbiol Immunol* **254**:117–136.
- Rastinejad F (2001) Retinoid X receptor and its partners in the nuclear receptor family. *Curr Opin Struct Biol* **11**:33–38.
- Rastinejad F, Wagner T, Zhao Q, and Khorasanizadeh S (2000) Structure of the RXR-RAR DNA-binding complex on the retinoic acid response element DR1. *EMBO (Eur Mol Biol Organ) J* **19**:1045–1054.
- Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, and Moras D (1995) Crystal structure of the RAR- $\gamma$  ligand-binding domain bound to all-*trans* retinoic acid. *Nature (Lond)* **378**:681–689.
- Resche-Rigon M and Gronemeyer H (1998) Therapeutic potential of selective modulators of nuclear receptor action. *Curr Opin Chem Biol* **2**:501–507.
- Rochette-Egly C (2003) Nuclear receptors: integration of multiple signalling pathways through phosphorylation. *Cell Signal* **15**:355–366.
- Rochette-Egly C and Chambon P (2001) F9 embryocarcinoma cells: a cell autonomous model to study the functional selectivity of RARs and RXRs in retinoid signaling. *Histol Histopathol* **16**:909–922.
- Schule R, Umesono K, Mangelsdorf DJ, Bolado J, Pike JW, and Evans RM (1990) Jun-Fos and receptors for vitamins A and D recognize a common response element in the human osteocalcin gene. *Cell* **61**:497–504.
- Smith WC, Nakshatri H, Leroy P, Rees J, and Chambon P (1991) A retinoic acid response element is present in the mouse cellular retinoid binding protein I (mCRBPI) promoter. *EMBO (Eur Mol Biol Organ) J* **10**:2223–2230.

- Sporn MB, Dunlop NM, Newton DL, and Smith JM (1976) Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). *Fed Proc* **35**: 1332–1338.
- Sporn MB, Roberts AB, and Goodman DS (1994) *The Retinoids: Biology, Chemistry and Medicine*. Raven Press, New York.
- Sun SY and Lotan R (2002) Retinoids and their receptors in cancer development and chemoprevention. *Crit Rev Oncol Hematol* **41**:41–55.
- Tabin C (1995) The initiation of the limb bud: growth factors, Hox genes, and retinoids. *Cell* **80**:671–674.
- Takeuchi M, Yano T, Omoto E, Takahashi K, Kibata M, Shudo K, Harada M, Ueda R, and Ohno R (1998) Relapsed acute promyelocytic leukemia previously treated with all-*trans* retinoic acid: clinical experience with a new synthetic retinoid, Am-80. *Leuk Lymphoma* **31**:441–451.
- Taneja R, Rochette-Egly C, Plassat JL, Penna L, Gaub MP, and 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 (Eur Mol Biol Organ) J* **16**:6452–6465.
- Taneja R, Roy B, Plassat JL, Zusi CF, Ostrowski J, Reczek PR, and Chambon P (1996) Cell-type and promoter-context dependent retinoic acid receptor (RAR) redundancies for RAR $\beta$ 2 and Hoxa-1 activation in F9 and P19 cells can be artefactually generated by gene knockouts. *Proc Natl Acad Sci USA* **93**:6197–6202.
- Thacher SM, Vasudevan J, and Chandraratna RA (2000) Therapeutic applications for ligands of retinoid receptors. *Curr Pharm Des* **6**:25–58.
- Tobita T, Takeshita A, Kitamura K, Ohnishi K, Yanagi M, Hiraoka A, Karasuno T, Takeuchi M, Miyawaki S, Ueda R, et al. (1997) Treatment with a new synthetic retinoid, Am80, of acute promyelocytic leukemia relapsed from complete remission induced by all-*trans* retinoic acid. *Blood* **90**:967–973.
- Vivat-Hannah V and Zusi FC (2005) Retinoids as therapeutic agents: today and tomorrow. *Mini Rev Med Chem* **5**:755–760.
- Westin S, Kurokawa R, Nolte RT, Wisely GB, McNerney EM, Rose DW, Milburn MV, Rosenfeld MG, and Glass CK (1998) Interactions controlling the assembly of nuclear-receptor heterodimers and co-activators. *Nature (Lond)* **395**:199–202.
- Weston AD, Blumberg B, and Underhill TM (2003) Active repression by unliganded retinoid receptors in development: less is sometimes more. *J Cell Biol* **161**:223–228.
- White JA, Beckett-Jones B, Guo YD, Dilworth FJ, Bonasoro J, Jones G, and Petkovich M (1997) cDNA cloning of human retinoic acid-metabolizing enzyme (hP450RAL) identifies a novel family of cytochromes P450. *J Biol Chem* **272**: 18538–18541.
- Widschwendter M, Berger J, Daxenbichler G, Muller-Holzner E, Widschwendter A, Mayr A, Marth C, and Zeimet AG (1997) Loss of retinoic acid receptor  $\beta$  expression in breast cancer and morphologically normal adjacent tissue but not in the normal breast tissue distant from the cancer. *Cancer Res* **57**:4158–4161.
- Wolbach SB and Howe PR (1925). Tissue changes following deprivation of fat-soluble A vitamin. *J Exp Med* **43**: 753–777.
- Xu XC, Sneige N, Liu X, Nandagiri R, Lee JJ, Lukmanji F, Hortobagyi G, Lippman SM, Dhingra K, and Lotan R (1997a) Progressive decrease in nuclear retinoic acid receptor  $\beta$  messenger RNA level during breast carcinogenesis. *Cancer Res* **57**: 4992–4996.
- Xu XC, Sozzi G, Lee JS, Lee JJ, Pastorino U, Pilotti S, Kurie JM, Hong WK, and Lotan R (1997b) Suppression of retinoic acid receptor  $\beta$  in non-small-cell lung cancer in vivo: implications for lung cancer development. *J Natl Cancer Inst* **89**:624–629.
- Zhu XJ, Tu P, Zhen J, and Duan YQ (2001) Adapalene gel 0.1%: effective and well tolerated in the topical treatment of acne vulgaris in Chinese patients. *Cutis* **68**:55–59.
- Zouboulis CC (2001) Retinoids—which dermatological indications will benefit in the near future? *Skin Pharmacol Appl Skin Physiol* **14**:303–315.
- Zusi FC, Lorenzi MV, and Vivat-Hannah V (2002) Selective retinoids and retinoids in cancer therapy and chemoprevention. *Drug Discov Today* **7**:1165–1174.

TABLE 1  
*RAR $\alpha$* 

Receptor Nomenclature	NR1B1
Receptor code	4.10.1:RA:1:B1
Molecular information	Hs: 462aa, P10276, chr. 17q21.1 <sup>1,2</sup> Rn: 459aa, chr. 10 <sup>1,3</sup> Mm: 462aa, P11416, 11 D <sup>1,4-6</sup>
DNA binding	
Structure	Heterodimer, RXR partner
HRE core sequence	PuG(G/T)TCA (DR1, DR2, DR5)
Partners	Cyclin H/cdk7/TFIIH (physical, functional): TFIIH phosphorylates RAR $\alpha$ 1 in its A/B region (Ser <sup>77</sup> ) by cdk7 subunit <sup>7-9</sup> ; AP-1 (physical, functional): RAR inhibits AP-1-driven transactivation, and AP-1 represses RAR-mediated transcription <sup>10-14</sup> ; CRABP1 (physical, functional): can enhance the transactivation by RAR $\alpha$ -RXR on DR5 element <sup>15</sup> ; PARP-1 (physical, functional): indispensable to RAR $\alpha$ -mediated transcription from the RAR $\alpha$ 2 <sup>16</sup>
Agonists	9- <i>cis</i> -Retinoic acid (0.3 nM),* all- <i>trans</i> -retinoic acid (0.4 nM),* AGN195183 (3 nM) [K <sub>d</sub> ] <sup>17-22</sup> ; Am580 (36 nM), TTNPB (36–72 nM), Am80 (124 nM) [IC <sub>50</sub> ] <sup>19-23</sup> ; BMS753 <sup>24</sup>
Antagonists	BMS614 (2 nM), BMS493 (4.2 nM), AGN193109 (2–16 nM), Ro-41-5253 (60 nM) [IC <sub>50</sub> ] <sup>19,22,24-28</sup>
Coactivators	NCOA1, NCOA2, NCOA3, PPARBP, CREBBP, p300 <sup>12,29-39</sup>
Corepressors	NCOR1, NCOR2 <sup>40-44</sup>
Biologically important isoforms	RAR $\alpha$ 1 (Hm, Mm): transcribed from the promoter P1 and differs from RAR $\alpha$ 2 in the A domain—RAR $\alpha$ 1 is phosphorylated by cdk7/TFIIH (Ser <sup>77</sup> ) <sup>5,45,46</sup> ; RAR $\alpha$ 2 (Hs, Mm): in contrast with the RAR $\alpha$ 1 isoform, RAR $\alpha$ 2 is transcribed from downstream promoter P2, which contains a DR5 and is inducible by retinoid <sup>5,47</sup>
Tissue distribution	Majority of tissues {Hs, Mm, Rn} [Northern blot, in situ hybridization, Western blot] <sup>6,48-54</sup>
Functional assays	Inhibition of cellular proliferation of the MCF-7 breast cancer cell line expressing the estrogen receptor {Hs} <sup>55</sup> ; induction of maturation of acute myeloid leukemia cell lines (NB4, PBL985, U937, HL60) using the histological nitro blue tetrazolium reaction and analysis of CD11c integrin expression by direct immunofluorescence {Hs} <sup>21,32,56,57</sup> ; parietal endodermal differentiation in the presence of cAMP of F9 murine embryonal carcinoma cell line {Mm} <sup>58</sup>
Main target genes	Activated: CYP26 {Hs, Mm, Rn}, <sup>59</sup> RAR $\beta$ 2 {Hs, Mm, Rn} <sup>26,57,60,61</sup> , Hoxa-1 {Mm} <sup>51,60,62</sup> , CRBP1 {Mm} <sup>60,63</sup> , CRABP1 {Mm} <sup>60,64</sup>
Mutant phenotype	Abnormalities observed: growth retardation, male sterility, impaired alveolar formation; congenital defects observed: webbed digits, homeotic transformations and malformations of cervical vertebrae, pterygoquadrate cartilage, malformations of the squamosal bone; note that both the specific RAR $\alpha$ 1-null and RAR $\alpha$ 2-null mutants are apparently normal {Mm} [knockout] <sup>34,65-67</sup>
Human disease	APL, a subtype of acute myelogenous leukemia: caused by several translocations that implicate the human RAR $\alpha$ gene; the reciprocal chromosomal translocation between RAR $\alpha$ and PML human genes produces a fusion protein PML-RAR $\alpha$ ; the use of supraphysiological doses of ATRA lead to remission in patients with APL; in contrast, the fusion protein resulting from the translocation between RAR $\alpha$ and the PLZF is insensitive to ATRA treatment <sup>68-70</sup>

aa, amino acids; chr., chromosome; HRE, hormone response element; PARP-1, poly(ADP-ribose) polymerase 1; TTNPB, 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid; PPARBP, peroxisome proliferator-activated receptor binding protein; CREBBP, cAMP response element-binding protein-binding protein.

\* Radioligand.

- Mattei MG, Riviere M, Krust A, Ingvansson S, Vennstrom B, Islam MQ, Levan G, Kautner P, Zelent A, Chambon P, et al. (1991) Chromosomal assignment of retinoic acid receptor (RAR) genes in the human, mouse, and rat genomes. *Genomics* **10**:1061–1069.
- Petkovich M, Brand NJ, Krust A, and Chambon P (1987) A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature* **330**:444–450.
- Akmal KM, Dufour JM, and Kim KH (1996) Region-specific localization of retinoic acid receptor- $\alpha$  expression in the rat epididymis. *Biol Reprod* **54**:1111–1119.
- Leid M, Kastner P, and Chambon P (1992) Multiplicity generates diversity in the retinoic acid signalling pathways. *Trends Biochem Sci* **17**:427–433.
- Leroy P, Krust A, Zelent A, Mendelsohn C, Garnier JM, Kastner P, Dierich A, and Chambon P (1991) Multiple isoforms of the mouse retinoic acid receptor  $\alpha$  are generated by alternative splicing and differential induction by retinoic acid. *EMBO (Eur Mol Biol Organ) J* **10**:59–69.
- Zelent A, Krust A, Petkovich M, Kastner P, and Chambon P (1989) Cloning of murine  $\alpha$  and  $\beta$  retinoic acid receptors and a novel receptor  $\gamma$  predominantly expressed in skin. *Nature* **339**:714–717.
- Bour G, Gaillard E, Bruck N, Lalevee S, Plassat JL, Busso D, Samama JP, and Rochette-Egly C (2005) Cyclin H binding to the RAR $\alpha$  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.
- Rochette-Egly C. (2003) Nuclear receptors: integration of multiple signalling pathways through phosphorylation. *Cell Signal* **15**:355–366.
- Rochette-Egly C, Adam S, Rossignol M, Egly JM, and 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.
- Chen JY, Penco S, Ostrowski J, Balaguer P, Pons M, Starrett JE, Reczek P, Chambon P, and Gronemeyer H (1995) RAR-specific agonist/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation. *EMBO (Eur Mol Biol Organ) J* **14**:1187–1197.
- Lafyatis R, Kim SJ, Angel P, Roberts AB, Sporn MB, Karin M, and Wilder RL (1990) Interleukin-1 stimulates and all-*trans*-retinoic acid inhibits collagenase gene expression through its 5' activator protein-1-binding site. *Mol Endocrinol* **4**:973–980.
- Nicholson RC, Mader S, Nagpal S, Leid M, Rochette-Egly C, and Chambon P (1990) Negative regulation of the rat stromelysin gene promoter by retinoic acid is mediated by an AP1 binding site. *EMBO (Eur Mol Biol Organ) J* **9**:4443–4454.
- Schule R, Umesono K, Mangelsdorf DJ, Bolado J, Pike JW, and Evans RM (1990) Jun-Fos and receptors for vitamins A and D recognize a common response element in the human osteocalcin gene. *Cell* **61**:497–504.
- Zhou XF, Shen XQ, and Shemshedini L (1999) Ligand-activated retinoic acid receptor inhibits AP-1 transactivation by disrupting c-Jun/c-Fos dimerization. *Mol Endocrinol* **13**:276–285.



15. Delva L, Bastie JN, Rochette-Egly C, Kraiba R, Balitrand N, Despouy G, Chambon P, and 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.
16. Pavri R, Lewis B, Kim TK, Dilworth FJ, Erdjument-Bromage H, Tempst P, de Murcia G, Evans R, Chambon P, and Reinberg D (2005) PARP-1 determines specificity in a retinoid signaling pathway via direct modulation of mediator. *Mol Cell* **18**:83–96.
17. Allenby G, Bocquel MT, Saunders M, Kazmer S, Speck J, Rosenberger M, Lovey A, Kastner P, Grippo JF, Chambon P, et al. (1993) Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids. *Proc Natl Acad Sci USA* **90**:30–34.
18. Beard RL, Duong TT, Teng M, Klein ES, Standevan AM, and Chandraratna RA (2002) Synthesis and biological activity of retinoic acid receptor- $\alpha$  specific amides. *Bioorg Med Chem Lett* **12**:3145–3148.
19. Keidel S, LeMotte P, and Apfel C (1994) Different agonist- and antagonist-induced conformational changes in retinoic acid receptors analyzed by protease mapping. *Mol Cell Biol* **14**:287–298.
20. Klaholz B, Mitschler A, and Moras D. (2000) Structural basis for isotype selectivity of the human retinoic acid nuclear receptor. *J Mol Biol* **302**:55–170.
21. Nagy L, Thomazy VA, Shipley GL, Fesus L, Lamph W, Heyman RA, Chandraratna RA, and Davies PJ (1995) Activation of retinoid X receptors induces apoptosis in HL-60 cell lines. *Mol Cell Biol* **15**:540–3551.
22. Thacher SM, Vasudevan J, and Chandraratna RA (2000) Therapeutic applications for ligands of retinoid receptors. *Curr Pharm Des* **6**:5–58.
23. Kagechika H, Kawachi E, Hashimoto Y, Himi T, and Shudo K (1988) Retinobenzoic acids. 1. Structure-activity relationships of aromatic amides with retinoid activity. *J Med Chem* **31**:2182–2192.
24. Gehin M, Vivat V, Wurtz JM, Losson R, Chambon P, Moras D, and Gronemeyer H (1999) Structural basis for engineering of retinoic acid receptor isotype-selective agonists and antagonists. *Chem Biol* **6**:519–529.
25. Bourguet W, Vivat V, Wurtz JM, Chambon P, Gronemeyer H, and Moras D (2000) Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. *Mol Cell* **5**:289–298.
26. Germain P, Iyer J, Zechel C, and Gronemeyer H (2002) Coregulator recruitment and the mechanism of retinoic acid receptor synergy. *Nature* **415**:187–192.
27. Klein ES, Pino ME, Johnson AT, Davies PJ, Nagpal S, Thacher SM, Krasinski G, and Chandraratna RA (1996) Identification and functional separation of retinoic acid receptor neutral antagonists and inverse agonists. *J Biol Chem* **271**:22692–22696.
28. Klein ES, Wang JW, Khalifa B, Gavigan SA, and Chandraratna RA (2000) Recruitment of nuclear receptor corepressor and coactivator to the retinoic acid receptor by retinoid ligands: influence of DNA-heterodimer interactions. *J Biol Chem* **275**:19401–19408.
29. Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, and Evans RM (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90**:569–580.
30. Farhoud B, Hauksdottir H, Wu Y, and 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.
31. Goodman RH and Smolik S (2000) CBP/p300 in cell growth, transformation, and development. *Genes Dev* **14**:1553–1577.
32. Lanotte M, Martin-Thouvenin V, Najman S, Balerini P, Valensi F, and Berger R (1991) NB4, a maturation inducible cell line with t(15;17) marker isolated from a human acute promyelocytic leukemia (M3). *Blood* **77**:1080–1086.
33. Li E, Sucov HM, Lee KF, Evans RM, and Jaenisch R (1993) Normal development and growth of mice carrying a targeted disruption of the  $\alpha$  1 retinoic acid receptor gene. *Proc Natl Acad Sci USA* **90**:1590–1594.
34. Mark M, Ghyselinck NB, and Chambon P (2006) Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signalling pathway during mouse embryogenesis. *Annu Rev Pharmacol Toxicol* **46**:451–480.
35. Onate SA, Tsai SY, Tsai MJ, and O'Malley BW (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270**:1354–1357.
36. Voegel JJ, Heine MJ, Tini M, Vivat V, Chambon P, and Gronemeyer H (1998) The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways. *EMBO (Eur Mol Biol Organ) J* **17**:507–519.
37. Voegel JJ, Heine MJ, Zechel C, Chambon P, and Gronemeyer H (1996) TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO (Eur Mol Biol Organ) J* **15**:3667–3675.
38. Walfish PG, Yoganathan T, Yang YF, Hong H, Butt TR, and Stallcup MR (1997) Yeast hormone response element assays detect and characterize GRIP1 coactivator-dependent activation of transcription by thyroid and retinoid nuclear receptors. *Proc Natl Acad Sci USA* **94**:3697–3702.
39. Westin S, Rosenfeld MG, and Glass CK (2000) Nuclear receptor coactivators. *Adv Pharmacol* **47**:89–112.
40. Chen JD, and Evans RM (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* **377**:454–457.
41. Horlein AJ, Naar AM, Heinzl T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK, et al. (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377**:397–404.
42. McKenna NJ, Lanz RB, and O'Malley BW (1999) Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev* **20**:321–344.
43. Sande S, and Privalsky ML (1996) Identification of TRACs (T3 receptor-associating cofactors), a family of cofactors that associate with, and modulate the activity of, nuclear hormone receptors. *Mol Endocrinol* **10**:813–825.
44. Wong CW and Privalsky ML (1998) Transcriptional silencing is defined by isoform- and heterodimer-specific interactions between nuclear hormone receptors and corepressors. *Mol Cell Biol* **18**:5724–5733.
45. Brand NJ, Petkovich M, and Chambon P (1990) Characterization of a functional promoter for the human retinoic acid receptor- $\alpha$  (hRAR- $\alpha$ ). *Nucleic Acids Res* **18**:6799–6806.
46. van der Leede BJ, Folkers GE, Kruyt FA, and van der Saag PT (1992) Genomic organization of the human retinoic acid receptor  $\beta$  2. *Biochem Biophys Res Commun* **188**:695–702.
47. Leroy P, Nakshatri H, and Chambon P (1991) Mouse retinoic acid receptor  $\alpha$  2 isoform is transcribed from a promoter that contains a retinoic acid response element. *Proc Natl Acad Sci USA* **88**:10138–10142.
48. Dolle P, Ruberte E, Kastner P, Petkovich M, Stoner CM, Gudas LJ, and Chambon P (1989) Differential expression of genes encoding  $\alpha$ ,  $\beta$  and  $\gamma$  retinoic acid receptors and CRABP in the developing limbs of the mouse. *Nature* **342**:702–705.
49. Dolle P, Ruberte E, Leroy P, Morriss-Kay G, and Chambon P (1990) Retinoic acid receptors and cellular retinoid binding proteins. I. A systematic study of their differential pattern of transcription during mouse organogenesis. *Development* **110**:1133–1151.
50. Giguere V, Ong ES, Segui P, and Evans RM (1987) Identification of a receptor for the morphogen retinoic acid. *Nature* **330**:624–629.
51. Laudet V, and Gronemeyer H (2002) *The Nuclear Receptor Facts Book*, Academic Press, San Diego.
52. Ruberte E, Dolle P, Chambon P, and Morriss-Kay G (1991) Retinoic acid receptors and cellular retinoid binding proteins. II. Their differential pattern of transcription during early morphogenesis in mouse embryos. *Development* **111**:45–60.
53. Ruberte E, Dolle P, Krust A, Zelent A, Morriss-Kay G, and Chambon P (1990) Specific spatial and temporal distribution of retinoic acid receptor  $\gamma$  transcripts during mouse embryogenesis. *Development* **108**:213–222.
54. Ruberte E, Friederich V, Chambon P, and Morriss-Kay G (1993) Retinoic acid receptors and cellular retinoid binding proteins. III. Their differential transcript distribution during mouse nervous system development. *Development* **118**:267–282.
55. Dawson MI, Chao WR, Pine P, Jong L, Hobbs PD, Rudd CK, Quick TC, Niles RM, Zhang XK, Lombardo A, et al. (1995) Correlation of retinoid binding affinity to retinoic acid receptor  $\alpha$  with retinoid inhibition of growth of estrogen receptor-positive MCF-7 mammary carcinoma cells. *Cancer Res* **55**:4446–4451.
56. Altucci L, Rossin A, Raffelsberger W, Reitmaier A, Chomienne C, and Gronemeyer H (2001) Retinoic acid-induced apoptosis in leukemia cells is mediated by paracrine action of tumor-selective death ligand TRAIL. *Nat Med* **7**:680–686.
57. Chen JY, Clifford J, Zusi C, Starrett J, Tortolani D, Ostrowski J, Reczek PR, Chambon P, and Gronemeyer H (1996) Two distinct actions of retinoid-receptor ligands. *Nature* **382**:819–822.
58. Taneja R, Rochette-Egly C, Plassat JL, Penna L, Gaub MP, and 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 (Eur Mol Biol Organ) J* **16**:6452–6465.
59. Loudig O, Babichuk C, White J, Abu-Abed S, Mueller C, and Petkovich M (2000) Cytochrome P450RAI(CYP26) promoter: a distinct composite retinoic acid response element underlies the complex regulation of retinoic acid metabolism. *Mol Endocrinol* **14**:1483–1497.
60. Chiba H, Clifford J, Metzger D, and Chambon P (1997) Distinct retinoid X receptor-retinoic acid receptor heterodimers are differentially involved in the control of expression of retinoid target genes in F9 embryonal carcinoma cells. *Mol Cell Biol* **17**:3013–3020.
61. de The H, Chomienne C, Lanotte M, Degos L, and Dejean A (1990) The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor  $\alpha$  gene to a novel transcribed locus. *Nature* **347**:558–561.
62. Dupe V, Davenne M, Brocard J, Dolle P, Mark M, Dierich A, Chambon P, and Rijli FM (1997) In vivo functional analysis of the Hoxa-1 3' retinoic acid response element (3'RARE). *Development* **124**:399–410.
63. Smith WC, Nakshatri H, Leroy P, Rees J, and Chambon P (1991) A retinoic acid response element is present in the mouse cellular retinoid binding protein I (mCRBPI) promoter. *EMBO (Eur Mol Biol Organ) J* **10**:2223–2230.

64. Durand B, Saunders M, Leroy P, Leid M, and Chambon P (1992) All-*trans* and 9-*cis* retinoic acid induction of CRABP II transcription is mediated by RAR-RXR heterodimers bound to DR1 and DR2 repeated motifs. *Cell* **71**:73–85.
65. Li H, Gomes PJ, and Chen JD (1997) RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. *Proc Natl Acad Sci USA* **94**:8479–8484.
66. Lufkin T, Lohnes D, Mark M, Dierich A, Gorry P, Gaub MP, LeMeur M, and Chambon P (1993) High postnatal lethality and testis degeneration in retinoic acid receptor  $\alpha$  mutant mice. *Proc Natl Acad Sci USA* **90**:7225–7229.
67. Mark M, and Chambon P (2003) Functions of RARs and RXRs in vivo: genetic dissection of the retinoid signaling pathway. *Pure Appl Chem* **75**:1709–1732.
68. de The H, Vivanco-Ruiz MM, Tiollais P, Stunnenberg H, and Dejean A (1990) Identification of a retinoic acid responsive element in the retinoic acid receptor  $\beta$  gene. *Nature* **343**:177–180.
69. Degos L, and Wang ZY (2001) All *trans* retinoic acid in acute promyelocytic leukemia. *Oncogene* **20**:7140–7145.
70. Lin RJ, Egan DA, and Evans RM (1999) Molecular genetics of acute promyelocytic leukemia. *Trends Genet* **15**:179–184.

TABLE 2  
RAR $\beta$ 

Receptor Nomenclature	NR1B2
Receptor code	4.10.1:RA:1:B2
Other names	Hap
Molecular information	Hs: 455aa, P10826, chr. 3p24 <sup>1,26</sup> Rn: chr. 15 <sup>26</sup> Mm: 482aa, P22605, chr. 14 A <sup>26,28,45,46</sup>
DNA binding	
Structure	Heterodimer, RXR partner
HRE core sequence	PuG(G/T)TCA (DR5)
Partners	AP-1 (functional): RAR $\beta$ inhibits AP-1-driven transactivation <sup>4,30</sup>
Agonists	9- <i>cis</i> -Retinoic acid (0.2 nM)*, all- <i>trans</i> -retinoic acid (0.4 nM)* [ $K_d$ ] <sup>14,15,29,37</sup> ; BMS641 (2.5 nM), TTNPB (5–22 nM) [ $IC_{50}$ ] <sup>10,14,15,29,37</sup>
Antagonists	BMS493 (2.9 nM), AGN193109 (2–7 nM) [ $IC_{50}$ ] <sup>9,16,17,37</sup>
Coactivators	NCOA1, NCOA2, NCOA3, PPARBP <sup>3,8,9,20–23,27,31,33,38,44</sup>
Biologically important isoforms	RAR $\beta$ 1 (Hs, Mm): differs from RAR $\beta$ 2 in the A domain <sup>46</sup> ; RAR $\beta$ 2 (Hs, Mm): in contrast to the RAR $\beta$ 1 isoform, RAR $\beta$ 2 is transcribed from promoter (the downstream one, P2) that contains a DR5 and is inducible by retinoid <sup>46</sup> ; RAR $\beta$ 3 (Mm): the RAR $\beta$ 3 isoform is generated from the promoter P1 and differs from RAR $\beta$ 1 by its N-terminal part—not detected in human <sup>46</sup> ; RAR $\beta$ 4 (Hs, Mm): RAR $\beta$ 4 is generated from the promoter P2 and differs from RAR $\beta$ 2 by its N terminus that initiates a non-AUG codon, CUG <sup>28</sup>
Tissue distribution	Brain, liver, kidney, heart, pituitary, colon, uterus, ovary, testis, prostate, adrenal, eye (Hs, Mm, Rn) [Northern blot, in situ hybridization, Western blot] <sup>5–7,13,19,35,36</sup>
Mutant phenotype	Abnormalities observed: growth retardation, behavioral defects, altered alveolar formation; congenital defects observed: homeotic transformations and malformations of cervical vertebrae, persistence and hyperplasia of the primary vitreous body; note that specific RAR $\beta$ 1/ $\beta$ 3-null mutants are apparently normal, and specific RAR $\beta$ 2/ $\beta$ 4-null mutants exhibited persistence and hyperplasia of the primary vitreous body (Mm) [knockout] <sup>11,12,18,24,25</sup>
Human disease	Human nonsmall cell lung cancer: associated with loss of RAR $\beta$ expression <sup>2,32,43</sup> ; human esophageal cancer: associated with loss of RAR $\beta$ expression <sup>34</sup> ; human breast cancer: associated with loss of RAR $\beta$ expression <sup>39–42</sup>

aa, amino acids; chr., chromosome; HRE, hormone response element; TTNPB, 4-[(*E*)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid; PPARBP, peroxisome proliferator-activated receptor binding protein.

\* Radioligand.

- Brand N, Petkovich M, Krust A, Chambon P, de The H, Marchio A, Tiollais P, and Dejean A (1988) Identification of a second human retinoic acid receptor. *Nature* **332**:850–853.
- Mattei MG, Riviere M, Krust A, Ingvarsson S, Vennstrom B, Islam MQ, Levan G, Kautner P, Zelent A, Chambon P, et al. (1991) Chromosomal assignment of retinoic acid receptor (RAR) genes in the human, mouse, and rat genomes. *Genomics* **10**:1061–1069.
- Nagpal S, Zelent A, and Chambon P (1992) RAR- $\beta$  4, a retinoic acid receptor isoform, is generated from RAR- $\beta$  2 by alternative splicing and usage of a CUG initiator codon. *Proc Natl Acad Sci USA* **89**:2718–2722.
- Zelent A, Krust A, Petkovich M, Kastner P, and Chambon P (1989) Cloning of murine  $\alpha$  and  $\beta$  retinoic acid receptors and a novel receptor  $\gamma$  predominantly expressed in skin. *Nature* **339**:714–717.
- Zelent A, Mendelsohn C, Kastner P, Krust A, Garnier JM, Ruffenach F, Leroy P, and Chambon P (1991) Differentially expressed isoforms of the mouse retinoic acid receptor  $\beta$  generated by usage of two promoters and alternative splicing. *EMBO (Eur Mol Biol Organ) J* **10**:71–81.
- Chen JY, Penco S, Ostrowski J, Balaguer P, Pons M, Starrett JE, Reczek P, Chambon P, and Gronemeyer H (1995) RAR-specific agonist/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation. *EMBO (Eur Mol Biol Organ) J* **14**:1187–1197.
- Nicholson RC, Mader S, Nagpal S, Leid M, Rochette-Egly C, and Chambon P (1990) Negative regulation of the rat stromelysin gene promoter by retinoic acid is mediated by an AP1 binding site. *EMBO (Eur Mol Biol Organ) J* **9**:4443–4454.
- Keidel S, LeMotte P, and Apfel C (1994) Different agonist- and antagonist-induced conformational changes in retinoic acid receptors analyzed by protease mapping. *Mol Cell Biol* **14**:287–298.
- Klaholz BP, Mitschler A, and Moras D (2000) Structural basis for isotype selectivity of the human retinoic acid nuclear receptor. *J Mol Biol* **302**:155–170.
- Nagy L, Thomazy VA, Shipley GL, Fesus L, Lamph W, Heyman RA, Chandraratna RA, and Davies PJ (1995) Activation of retinoid X receptors induces apoptosis in HL-60 cell lines. *Mol Cell Biol* **15**:3540–3551.
- Thacher SM, Vasudevan J, and Chandraratna RA (2000) Therapeutic applications for ligands of retinoid receptors. *Curr Pharm Des* **6**:25–58.
- Germain P, Kammerer S, Perez E, Peluso-Itlis C, Tortolani D, Zusi FC, Starrett J, Lapointe P, Daris JP, Marinier A, et al. (2004) Rational design of RAR-selective ligands revealed by RAR $\beta$  crystal structure. *EMBO (Eur Mol Biol Organ) Rep* **5**:877–882.
- Germain P, Iyer J, Zechel C, and Gronemeyer H (2002) Coregulator recruitment and the mechanism of retinoic acid receptor synergy. *Nature* **415**:187–192.
- Klein ES, Pino ME, Johnson AT, Davies PJ, Nagpal S, Thacher SM, Krasinski G, and Chandraratna RA (1996) Identification and functional separation of retinoic acid receptor neutral antagonists and inverse agonists. *J Biol Chem* **271**:22692–22696.
- Klein ES, Wang JW, Khalifa B, Gavigan SA, and Chandraratna RA (2000) Recruitment of nuclear receptor corepressor and coactivator to the retinoic acid receptor by retinoid ligands: influence of DNA-heterodimer interactions. *J Biol Chem* **275**:19401–19408.
- Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, and Evans RM (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90**:569–580.
- Farboud B, Hauksdottir H, Wu Y, and 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.
- Laudet V and Gronemeyer H (2002) *The Nuclear Receptor Facts Book*. Academic Press, San Diego.
- Lemon BD and Freedman LP (1999) Nuclear receptor cofactors as chromatin remodelers. *Curr Opin Genet Dev* **9**:499–504.
- Li H, Gomes PJ, and Chen JD (1997) RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. *Proc Natl Acad Sci USA* **94**:8479–8484.
- McKenna NJ, Lanz RB, and O'Malley BW (1999) Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev* **20**:321–344.
- Onate SA, Tsai SY, Tsai MJ, and O'Malley BW (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270**:1354–1357.
- Pogenberg V, Guichou JF, Vivat-Hannah V, Kammerer S, Perez E, Germain P, de Lera AR, Gronemeyer H, Royer CA, and Bourguet W (2005) Characterization of the interaction between retinoic acid receptor/retinoid X receptor (RAR/RXR) heterodimers and transcriptional coactivators through structural and fluorescence anisotropy studies. *J Biol Chem* **280**:1625–1633.
- Voegel JJ, Heine MJ, Zechel C, Chambon P, and Gronemeyer H (1996) TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO (Eur Mol Biol Organ) J* **15**:3667–3675.

25. Yuan CX, Ito M, Fondell JD, Fu ZY, and Roeder RG (1998) The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. *Proc Natl Acad Sci USA* **95**:7939–7944.
26. de The H, Marchio A, Tiollais P, and Dejean A (1989) Differential expression and ligand regulation of the retinoic acid receptor  $\alpha$  and  $\beta$  genes. *EMBO (Eur Mol Biol Organ) J* **8**:429–433.
27. Dolle P, Ruberte E, Kastner P, Petkovich M, Stoner CM, Gudas LJ, and Chambon P (1989) Differential expression of genes encoding  $\alpha$ ,  $\beta$  and  $\gamma$  retinoic acid receptors and CRABP in the developing limbs of the mouse. *Nature* **342**:702–705.
28. Dolle P, Ruberte E, Leroy P, Morriss-Kay G, and Chambon P (1990) Retinoic acid receptors and cellular retinoid binding proteins. I. A systematic study of their differential pattern of transcription during mouse organogenesis. *Development* **110**:1133–1151.
29. Krust A, Kastner P, Petkovich M, Zelent A, and Chambon P (1989) A third human retinoic acid receptor, hRAR- $\gamma$ . *Proc Natl Acad Sci U S A* **86**:5310–5314.
30. Ruberte E, Dolle P, Chambon P, and Morriss-Kay G (1991) Retinoic acid receptors and cellular retinoid binding proteins. II. Their differential pattern of transcription during early morphogenesis in mouse embryos. *Development* **111**:45–60.
31. Ruberte E, Dolle P, Krust A, Zelent A, Morriss-Kay G, and Chambon P (1990) Specific spatial and temporal distribution of retinoic acid receptor  $\gamma$  transcripts during mouse embryogenesis. *Development* **108**:213–222.
32. Ghyselinck NB, Dupe V, Dierich A, Messaddeq N, Garnier JM, Rochette-Egly C, Chambon P, and Mark M (1997) Role of the retinoic acid receptor  $\beta$  (RAR $\beta$ ) during mouse development. *Int J Dev Biol* **41**:425–447.
33. Grondona JM, Kastner P, Gansmuller A, Decimo D, Chambon P, and Mark M (1996) Retinal dysplasia and degeneration in RAR $\beta$  2/RAR $\gamma$  2 compound mutant mice. *Development* **122**:2173–2188.
34. Krezel W, Ghyselinck N, Samad TA, Dupe V, Kastner P, Borrelli E, and Chambon P (1998) Impaired locomotion and dopamine signaling in retinoid receptor mutant mice. *Science* **279**:863–867.
35. Mark M and Chambon P (2003) Functions of RARs and RXRs in vivo: genetic dissection of the retinoid signaling pathway. *Pure Appl Chem* **75**:1709–1732.
36. Mark M, Ghyselinck NB, and Chambon P (2006) Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signalling pathway during mouse embryogenesis. *Annu Rev Pharmacol Toxicol* **46**:451–480.
37. Castillo L, Milano G, Santini J, Demard F, and Pierrefite V (1997) Analysis of retinoic acid receptor  $\beta$  expression in normal and malignant laryngeal mucosa by a sensitive and routine applicable reverse transcription-polymerase chain reaction enzyme-linked immunosorbent assay method. *Clin Cancer Res* **3**:2137–2142.
38. Picard E, Seguin C, Monhoven N, Rochette-Egly C, Siat J, Borrelly J, Martinet Y, Martinet N, and Vignaud JM (1999) Expression of retinoid receptor genes and proteins in non-small-cell lung cancer. *J Natl Cancer Inst* **91**:1059–1066.
39. Xu XC, Sozzi G, Lee JS, Lee JJ, Pastorino U, Pilotti S, Kurie JM, Hong WK, and Lotan R (1997) Suppression of retinoic acid receptor  $\beta$  in non-small-cell lung cancer in vivo: implications for lung cancer development. *J Natl Cancer Inst* **89**:624–629.
40. Qiu H, Zhang W, El-Naggar AK, Lippman SM, Lin P, Lotan R, and Xu XC (1999) Loss of retinoic acid receptor- $\beta$  expression is an early event during esophageal carcinogenesis. *Am J Pathol* **155**:1519–1523.
41. Widschwendter M, Berger J, Daxenbichler G, Muller-Holzner E, Widschwendter A, Mayr A, Marth C, and Zeimet AG (1997) Loss of retinoic acid receptor  $\beta$  expression in breast cancer and morphologically normal adjacent tissue but not in the normal breast tissue distant from the cancer. *Cancer Res* **57**:4158–4161.
42. Widschwendter M, Berger J, Hermann M, Muller HM, Amberger A, Zeschnick M, Widschwendter A, Abendstein B, Zeimet AG, Daxenbichler G, et al. (2000) Methylation and silencing of the retinoic acid receptor- $\beta$  2 gene in breast cancer. *J Natl Cancer Inst* **92**:826–832.
43. Widschwendter M, Berger J, Muller HM, Zeimet AG, and Marth C (2001) Epigenetic downregulation of the retinoic acid receptor- $\beta$  2 gene in breast cancer. *J Mamm Gland Biol Neoplasia* **6**:193–201.
44. Xu XC, Sneige N, Liu X, Nandagiri R, Lee JJ, Lukmanji F, Hortobagyi G, Lippman SM, Dhingra K, and Lotan R (1997) Progressive decrease in nuclear retinoic acid receptor  $\beta$  messenger RNA level during breast carcinogenesis. *Cancer Res* **57**:4992–4996.
45. Zelent A, Krust A, Petkovich M, Kastner P, and Chambon P (1989) Cloning of murine alpha and beta retinoic acid receptors and a novel receptor gamma predominantly expressed in skin. *Nature (Lond)* **339**:714–717.
46. Zelent A, Mendelsohn C, Kastner P, Krust A, Garnier JM, Ruffenach F, Leroy P, and Chambon P (1991) Differentially expressed isoforms of the mouse retinoic acid receptor beta generated by usage of two promoters and alternative splicing. *EMBO (Eur Mol Biol Org) J* **10**:71–81.

TABLE 3  
RAR $\gamma$ 

Receptor Nomenclature	NR1B3
Receptor code	4.10.1:RA:1:B3
Molecular information	Hs: 454aa, P13631, chr. 12q13 <sup>1-3</sup> Rn: chr. 7 <sup>3-5</sup> Mm: 458aa, P18911, chr. 15 F <sup>3,6</sup>
DNA binding	
Structure	Heterodimer, RXR partner
HRE core sequence	PuG(G/T)TCA (DR2, DR5)
Partners	AP-1 (functional): RAR $\gamma$ inhibits AP-1-driven transactivation <sup>7-11</sup> ; cdk7/TFIIH (physical, functional): TFIIH phosphorylates RAR $\gamma$ 2 in its A/B region (Ser <sup>68</sup> ) by cdk7 subunit <sup>12,13</sup> ; p38 MAPK (functional): required for RA-induced RAR $\gamma$ degradation and transactivation <sup>13-15</sup> ; SUG1 (physical, functional): required for RA-induced RAR $\gamma$ degradation and transactivation <sup>13-15</sup> ; vinexin $\beta$ (physical, functional): interacts with AF-1 domain of RAR $\gamma$ and represses RAR-mediated transcription <sup>16</sup>
Agonists	All- <i>trans</i> -retinoic acid (0.2 nM)*, 9- <i>cis</i> -retinoic acid (0.8 nM)* [K <sub>d</sub> ] <sup>17-21</sup> ; TTNPB (15-26 nM), CD666 (68 nM), BMS270394 (528 nM), BMS961 (500 nM) [IC <sub>50</sub> ] <sup>18-23</sup>
Antagonists	AGN193109 (3-7 nM), BMS493 (98 nM), CD2665 (81 nM) [IC <sub>50</sub> ] <sup>21,24-27</sup>
Coactivators	NCOA1, NCOA2, NCOA3 <sup>4,28-34</sup>
Corepressors	NCOR1, NCOR2 <sup>29,31,35-38</sup>
Biologically important isoforms	RAR $\gamma$ 1 (Hs, Mm): differs from RAR $\gamma$ 2 in its N-terminal domain <sup>6,39</sup> ; RAR $\gamma$ 2 (Hs, Mm): the expression of RAR $\gamma$ 2 is regulated through a specific RARE element; RAR $\gamma$ 2 is phosphorylated by p38 MAPK (Ser <sup>66</sup> ) and by cdk7/TFIIH (Ser <sup>68</sup> ) (Hs, Mm) <sup>6,12,14,15,40-43</sup>
Tissue distribution	Highly expressed in the epidermis (Hs, Mm) [Northern blot, in situ hybridization, Western blot] <sup>2,6,41,44-47</sup>
Functional assays	Primitive endodermal differentiation and morphological differentiation of the F9 murine embryonal carcinoma cell line {Mm} <sup>48-50</sup>
Main target genes	Activated: laminin B1 {Mm} <sup>50</sup> , RAR $\beta$ 2 (Hs, Mm, Rn) <sup>48,51,52</sup> , Hoxa-1 {Mm} <sup>4,48,53</sup> , CRBP1 {Mm} <sup>48,54</sup> , CRABP1 {Mm} <sup>48,55</sup> ; repressed:
Mutant phenotype	Abnormalities observed: growth deficiency, male sterility, squamous epithelia of various epithelia, impaired alveolar formation; congenital defects observed: webbed digits, homeotic transformations and malformations of cervical vertebrae, malformed laryngeal cartilages and tracheal rings, agenesis of the Harderian glands, agenesis of the metopic pillar of the skull, abnormal differentiation of granular keratinocytes; note that specific RAR $\gamma$ 2-null mutants are apparently normal, and specific RAR $\gamma$ 1-null mutants exhibited a growth deficiency, malformations of cervical vertebrae, and abnormal differentiation of granular keratinocytes {Mm} [knockout] <sup>56-60</sup>
Human disease	Photoaging: level of RAR $\gamma$ is reduced after UV treatment of human skin <sup>61-63</sup>

aa, amino acids; chr., chromosome; HRE, hormone response element; TFIIH, transcription factor IIIH; TTNPB, 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid.

\* Radioligand.

- Ishikawa T, Umehara K, Mangelsdorf DJ, Aburatani H, Stanger BZ, Shibasaki Y, Imawari M, Evans RM, and Takaku F (1990) A functional retinoic acid receptor encoded by the gene on human chromosome 12. *Mol Endocrinol* **4**:837-844.
- Krust A, Kastner P, Petkovich M, Zelent A, and Chambon P (1989) A third human retinoic acid receptor, hRAR- $\gamma$ . *Proc Natl Acad Sci USA* **86**:5310-5314.
- Mattei MG, Riviere M, Krust A, Ingvarsson S, Vennstrom B, Islam MQ, Levan G, Kautner P, Zelent A, Chambon P, et al. (1991) Chromosomal assignment of retinoic acid receptor (RAR) genes in the human, mouse, and rat genomes. *Genomics* **10**:1061-1069.
- Laudet V and Gronemeyer H (2002) *The Nuclear Receptor Facts Book*, Academic Press, San Diego.
- Lopes da Silva S, Van Horssen AM, Chang C, and Burbach JP (1995) Expression of nuclear hormone receptors in the rat supraoptic nucleus. *Endocrinology* **136**:2276-2283.
- Kastner P, Krust A, Mendelsohn C, Garnier JM, Zelent A, Leroy P, Staub A, and Chambon P (1990) Murine isoforms of retinoic acid receptor  $\gamma$  with specific patterns of expression. *Proc Natl Acad Sci USA* **87**:2700-2704.
- Chen JY, Penco S, Ostrowski J, Balaguer P, Pons M, Starrett JE, Reczek P, Chambon P, and Gronemeyer H (1995) RAR-specific agonist/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation. *EMBO (Eur Mol Biol Organ) J* **14**:1187-1197.
- Lafyatis R, Kim SJ, Angel P, Roberts AB, Sporn MB, Karin M, and Wilder RL (1990) Interleukin-1 stimulates and all-*trans*-retinoic acid inhibits collagenase gene expression through its 5' activator protein-1-binding site. *Mol Endocrinol* **4**:973-980.
- Nicholson RC, Mader S, Nagpal S, Leid M, Rochette-Egly C, and Chambon P (1990) Negative regulation of the rat stromelysin gene promoter by retinoic acid is mediated by an AP1 binding site. *EMBO (Eur Mol Biol Organ) J* **9**:4443-4454.
- Schule R, Rangarajan P, Yang N, Kliewer S, Ransone LJ, Bolado J, Verma IM, and Evans RM (1991) Retinoic acid is a negative regulator of AP-1-responsive genes. *Proc Natl Acad Sci USA* **88**:6092-6096.
- Schule R, Umehara K, Mangelsdorf DJ, Bolado J, Pike JW, and Evans RM (1990) Jun-Fos and receptors for vitamins A and D recognize a common response element in the human osteocalcin gene. *Cell* **61**:497-504.
- Bastien J, Adam-Stitah S, Riedl T, Egly JM, Chambon P, and 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.
- Rochette-Egly C (2003) Nuclear receptors: integration of multiple signalling pathways through phosphorylation. *Cell Signal* **15**:355-366.
- Gianni M, Bauer A, Garattini E, Chambon P, and Rochette-Egly C (2002) Phosphorylation by p38MAPK and recruitment of SUG-1 are required for RA-induced RAR  $\gamma$  degradation and transactivation. *EMBO (Eur Mol Biol Organ) J* **21**:3760-3769.
- Gianni M, Kopf E, Bastien J, Oulad-Abdelghani M, Garattini E, Chambon P, and Rochette-Egly C (2002) 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* **277**:24859-24862.
- Bour G, Plassat JL, Bauer A, Lalevee S, and Rochette-Egly C (2005) Vinexin  $\beta$  interacts with the non-phosphorylated AF-1 domain of retinoid receptor  $\gamma$  (RAR $\gamma$ ) and represses RAR $\gamma$ -mediated transcription. *J Biol Chem* **280**:17027-17037.
- Allenby G, Bocquel MT, Saunders M, Kazmer S, Speck J, Rosenberger M, Lovey A, Kastner P, Grippo JF, Chambon P, et al. (1993) Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids. *Proc Natl Acad Sci USA* **90**:30-34.
- Keidel S, LeMotte P, and Apfel C (1994) Different agonist- and antagonist-induced conformational changes in retinoic acid receptors analyzed by protease mapping. *Mol Cell Biol* **14**:287-298.
- Klaholz BP, Mitschler A, and Moras D (2000) Structural basis for isotype selectivity of the human retinoic acid nuclear receptor. *J Mol Biol* **302**:155-170.

20. Nagy L, Thomazy VA, Shipley GL, Fesus L, Lamph W, Heyman RA, Chandraratna RA, and Davies PJ (1995) Activation of retinoid X receptors induces apoptosis in HL-60 cell lines. *Mol Cell Biol* **15**:3540–3551.
21. Thacher SM, Vasudevan J, and Chandraratna RA (2000) Therapeutic applications for ligands of retinoid receptors. *Curr Pharm Des* **6**:25–58.
22. Bernard BA, Bernardon JM, Delescluse C, Martin B, Lenoir MC, Maignan J, Charpentier B, Pilgrim WR, Reichert U, and Shroet B (1992) Identification of synthetic retinoids with selectivity for human nuclear retinoic acid receptor  $\gamma$ . *Biochem Biophys Res Commun* **186**:977–983.
23. Klaholz BP, Mitschler A, Belema M, Zusi C, and Moras D (2000) Enantiomer discrimination illustrated by high-resolution crystal structures of the human nuclear receptor hRAR $\gamma$ . *Proc Natl Acad Sci USA* **97**:6322–6327.
24. Germain P, Iyer J, Zechel C, and Gronemeyer H (2002) Coregulator recruitment and the mechanism of retinoic acid receptor synergy. *Nature* **415**:187–192.
25. Klein ES, Pino ME, Johnson AT, Davies PJ, Nagpal S, Thacher SM, Krasinski G, and Chandraratna RA (1996) Identification and functional separation of retinoic acid receptor neutral antagonists and inverse agonists. *J Biol Chem* **271**:22692–22696.
26. Klein ES, Wang JW, Khalifa B, Gavigan SA, and Chandraratna RA (2000) Recruitment of nuclear receptor corepressor and coactivator to the retinoic acid receptor by retinoid ligands: influence of DNA-heterodimer interactions. *J Biol Chem* **275**:19401–19408.
27. Szondy Z, Reichert U, Bernardon JM, Michel S, Toth R, Ancian P, Ajzner E, and Fesus L (1997) Induction of apoptosis by retinoids and retinoic acid receptor  $\gamma$ -selective compounds in mouse thymocytes through a novel apoptosis pathway. *Mol Pharmacol* **51**:972–982.
28. Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, and Evans RM (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90**:569–580.
29. Farboud B, Hauksdottir H, Wu Y, and 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.
30. Li H, Gomes PJ, and Chen JD (1997) RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. *Proc Natl Acad Sci USA* **94**:8479–8484.
31. McKenna NJ, Lanz RB, and O'Malley BW (1999) Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev* **20**:321–344.
32. Onate SA, Tsai SY, Tsai MJ, and O'Malley BW (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270**:1354–1357.
33. Voegel JJ, Heine MJ, Tini M, Vivat V, Chambon P, and Gronemeyer H (1998) The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways. *EMBO (Eur Mol Biol Organ) J* **17**:507–519.
34. Voegel JJ, Heine MJ, Zechel C, Chambon P, and Gronemeyer H (1996) TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO (Eur Mol Biol Organ) J* **15**:3667–3675.
35. Chen JD and Evans RM (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* **377**:454–457.
36. Horlein AJ, Naar AM, Heinzl T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK, et al. (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377**:397–404.
37. Sande S and Privalsky ML (1996) Identification of TRACs (T3 receptor-associating cofactors), a family of cofactors that associate with, and modulate the activity of, nuclear hormone receptors. *Mol Endocrinol* **10**:813–825.
38. Wong CW and Privalsky ML (1998) Transcriptional silencing is defined by isoform- and heterodimer-specific interactions between nuclear hormone receptors and corepressors. *Mol Cell Biol* **18**:5724–5733.
39. Lehmann JM, Hoffmann B, and Pfahl M (1991) Genomic organization of the retinoic acid receptor  $\gamma$  gene. *Nucleic Acids Res* **19**:573–578.
40. Bastien J, Plassat JL, Payrastra B, and Rochette-Egly C (2006) The phosphoinositide 3-kinase/Akt pathway is essential for the retinoic acid-induced differentiation of F9 cells. *Oncogene* **25**:2040–2047.
41. Giguere V, Shago M, Zirngibl R, Tate P, Rossant J, and Varmuza S (1990) Identification of a novel isoform of the retinoic acid receptor  $\gamma$  expressed in the mouse embryo. *Mol Cell Biol* **10**:2335–2340.
42. Lehmann JM, Zhang XK, and Pfahl M (1992) RAR  $\gamma$  2 expression is regulated through a retinoic acid response element embedded in Sp1 sites. *Mol Cell Biol* **12**:2976–2985.
43. Taneja R, Rochette-Egly C, Plassat JL, Penna L, Gaub MP, and 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 (Eur Mol Biol Organ) J* **16**:6452–6465.
44. Dolle P, Ruberte E, Kastner P, Petkovich M, Stoner CM, Gudas LJ, and Chambon P (1989) Differential expression of genes encoding  $\alpha$ ,  $\beta$  and  $\gamma$  retinoic acid receptors and CRABP in the developing limbs of the mouse. *Nature* **342**:702–705.
45. Dolle P, Ruberte E, Leroy P, Morriss-Kay G, and Chambon P (1990) Retinoic acid receptors and cellular retinoid binding proteins. I. A systematic study of their differential pattern of transcription during mouse organogenesis. *Development* **110**:1133–1151.
46. Haq R, Pfahl M, and Chytil F (1991) Retinoic acid affects the expression of nuclear retinoic acid receptors in tissues of retinol-deficient rats. *Proc Natl Acad Sci USA* **88**:8272–8276.
47. Ruberte E, Dolle P, Krust A, Zelent A, Morriss-Kay G, and Chambon P (1990) Specific spatial and temporal distribution of retinoic acid receptor  $\gamma$  transcripts during mouse embryogenesis. *Development* **108**:213–222.
48. Chiba H, Clifford J, Metzger D, and Chambon P (1997) Distinct retinoid X receptor-retinoic acid receptor heterodimers are differentially involved in the control of expression of retinoid target genes in F9 embryonal carcinoma cells. *Mol Cell Biol* **17**:3013–3020.
49. Rochette-Egly C and Chambon P (2001) F9 embryocarcinoma cells: a cell autonomous model to study the functional selectivity of RARs and RXRs in retinoid signaling. *Histol Histopathol* **16**:909–922.
50. Vasio GW, Gold JD, Petkovich M, Chambon P, and Gudas LJ (1989) A retinoic acid-responsive element is present in the 5' flanking region of the laminin B1 gene. *Proc Natl Acad Sci USA* **86**:9099–9103.
51. de The H, Vivanco-Ruiz MM, Tiollais P, Stunnenberg H, and Dejean A (1990) Identification of a retinoic acid responsive element in the retinoic acid receptor  $\beta$  gene. *Nature* **343**:177–180.
52. Ferrari N, Pfahl M, and Levi G (1998) Retinoic acid receptor  $\gamma$  1 (RAR $\gamma$  1) levels control RAR $\beta$  2 expression in SK-N-BE2(c) neuroblastoma cells and regulate a differentiation-apoptosis switch. *Mol Cell Biol* **18**:6482–6492.
53. Dupe V, Davenne M, Brocard J, Dolle P, Mark M, Dierich A, Chambon P, and Rijli FM (1997) In vivo functional analysis of the Hoxa-1 3' retinoic acid response element (3'RARE). *Development* **124**:399–410.
54. Smith WC, Nakshatri H, Leroy P, Rees J, and Chambon P (1991) A retinoic acid response element is present in the mouse cellular retinol binding protein I (mCRBPI) promoter. *EMBO (Eur Mol Biol Organ) J* **10**:2223–2230.
55. Durand B, Saunders M, Leroy P, Leid M, and Chambon P (1992) All-*trans* and 9-*cis* retinoic acid induction of CRABP II transcription is mediated by RAR-RXR heterodimers bound to DR1 and DR2 repeated motifs. *Cell* **71**:73–85.
56. Chapellier B, Mark M, Messaddeq N, Calleja C, Warot X, Brocard J, Gerard C, Li M, Metzger D, Ghyselinck NB, et al. (2002) Physiological and retinoid-induced proliferations of epidermis basal keratinocytes are differentially controlled. *EMBO (Eur Mol Biol Organ) J* **21**:3402–3413.
57. Ghyselinck NB, Dupe V, Dierich A, Messaddeq N, Garnier JM, Rochette-Egly C, Chambon P, and Mark M (1997) Role of the retinoic acid receptor  $\beta$  (RAR $\beta$ ) during mouse development. *Int J Dev Biol* **41**:425–447.
58. Lohnes D, Kastner P, Dierich A, Mark M, LeMeur M, and Chambon P (1993) Function of retinoic acid receptor  $\gamma$  in the mouse. *Cell* **73**:643–658.
59. Mark M, and Chambon P (2003) Functions of RARs and RXRs in vivo: genetic dissection of the retinoid signaling pathway. *Pure Appl Chem* **75**:1709–1732.
60. Mark M, Ghyselinck NB, and Chambon P (2006) Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signalling pathway during mouse embryogenesis. *Annu Rev Pharmacol Toxicol* **46**:451–480.
61. Fisher GJ, Datta SC, Talwar HS, Wang ZQ, Varani J, Kang S, and Voorhees JJ (1996) Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature* **379**:335–339.
62. Fisher GJ, and Voorhees JJ (1996) Molecular mechanisms of retinoid actions in skin. *FASEB J* **10**:1002–1013.
63. Wang Z, Boudjelal M, Kang S, Voorhees JJ, and Fisher GJ (1999) Ultraviolet irradiation of human skin causes functional vitamin A deficiency, preventable by all-*trans* retinoic acid pre-treatment. *Nat Med* **5**:418–422.