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REGULATION OF GENE EXPRESSION BY VITAMIN A: The Role of Nuclear Retinoic Acid Receptors

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PERSPECTIVES

Determination of the genetic interactions responsible for definition of the body plan during embryogenesis remains one of the most interesting problems in developmental biology. Different species employ rather diverse strategies

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in establishing ultimate body plan or pattern. One basic mechanism found in many phylogenetically unrelated invertebrates such as echinoderm and nematode (28) is asymmetric cell division involving canonical patterns of cell cleavage during blastula formation; cleavage planes formed during cell division define spatial domains restricted to specific cell fates. Although some of the differentiated cell types in these organisms depend on cell-cell interactions (48), certain cell lineages appear to be established by the inheritance of regionally sequestered maternal factors (28).

In another strategy, which is the predominant mechanism in higher vertebrates, the establishment of axial polarity and the patterning of cells depend on local signals distributed in the form of gradients that derive from restricted regions of the embryo (93). In addition, identity of cell lineages, and the ultimate patterns of differentiation in their progeny, depend qualitatively on contact with other cells, either directly or via signal molecules. In vertebrates, early cell lineages arising from blastomeres are for the most part indeterminate (28, 73, 115). Lineage indeterminacy suggests that the establishment of cell fate requires position-dependent cell interactions that may begin as early as cleavage (28, 32). However, positional information is required not only during the very early stages of development but as long as cells remain multipotent, even throughout adulthood. Consequently, numerous signal molecules have been identified, such as retinoids, fibroblast growth factors (FGFs), and transforming growth factors (TGFβs), which play important roles in the determination of cell fate both in the embryo and in the adult (2, 20, 32, 92).

Retinoids and Development

A major breakthrough in the study of signal molecules and pattern formation in vertebrates has come from recognition that certain metabolites of vitamin A,[1] retinoic acid (RA) in particular, are able to re-specify positional cues in the chick limb bud in a dose-dependent manner (41). More recently it has been shown that retinoic acid may also play an essential role in the establishment of the primary embryonic axes (1a, 40, 42, 129, 149).[2] The effectiveness of RA as a potent inducer of cell differentiation is not limited to embryonic tissues; the epithelium, connective tissues, including bone and cartilage, as well as hematopoetic tissues are some of the better known RA targets in the adult (99, 107, 113, 133, 134, 158, and references therein). The pleiotropy of RA activity has supported the hypothesis that RA may function

[1]A comprehensive review on retinoid metabolism can be found elsewhere in this volume.

[2]A complete discussion of the physiological effects of RA is beyond the scope of this review; several excellent reviews have been published recently on the effects of retinoids on vertebrate development (12–14, 41, 63, 72, 138).

through several different pathways. However, the identification of RA nuclear receptors, which belong to the steroid hormone receptor superfamily (50, 106), suggests that RA may effect cellular responses mainly through the regulation of gene transcription (43, 56). Can the ability of RA to regulate gene expression through these receptors alone account for the diversity of RA responses? In this review, an attempt has been made to summarize what is known about retinoic acid receptors and to describe how they might directly or indirectly interact with other cellular components to generate diversity in cellular responsiveness to RA.

RETINOIC ACID RECEPTORS

Nuclear Receptor Structure and Function

At present, more than 30 members of the nuclear receptor family have been identified (43, 56). Specific ligands for many of these receptors include the steroid hormones estrogen, androgen, mineralocorticoid, glucocorticoid, vitamin D_3, and progesterone (43, 56). However, the discoveries that the actions of thyroid hormone and retinoic acid are also mediated through such nuclear receptors indicated that this class of transcription factors has evolved to recognize many structurally unrelated ligands (43, 50, 56, 106). Indeed, given the growing number of orphan receptors (nuclear receptors for which ligands have not yet been identified) and the present lack of obvious prospective ligands, it is likely that the molecular nature of activating ligands will further diversify. Despite the structural unrelatedness of ligands, nuclear receptors are remarkably well conserved (43, 56). Little variation is found in the linear arrangement of their functional elements, which typically comprise six domains (43, 52, 56), denoted A–F in Figure 1. Also the degree of amino acid identity between physiologically unrelated receptors is striking, particularly in the DNA- and ligand-binding regions. Although each nuclear receptor exhibits peculiarities distinguishing it from others in the family, the conservation of basic structure suggests conservation of basic function as well (43, 45, 47, 56, 77). Consequently, structure/function relationships for one subclass of receptors can in many cases be inferentially applied to another, but always with due caution. In many respects (described below), the retinoic acid receptors resemble other steroid receptors; however, they also display features unique to this subclass.

The DNA-Binding Domain

The DNA-binding domain, region C, is the hallmark of the nuclear receptor family. Of the 66–68 amino acids that make up this domain, 19 residues are invariant, including 8 cysteine residues involved in chelation of 2 Zn^{2+} ions, thus forming the base of the two so-called Zn-binding fingers (60, 86). X-ray

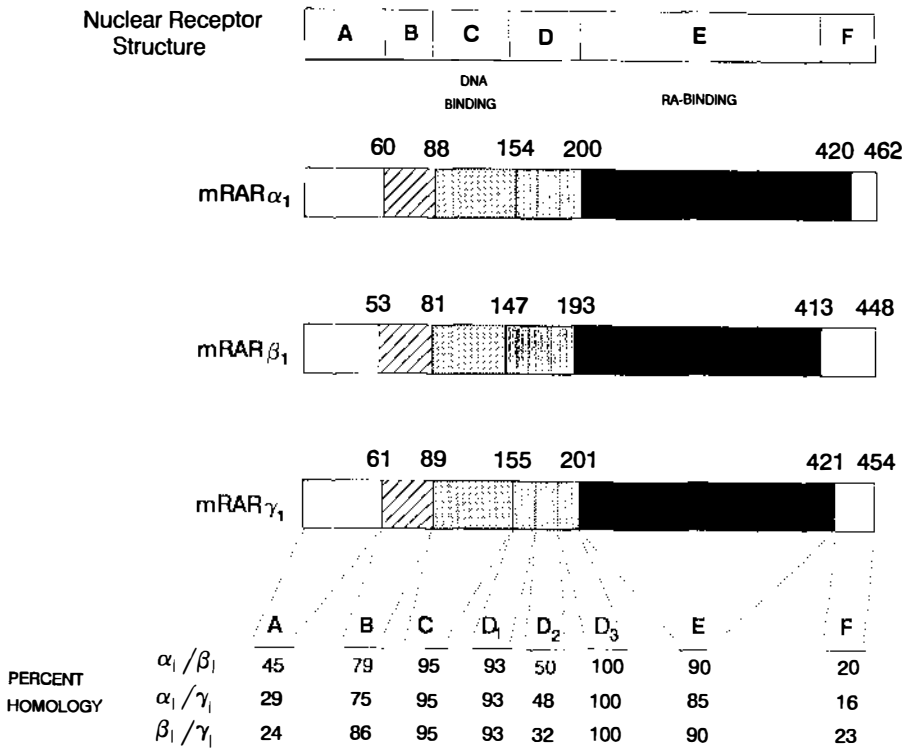

Figure 1 The retinoic acid receptor family (RARs). Schematic comparison of the murine RAR proteins. The basic linear organization of nuclear receptor functional domains is shown at the *top* of the figure (see text). The primary amino acid sequences (numbered above the protein structure) have been aligned on the basis of sequence identity, the percentage of which is indicated in the table at the *bottom* of the figure. The DNA- and hormone-binding domains are indicated. The subdomains of region D in the RARs are indicated by the *hatched* lines (see text for details).

crystallographic studies of the glucocorticoid receptor DNA-binding domain indicate that unlike other finger-containing proteins such as TFIIIA, whose fingers act as independent units, each contributing to DNA binding, the zinc fingers of nuclear receptors fold together as part of a larger, more integrated globular domain (86). These studies further indicate that the most N-terminal finger (C1) of the glucocorticoid receptor (GR) DNA-binding domain adopts a configuration allowing its close apposition to key nucleotide residues in the cognate hormone responsive element (HRE; discussed below) that are important for binding selectivity (86 and references therein). The second finger (C2) may stabilize this binding through nonspecific interactions. Crystallographic analysis of the GR, and NMR studies on the estrogen receptor (ER) and GR DNA-binding regions, confirm earlier studies showing (*a*) that swapping the C1 finger between the ER and GR was sufficient to trade specificities for the

estrogen and glucocorticoid responsive elements and (*b*) that three amino acid residues in the C1 finger were implicated in this specificity (26, 77, 87, 144).

Retinoic acid receptors (RARs) were first isolated by virtue of their sequence similarity to other nuclear receptors in the DNA-binding region (50, 106). Subsequently, three types of retinoic acid receptors (RARs), RAR-α (50, 106), RAR-β (11), and RAR-γ (67, 75), have been isolated from several species of vertebrates. When compared with other members of the nuclear receptor family, the retinoic acid receptors exhibit a similar structure showing the highest degree of homology in the DNA (C) binding region. For example the 66 amino acid RAR-α region C (see Figure 1) is 62%, 58%, and 45% homologous to the corresponding domains of the thyroid hormone receptor (c-ErbA), ER, and GR, respectively. When compared with each other, the RARs are almost completely identical in region C (see Figure 1). Figure 2 shows the hypothetical structure of the human RAR-α region C as it might be

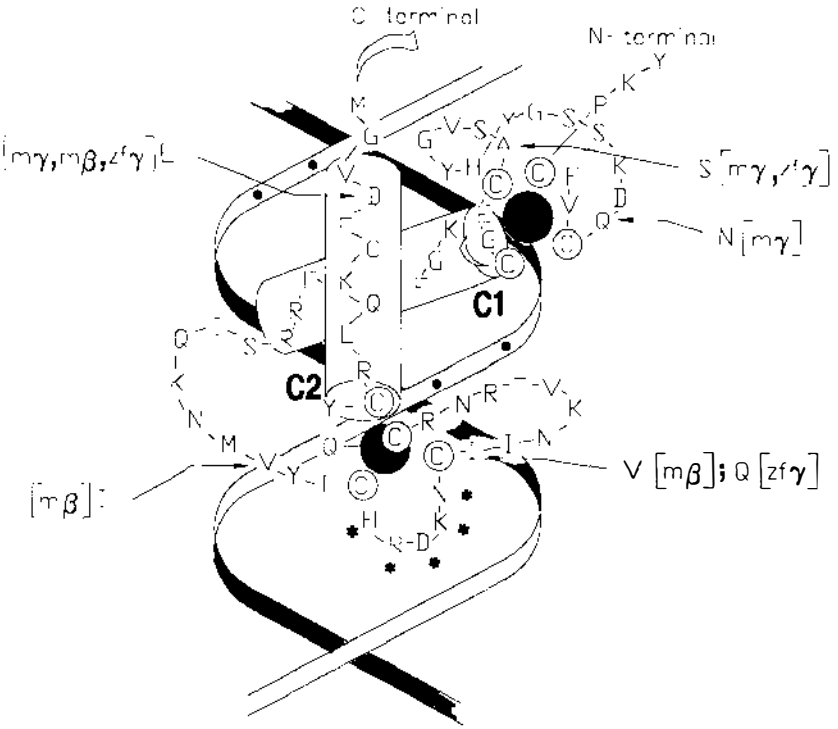

Figure 2 Schematic representation of the mouse RAR-α DNA-binding domain bound to DNA. Hypothetical modelling of the DNA-binding domain based on the resolution of the glucocorticoid receptor DNA-binding domain (see text for references). The amino- and carboxy-terminal ends (residues 88 and 153, respectively) are indicated as are the two "zinc-finger" domains C1 and C2 (see text). Zinc ions are depicted by solid discs. Residues marked by asterisks correspond to the D-box referred to in the text. Arrows indicate amino acid residue substitutions found in mouse RAR-β and -γ (mβ and mγ respectively) and zebrafish RAR-γ; zfγ.

folded together to contact DNA. The few differences in amino acid sequence between RAR α, β, and γ are noted in the diagram. In addition, the conservation between region C of zebrafish RAR-γ and mouse RARs is also noted. This comparison illustrates that this sequence has remained essentially unchanged for over 450 million years.

Responsive Elements

DNA sequences required for the action of particular classes of nuclear receptors have been identified. For example, several natural glucocorticoid responsive elements (GREs) contain an imperfect inverted repeat of the conserved hexanucleotide TGTTCT, separated by a spacer of three nonconserved nucleotides (26, 87, 144, and references therein). Receptors for androgens, mineralocorticoids, and progestins, each of which on the basis of amino acid sequence homology have been classified as belonging to the GR subfamily, also appear to share specificity for this target site motif (26, 87, 90, 144). The estrogen responsive element (ERE), though similar in structure to the GRE, contains the conserved hexanucleotide TGACCT (56). Most of the other members of the nuclear receptor family, including the RARs, belong to the estrogen receptor/thyroid hormone receptor (ER/TR) subfamily (also on the basis of amino acid homology) and display varying degrees of cross-recognition of palindromic responsive elements containing canonical or degenerate forms of the ER-type half-site TGACCT (90). However, as first noted for the RARs, the spacing between ER-type response element half-sites as well as their relative orientation appears to be important for the selective discrimination of target responsive elements by members of the ER/TR subfamily (26, 145). For example, whereas palindromic thyroid hormone responsive elements (TREs) (e.g. TGACCGGTCA) confer retinoic acid responsiveness to heterologous promoters, direct repeat TREs do not (26, 145). Recent studies indicate that the presence of 3, 4, or 5 spacer nucleotides between direct repeats of the TGACCT (or inverted AGGTCA) half-site will determine the specificity of response to vitamin D_3, T_3, and RA, respectively (26, 145). However, response element configuration alone is insufficient to specify receptor-target gene interactions. How do GR target sites for the GREs select for response to the GR when progesterone receptors (PR) and androgen receptors (AR) appear to recognize the target element equally well? Even more puzzling, do RA responsive elements (RAREs) exist that are specific for RARα, β, and γ subtypes?

RAREs and RARs

Minor differences between the DNA-binding domains of the three RARs appear to be conserved between species (e.g. see Figures 1 and 2). Could these minor changes target subtle differences in RAREs? Co-transfection of

the RAR subtypes α, β, and γ with various RARE promoter constructs has thus far failed to reveal selectivity for one subtype over another (116, 136). Unfortunately, co-transfection experiments in which receptor and target copy numbers greatly exceed physiological levels might create conditions that favor nonspecific interactions between receptor and target and thus obscure the more subtle protein-DNA and/or protein-protein contacts required for specificity. In this regard, it is interesting to note that in transgenic mice harboring a construct containing the RARE from the RAR-β promoter and a "neutral" promoter (hsp 68) spliced to a β-galactosidase *(lacZ)* encoding reporter gene, the resulting pattern of *lacZ* expression closely resembles that of the RAR-β gene (116), even though in transient transfection experiments the same RARE was recognized by all three RARs (116, 136). Since RAR-α gene expression is ubiquitous throughout most developmental stages, and RAR-γ is expressed in regions of the embryo different from RAR-β, this particular RARE apparently displays a preference for the latter (116, 119, 130). However, these results may also indicate the requirement for the coexpression of factors other than RARs in tissues in which the RARE is active (see below).

Although the expression of many genes appears to be regulated by RA (e.g. 21, 23, 29, 35, 57, 65, 68, 78, 84, 85, 107, 133, 137, 142, 146), relatively few RA-responsive elements (RAREs) have been characterized. One of the first naturally occurring RAREs to be characterized was found in the RAR-β promoter (23, 64). In both the human (23) and mouse (64) forms of this promoter, which exhibit extensive homology with each other, the RARE is a direct repeat of the motif GTTCA separated by a gap of 6 nucleotides. Related RAREs have been identified in other genes, including the human alcohol/dehydrogenase promoter (145), the mouse complement factor H gene promoter (97, 145), the mRAR-α2 isoform promoter (81), and the mouse cellular retinol-binding protein type-I (mCRBP1) (132). For the RAR-β2 RARE, mutagenesis has demonstrated that both repeats are essential for RAR binding (98, 122). Moreover, the 6 nucleotide spacing is optimal, although a spacing of 3 nucleotides was also shown to be effective both in binding RAR and in conferring RA responsiveness to a heterologous promoter (122).

Although the kinetics of receptor binding to response elements has not been thoroughly investigated, within receptor subclasses some degeneracy in response elements clearly is permissible. As mentioned above, the ER will not bind detectably to a GRE but will bind to certain degenerate forms of the ERE. Similarly, the GR does not bind detectably to EREs. In some instances an ERE half-site (TGACCT) has been shown capable of confering estrogen inducibility to a promoter. However, if the formation of an ER dimer is required for estrogen-inducible transactivation, and one ER monomer binds to one half-site, how is this possible? Perhaps a receptor dimer can still func-

tionally bind to a half-site. In this case, one ER monomer would make specific contacts with the half-site while the second would make nonspecific contacts with adjacent nucleotides. Results from crystallographic studies of the GR suggest that this may indeed be the case (86). These studies show that the DNA-binding domain of the GR is capable of making contacts with non-GRE motifs as long as there is a stabilizing interaction with another monomer bound to a half-site (86). Thus if this kind of interaction is widespread, isolated half-sites that confer hormone responsiveness may in fact be binding receptor dimers. Furthermore, receptors may functionally interact with "poor" or "nonconsensus" responsive elements provided that this interaction is stabilized by "strong" or "specific" protein-protein contacts with other proteins bound to DNA. Although this latter possibility has yet to be demonstrated experimentally, some evidence suggests that certain nuclear receptors interact specifically with other proteins bound to DNA (see below).

The laminin B1 (LamB1) promoter contains several degenerate forms of isolated ERE half sites that have been shown to be important for the RA responsiveness of this promoter (147, 148). The LamB1 RAREs appear not to be selective for a particular RAR subtype in transient transfection experiments (147, 148). Do RARs bind to these sites as monomers, homodimers, or heterodimers? Furthermore, since the most efficient RARE is in the form of a direct repeat of half-sites rather than inverted, as would be the case for the ER or GR, how might RAR monomers be oriented with respect to each other? Since evidence suggests that other nuclear receptors may form heterodimers with RARs (Ref. 54 and see below), it will be interesting to see how RAR-RAR or RAR-nuclear receptor contacts differ from those of ER and GR homodimers.

The Ligand-Binding Domain

The ligand-binding region of nuclear receptors, characterized by the moderate degree of conservation it displays with other members of the receptor family, fulfils numerous functions. For those receptors known to bind specific ligands, mutational analyses have unequivocally demonstrated the importance of this region for ligand binding (43, 56, 77). Other functions ascribed to this region, such as receptor dimerization and transcription activation, have been shown to be ligand dependent (44, 45, 58, 151). The molecular nature of the ligand dependency of these functions has not yet been defined. For receptors such as the PR, GR, and ER, one model suggests that the binding of ligand dissociates the receptor from the protein-inactivating function of the heat-shock protein HSP 90, thus permitting receptor dimerization, high affinity DNA binding, and transcriptional activation to occur (92, 108–110). However, recent studies using in vitro transcription systems indicate that hormone-dependent transcription activation can occur in the absence of heat shock

proteins (4). Furthermore, there is no evidence that nuclear receptors such as RAR, TRs, and vitamin D_3 receptor (VDR) form complexes with Hsp 90 (25, 107). The ligand binding domains of receptors for structurally unrelated ligands share significant regions of homology (43, 56), Perhaps the key to the mechanism of ligand activation is formed in the amino acid residues that are conserved among these different receptors.

The retinoic acid receptors exhibit almost complete conservation in their retinoic acid binding domains (see Figure 1). Of the 220 amino acids that make up this domain, all but 35 residues are identical when RAR-α, -β, and -γ are compared with each other. In addition, most of these subtype specific residues are also conserved when these receptors are compared with their respective counterparts in other species. Therefore, it seems likely that these amino acid differences are indicative of important functional differences between the three RAR subtypes.

The ligand-binding region (E) of the RARs may, like other nuclear receptors, be involved in several functions: (*a*) ligand binding, (*b*) receptor dimerization, and (*c*) transactivation.

LIGAND BINDING The RAR retinoic acid binding domain has been defined as a 220 amino acid region in the C-terminal half of these receptors. This region was originally designated as the RA-binding region because of its relative homology to ligand-binding domains of other steroid receptors (92, 106). Investigators have now shown that when the C-terminal portions of RAR-α and RAR-β, extending from the beginning of region D to the C-termini, are expressed in *Escherichia coli,* they bind retinoic acid apparently as efficiently as do whole receptors (16, 24, 136, 156).

When human (hRAR-α) and mouse (mRAR-β) were compared with respect to their abilities to activate reporter gene transcription, approximately 5–10 fold higher concentrations of RA were required to achieve the same level of activation with RAR-α as was achieved with RAR-β (11). This study provided the first evidence that the abilities of the three receptors to bind retinoic acid could be different. With the identification of RAR-γ, transcription activation studies suggested that, of the three receptors, RAR-γ might have the highest affinity for RA (51). The notion that these differences might be exploited to identify synthetic subtype-specific retinoid analogs has led to the identification of several such compounds exhibiting subtype preference (3, 16, 61, 113, 141). One example is the retinobenzoic acid compound Am80 (24, 139), which activates RAR-α with a higher apparent affinity than it does RAR-β, the converse of observations with RA. Interestingly, Am80, which binds cellular retinoic acid binding protein (CRABP) with a much lower affinity than does RA, mimics exactly the effects of RA in causing digit duplications in chick wing bud experiments (139).

Tagged onto the C-terminal end of the ligand-binding domain is region F. Little is known about the function, if any, of this region of the RARs. Although it is relatively well conserved between man and mouse, little homology is seen in more divergent species such as *Xenopus* (42), newt (111), and zebra fish (J. White and M. Petkovich, unpublished).

RAR DIMERS AND HETERODIMERS Receptor dimerization has been shown to occur for many receptors (44, 45, 80, 86). Crystallographic and NMR analyses of the ER and GR indicate that dimerization occurs at least in part through interactions between DNA-binding domains (86). Several structure/function studies have also indicated an important role for the ligand-binding domain in the dimerization process. The identification of a conserved motif in ligand-binding domains of various nuclear receptors including the TR and RARs, which is reminiscent of the α-helical "zipper" motifs (47, 79) that mediate homo- and heterodimer formation between the transcription factors c-jun, C/EBP, DBP, and c-myc, indicated a possible point of interaction between receptors (44–46, 62, and references therein).

The formation of homodimers is essential for the function of many transcription-regulating proteins. This appears to be the case for several nuclear receptors including the ER and GR (44, 76, 135, and references therein). The existence of multiple genes encoding RARs, each of which through alternate exon usage can give rise to several different isoforms (see below), raises the possibility that RAR homo/hetero dimer formation may be an important regulatory mechanism. Co-transfection studies indicate that the RAR-γ2 isoform can inhibit the activity of RAR-β (66). Whether this antagonism is the result of heterodimer formation or due to competition for limiting transcription factors is not clear.

Cross-linking studies have shown that the TR and RAR-α may form complexes with several distinct nuclear proteins (co-regulators) that are expressed in a cell-dependent fashion (27, 46, 54, 55). These interactions are apparently mediated by protein-protein interactions occurring through the ligand-binding domain "zipper-like" motif (44–46, 54, 103). Although the identities of these co-regulators are under investigation in several laboratories, at least one of them corresponds to another nuclear receptor, TR (54). This relationship between RAR and TR has been quite revealing in terms of the oncogenic activity of the v-erbA TR derivate and is discussed in further detail below. Nuclear receptors other than TR are likely to form complexes with RARs. Most recently, a novel class of retinoid receptors (RXRs) has been characterized (88). Interestingly, RXRs have been shown to form heterodimers with RARs, thereby enhancing manyfold RAR binding to RAREs (see below). Furthermore, transcription activation studies indicate that cer-

tain intermediary factors functionally, and perhaps physically, link the nuclear receptors to the transcriptional machinery (see below). The nature of these intermediary factors remains to be determined.

TRANSCRIPTION ACTIVATION FUNCTIONS Several classes of domains are capable of mediating transcriptional activation; many of them are acidic in nature, such as the yeast transcription factor Gal4 and the herpes simplex virus protein VP16 (151, 152, 154, and references therein). Studies with hormone receptors have shown that regions important for transcription activation can be found both in the N-terminal A/B domains as well as in the ligand-binding domain (region E) (140, 143, 151). Interestingly, the A/B region activation domain can function independently of ligand, whereas the C-terminal activation domain appears to be hormone dependent (140, 151). Neither of these domains in the estrogen receptor contains stretches of acidic amino acids whereas both comparable domains in the glucocorticoid receptor (τ1, located in the A/B domain, and τ2, in the N-terminal regiona of the glucocorticoid-binding domain) are acidic in content.

By expressing portions of these receptors either alone or as chimeras with other transcription factors, researchers have demonstrated that the two separate transactivation domains in steroid receptors can function independently by interacting with different, specific intermediary factors (140, 151, and references therein). These intermediary factors are currently uncharacterized.

N-TERMINAL REGION ISOFORMS Several classes of steroid receptors including those for progesterone, retinoic acid, and thyroid hormone exhibit alternative exon usage in the most N-terminal or A/B region of receptors (71, 75, 140, 143, 155). The PR exists in two forms, which differ in their A domains and exhibit both cell-type and promoter specificity in their ability to activate reporter gene transcription (143). Possibly, each of the two different N-terminal alternate regions of the PR interacts with different intermediary factors that exhibit cell-specific patterns of expression (140). This possibility is particularly interesting given that several alternate A regions for each of the three RAR subtypes (α, β, and γ) as well as for TR-β have been characterized. Expression of these alternative A regions, at least for the RAR and PR, appears to be regulated independently by different promoters in a tissue-specific manner (81, 159).

At least seven different isoforms have been identified for mouse RAR-α, three for RAR-β, and seven for RAR-γ (51, 71, 159). For most of these isoforms, regions B through F (Figure 1) are constant (10). The functional importance of the A region for RARs is not know. However, sequence

comparisons with homologous isoforms found in species other than mouse, chicken (100, 131), man (75), newt (111), *Xenopus* (42), and zebra fish (J. White and M. Petkovich, unpublished) reveal a high degree of conservation. Although the tissue-specific distribution of most of these isoforms is not presently known, several of the isoforms have been expressed in a temporally and spatially restricted manner. For example, whereas the RAR-α1 isoform is ubiquitously expressed, RAR-α2 appears to be specifically expressed in intestine and lung with only trace amounts of transcript detectable in other tissues (81). Similarly, RAR-β2 is found in kidney, heart, and skeletal muscle, whereas the RAR-β1 and -β3 isoforms are expressed more abundantly in brain, lung, and skin. RAR-γ1 is the predominant RAR-γ isoform expressed in skin, and both RAR-γ1 and -γ2 isoforms are expressed during embryogenesis; the RAR-γ2 isoform is expressed more abundantly during early stages of development (71). Interestingly, certain isoforms of RAR-α and -β appear to be regulated by different promoters (81, 159). The expression of RAR-α2, for example, could be induced by retinoic acid in embryonal carcinoma cells, whereas RAR-α1 appears constitutively expressed (81). In addition, each different isoform mRNA contains a different 5'-stretch of noncoding mRNA that may affect its stability or translation efficiency in a tissue-specific manner (74).

Sequence comparisons between RAR-α1, RAR-β1, and RAR-γ1 or between RAR-α2, RAR-β2, and RAR-γ2 reveal significant homologies between these different subtype isoforms. These homologies indicate that the related subtypes may have functional similarities (17). The functional differences between A regions remain to be determined, yet the tissue-specific distribution, independent regulation of different isoforms, and evolutionary sequence conservation strongly suggest that specific differences do exist (17).

Hinge Region: Region D

The region joining the ligand- and DNA-binding domains (region D) has gained little attention, because its sole purpose for the ER and GR appears to be to serve as a structural joint between the two functional domains (43, 56). The RARs, however, exhibit an unusual degree of subtype-specific sequence conservation in this region (158). On the basis of homology, this region can be subdivided into three separate regions: D_1, D_2, and D_3 (158) (Figure 1). The most N-terminal subdivision is conserved 14 out of 15 residues between RAR-α, -β, and -γ (Figure 1) and contains a sequence resembling the nuclear localization signal for the large T antigen (106) while the most C-terminal region, D_3, is completely conserved (7/7 residues). The central region D_2 is less than 50% conserved when RAR-α, -β, and -γ are compared with each

other but is almost entirely conserved in cross species comparisons (17). For example the hRAR-γ D_2 is identical in 17 out of 24 residues with respect to its zebra fish counterpart (J. White and M. Petkovich, unpublished). This degree of conservation indicates that the D region plays a specific functional role that possibly is unique to this class of receptors. The importance of this region remains to be determined.

RAR Phosphorylation

Several classes of nuclear receptors including the PR, GR, ER, VDR, and TR are posttranslationally modified by phosphorylation (31, 69, 114). Recently, RAR-γl has also been shown to be phosphorylated (114). Although the phosphorylated amino acid residues were not identified, regions A/B and D were shown to be targets of phosphorylation (114). Both regions A/B and D of RAR-γ contain several kinase recognition motifs for serine phosphorylation (see Table II in Ref. 114). Although the phosphorylation of PR, GR, and VDR could be increased by the presence of their respective ligands, this was apparently not the case with RAR-γ (31, 69, 114). What function phosphorylation serves for nuclear receptor function has yet to be determined; however, D region phosphorylation may be important in nuclear localization. Furthermore, amino-terminal phosphorylation would not be inconsistent with the possibility that phosphorylation may modulate the efficiency of transcription activation (114).

Developmental Expression of RARs

The three genes encoding RARs are differentially expressed during mouse development. Several comprehensive studies comparing the temporal and spatial distribution of RAR, CRBP, and CRABP mRNA transcripts have been carried out in mouse (15, 38, 39, 94, 119, 120) and chicken (123, 131), and the reader is directed to these studies for detailed accounts of expression patterns. These studies show that RAR expression is widespread throughout various developing tissues and organs but is topographically restricted in a subtype-specific manner (38, 39, 119, 120). RAR-α in general is rather ubiquitously expressed. However, as previously discussed, at least one isoform of RAR-α exhibits a more tissue-specific pattern of expression (81). Tracheal epithelium has long been suspected as a prime target for the effects of RA. The expression of RAR-β in trachea, among other epithelial tissues, implies that this subtype has a particular role in mediating retinoid effects. Skin is an important RA target tissue and RAR-γ appears to be the predominantly expressed RAR in this organ (102, 158). Similarly, the expression of RAR-γ in chondrogenic regions is consistent with previous studies describing effects of retinoids on bone growth and development (107, 120). This is

particularly noteworthy in the developing vertebrate limb where RAR-γ expression appears to be restricted to precartilagenous condensations that will eventually form the bone models (38, 120).

Regulating RAR Function: Interactions with Other Gene Products

OTHER TRANSCRIPTION FACTORS Several lines of evidence indicate that membrane and nuclear receptor signalling pathways converge on common regulatory elements and can exert opposite effects on gene transcription. For example, it has been shown that a site in the human osteocalcin promoter can be recognized both by the VDR and RAR as well as by the AP1 site binding proteins c-jun and c-fos. Whereas this responsive element can mediate induction of osteocalcin gene transcription by RA and vitamin D_3, the coexpression of c-jun and c-fos suppresses basal transcription of this gene as well as induction by the two vitamins (125). Whether these opposing effects of the receptors and fos/jun complex are the result of direct protein-protein interactions or of competition for the common DNA motif is not clear. Conversely, studies have shown that the collagenase promoter is positively regulated by the AP-1 site, which binds the fos/jun complex, and that this positive regulation can be blocked by the GR (37, 124, 157). DNA binding and protein cross-linking experiments indicate that a direct interaction between the GR and either fos or jun is responsible for the mutual corepression (157).

Interestingly, the promoter of interstitial collagenase and a synthetic AP-1-dependent promoter were repressed both by RAR-α and the thyroid hormone receptor (c-ErbAα) in a ligand-dependent manner. The mechanism of this repression is like that of the GR and is also thought to be through a specific receptor-mediated decrease in activity of c-fos/c-jun, which positively regulate these promoters (33). Without the AP-1 site, RAR-α and c-ErbA-α do not affect promoter activity (33, 99). Although there is no evidence as yet that RAR-α and c-Erb-α are directly interacting with c-fos and/or c-jun proteins, the v-erbA oncoprotein (126), in a dominant negative fashion, abrogates the RAR-α mediated repression of AP-1 activity. Possibly, v-erbA competes with RAR-α and c-ErbA for receptor-binding sites, but due to specific mutations in its C-terminal region, v-erbA is unable to inactivate or repress AP-1 activity. Such a mechanism may account for the oncogenic activity of v-erbA. Since c-ErbAα has been shown to form heterodimers with RAR-α (53, 54), a similar heterodimer formed between v-erbA and RARα might be inactive (33, 53, 54).

Although the exact nature of receptor-fos/jun interactions is not known, such interactions may be essential for the reciprocal control of cell prolifera-

tion and differentiation. Such interactions may also provide a basis for understanding some of the mechanisms underlying the hormonal responsiveness of tumors.

CELLULAR RETINOIC ACID BINDING PROTEINS In addition to the RARs, small intracellular retinoic acid binding proteins (CRABPs) have also been identified (5, 49, 127, 128, 134, 153). CRABPs belong to a multigene family of proteins that includes the retinol binding proteins CRBPI and II (5, 49) as well the myelin protein P2 (91) and fatty acid binding protein (FABP) (91). These proteins, which are approximately 15 kd in size, do not bear an obvious similarity to any known transcription factors (128), nor is there any similarity in the structure of these proteins with the RAR ligand-binding domain; yet they bind RA selectively and with high affinity. The function of these proteins is unknown; however, the lack of expression of these proteins in certain RA-responsive target tissues and cells suggests that CRABPs are not directly involved in mediating RA effects (15, 134). Recent studies have shown that CRABP-I, when stably transfected into F9 teratocarcinoma stem cell lines, can inhibit RA-induced differentiation into extraembryonic endoderm (9). This finding supports the notion that CRABPs may limit the amount of RA available to the nuclear receptors, thus attenuating RA effects on gene regulation (87). This model is consistent with the observation that sites of expression of CRABPs, such as in the developing central nervous system, craniofacial area, and limbs, are targets for retinoid teratogenicity (6, 15, 112).

RETINOID X RECEPTORS

In addition to the RARs, a second family of nuclear receptors, the retinoid X receptors (RXRs), appear to be involved in mediating cellular responses to retinoids (59, 88, 118). Their structures are shown in Figure 3. RXR-α was identified by low stringency cross-hybridization with the RAR-α DNA-binding domain (88) while RXR-β (H-2R11BP) was identified as a transcription factor binding to the region II enhancer of major histocompatability complex (MHC) class I genes (59). A closely related receptor has recently been identified in *Drosophila melanogaster;* however, this RXR-like receptor neither binds retinoids nor activates gene expression in a RA-dependent manner (104). Although RARs and RXRs differ substantially in primary structure (see Figure 3), RXRs respond specifically to RA (88, 89). The degree of homology between RXR-α and RXR-β in both the DNA- and ligand-binding domains is greater than 85% (Figure 3). RXR-α requires relatively higher concentrations of RA for activation than do the RARs (88).

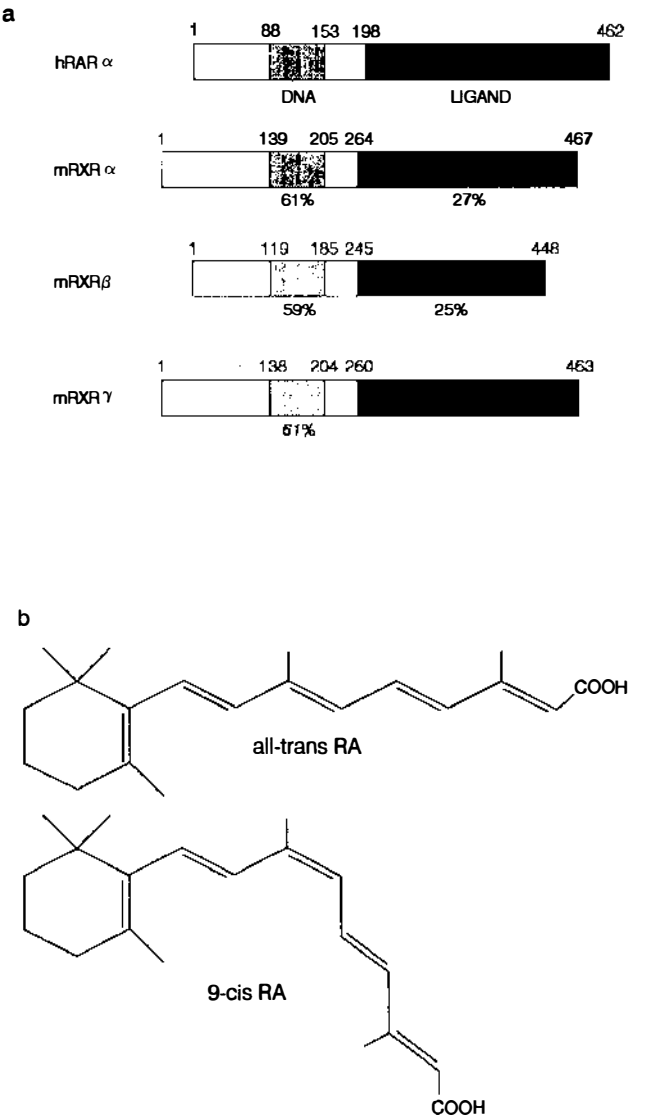

Figure 3 (*a*) The retinoid-X-receptor (RXR) family. Schematic comparison of the amino acid sequence homology between RAR-α and mouse RXR-α, RXR-β, and RXR-γ. Protein residues are indicated above the protein structures. Only the DNA-binding and ligand-binding domains of RXRs exhibit significant amino acid identity with RAR-α (shown by the *shaded* and *solid* regions respectively). Sequence comparisons between the three RXRs indicate almost complete conservation of DNA- and ligand-binding regions (>90%) (see reference 80a). (*b*) Structural representations of all-*trans (top)* and 9-*cis (bottom)* isomers of retinoic acid.

In co-transfection experiments, well-characterized retinoid analogues (e.g. TTNBP) were much less effective at stimulating a transcriptional response with RXR-α than with RAR-α (88). These results suggest that RXR-α might be specific for a retinoid other than RA (7, 88). Recently, 9-*cis* isomer of RA has been identified as a likely candidate for the RXR ligand (Figure 8*b*) (62a). This naturally occurring retinoid binds to RXRs with higher affinity than does all-*trans* RA, the metabolite from which it is derived (62a, 81a).

RXR-α and RXR-β exhibit unique tissue-specific patterns of expression distinct from those seen for the RARs (59, 88, 118). Interestingly, the tissue distribution of the chicken RXR-α (cRXR-α) overlaps extensively with that for CRABP (118) in the developing peripheral nervous system; however, it is not clear whether RXR and CRABP functions are in any way integrated (118). Comparatively high levels of RXR-α expression have been observed in both chicken and rat liver (88). This high level of expression in the liver may suggest a regulatory role for RXR-α in retinoid metabolism and transport (88). Note that RXR-α is also expressed in the intestinal villi (118), where it may be involved in regulating the genes involved in retinol transport (118, and see below). Less is presently known about the expression of RXR-β; however, Northern blot analysis indicates that unlike RXR-α, RXR-β is not highly expressed in the liver and may be specific for the central nervous system (59, 88). A third RXR, RXR-γ has recently been identified (80a); however, little is presently known about its tissue-specific expression.

Although the RXRs, like RARs, can recognize several ERE and thyroid hormone responsive element (TRE) related elements, at least two responsive elements that confer differential regulation by RARs and RXRs have been identified (89, 117). One of these elements is located in the upstream region of the cellular retinol-binding protein-type II (CRBPII), which is thought to be important in the movement of vitamin A across the intestinal lumen (89). This element is unusual because it contains five nearly perfect tandem repeats of the sequence AGGTCA that are separated by single spacer nucleotides (89). Furthermore, although both RAR and RXR can bind to this responsive element, only RXR can activate reporter gene expression in co-transfection experiments (89). Co-expression of RAR with RXR in these experiments resulted in almost complete inhibition of transcription activation (89). A second RXR-specific responsive element was recently identified in the apolipoprotein A1 (apoA1) gene. ApoA1 encodes a plasma protein involved in lipid transport and it is synthesized in both the liver and intestine (117). Site A in this promoter (–214 to –192) can bind in vitro to RXR-α but only weakly to RAR-α and RAR-β. Accordingly, it can confer a RA response to a reporter gene when co-transfected with RXR-α but not with RAR-α and -β (117).

RXRs Interact with Several Classes of Nuclear Receptors

In a remarkable twist of scientific fate, researchers seeking the identity of a factor essential for the efficient binding of RARs to RAREs purified and identified what turned out to be an RXRβ (80a, 157a). Thus, heterodimers of RARs and RXRs appear to be more efficient at binding to RAREs than do homodimers of these receptors. This linkage between two receptor subclasses unifies two RA signal transduction pathways; different RAR/RXR combinations may exhibit unique specificities in target gene activation. A better picture of RXR/RAR overlap, in terms of their domains of tissue specific expression, will tell us much about the particular roles of individual RXR/RAR complexes. Even more surprising is the degree of promiscuity of RXRs; RXRs interact not only with RARs but also with thyroid hormone receptors and vitamin D_3 receptors (73a, 80a, 157a, 160). These interactions indicate that RXRs may play a more central role in nuclear receptor signalling pathways (73a). The extent to which RXRs interact with other members of the superfamily of nuclear receptors remains to be determined.

RAR-α AND PROMYELOCYTIC LEUKEMIA

The assignment of the RAR-α locus to chromosome 17 (see Table 1) led to speculation that the RAR-α might be involved in the pathogenesis of acute promyelocytic leukemia (APL) (8, 18, 19, 22, 34, 82). This hypothesis was substantiated by the fact that APL patients could be effectively treated by RA (18, 19, 22, 82, 95). Several groups have shown that the chromosome 15;17 translocation t(15;17), which has become essentially pathonomic for APL, fuses most of the coding portion of the RAR-α, from breakpoints clustered within the second intron of the gene, to the promyelocytic leukemia locus (PML) on chromosome 15 (1, 30, 35, 36, 83). PML, first identified by characterization of the translocation locus on chromosome 15, also appears to encode a transcription factor (36, 70, 105). The translocation gives rise to a novel protein comprising most of RAR-α (regions B through F) fused in frame and downstream of a large portion of PML (36, 70). Expression of the RAR-α PML chimera in co-transfection studies indicates that it may act as a dominant negative regulation of promyelocyte differentiation (36). However, depending on the exact point of fusion which can vary from one APL patient to another (36, 70, 105), certain PML-RAR fusions can function as transcription activators with greater or lesser efficiencies than those of wild-type RAR-α. Although it is presently unclear whether the PML-RAR-α protein is causally related to the pathogenesis of APL, the mechanism of PML-RARα function may be consistent with that proposed for v-erb A (see above) (33). As in the case where v-erb A expression appears to abrogate the normal

inhibitory actions of RAR-α with the AP1 element (see above), PML-RAR-α may prove to be ineffective in suppressing AP1-mediated transcription activation on those target genes where this function is necessary for the normal differentiation of promyelocytes (33).

CONCLUSIONS

Modulation of Retinoid Signal Transduction

The recent identification of a number of interacting components of the retinoid signal transduction system supports the view that the cellular response to RA depends on numerous factors. Below are listed some of the critical factors that may determine the biological activity of RA in a given tissue.

RETINOID METABOLISM Relatively little is known about the physiological regulation of retinoid concentrations (7). Whether or not RA concentrations are modulated at the level of the target tissue is of particular interest (7). In recent years, there has been considerable interest in the role of RA in chick limb pattern formation (41, 138). When RA is locally applied in the form of a RA-soaked bead to the anterior margin of the wing bud, a minor duplication of digit pattern develops, exactly mimicking the pattern resulting from grafts from the zone of polarizing activity (ZPA) located in the posterior margin of the limb bud (41, 138). Although RA may be a morphogen in the limb bud that provides graded spatial clues in the form of a concentration gradient emanating either from the ZPA or from RA-soaked beads, recent studies suggest that RA may act to convert cells into ZPA-like regions that release a morphogen other than RA (101, 150). In either case, the regulation of local levels of RA is likely to be important. Perhaps the graded distribution of CRABP(S) (41, 138), which appears to be opposite in polarity to the RA gradient presumed to exist in the limb bud, may be important to ensure that only one specified region of the limb forms a ZPA. In any case, modulation of RA concentration in the limb bud results in a very discrete change in tissue response. Thus, the maintenance of normal tissue responses may require factors such as CRBPs, CRABPs as well as retinoid-metabolizing enzymes (7, 138). The discovery that 9-*cis* RA is a more potent ligand for RXRs than all-*trans* RA indicates that retinoid isomerization may be a pivotal point for regulating target tissue responses to retinoids (62a, 81a). There are, of course, other metabolites of RA, and it will be important to determine if any of these other metabolites are receptor selective (141).

RARs The three subtypes of retinoic acid receptors are RAR-α, RAR-β, and RAR-γ (11, 50, 67, 75, 106). These receptors, by regulating target gene

expression in a RA-dependent manner, play a pivotal role in the cellular response to RA. Although these nuclear receptors are highly similar in structure, the minor differences between them are well conserved throughout evolution, suggesting that each RAR may serve different functions. This observation is supported by the finding that the efficiency of transactivation by RA and other synthetic retinoids appears to be dependent on the RAR subtype mediating the effect (see above). Moreover, the different subtypes are differentially expressed in retinoid target tissues. Little is known about the functional significance of the RAR isoforms that differ in their A regions (see Figure 1). These different A regions (see above), by interacting specifically with transcription intermediary factors (TIFs), may play a role in determining promoter and cell selective transactivation, as appears to be the case for other nuclear receptors (143, 151, 154). Thus, the range of possible responses to RA may vary greatly depending on (*a*) which isoforms are present and (*b*) what intermediary factors (TIFs) are available in a given cell-type. The possibility that RARs may form homo- and heterodimers would further increase the possible RAR configurations on target gene RAREs.

RXRs The discovery of a second subclass of RA-activated nuclear receptors, the RXRs, has added a further level of control in regulating the cellular response to RA. In this parallel RA signal transduction pathway are also three receptor subtypes: RXR-α, RXR-β, and RXR-γ (80a, 157a). Because of the comparatively high levels of RA required to activate RXRs, some studies have suggested that other retinoids may specifically act through these receptors (88), and indeed 9-*cis* RA appears to be the likely candidate (62a, 81a). The finding that RXRs interact with RARs to enhance their specific activities indicates that the effect of RA on the expression of a particular target gene will depend on (*i*) which combinations of RAR/RXRs are present and (*ii*) whether all-*trans* RA, 9-*cis* RA, or both isomers are present in a given cell.

RAREs RARs have been shown to activate gene transcription through a number of different types of responsive elements including EREs, TREs, RAREs, as well as ERE half-sites (see above), consisting of direct repeats, inverted repeats, and isolated half-sites of the motif G(G/T)TCA. How effectively do these sites promote the RA activation of gene expression by binding RARs? The affinity of the RARs for these sites will likely affect the fold induction of the target gene. How does the orientation of half-sites (direct, or inverted) affect the ability of RAR subtypes and isoforms to bind and activate transcription from these response elements? Do different combinations of RAR/RXR complexes exhibit different response element selectivities? The answer to this latter question appears to be yes. Consistent with this notion are the results of in vitro DNA binding experiments using different response

elements and, indirectly, the results of co-transfection experiments (73a, 80a, 157a, 160). The answers to these fundamental questions will become clearer as additional RAREs become characterized.

OTHER TRANSCRIPTION FACTORS The possibility that RARs form heterodimers with c-ErbA suggests that associations similar to that observed for RARs and RXRs may also exist between RARs and other nuclear receptors. RAR mutants such as PML-RAR-α observed in acute promyelocytic leukemias may also exert deleterious effects on RA response through heterodimer formation (33, 43, 54). Conceivably, such interactions would result not only in different levels of transcription activation but could also affect target gene specificity (53, 54, 80, 103).

Finally, the contextual location of an RARE in a promoter may significantly influence whether or not a particular RA target gene is expressed. For example, the pattern of expression of a β-galactosidase marker gene under the control of the RAR-β2 promoter in transgenic mice suggests that the mere presence of RA is insufficient to activate the RARE-bearing promoter (94). The activity of a RARE, nestled among other *cis*-acting elements, may depend on the presence or absence of the corresponding *trans*-acting factors that bind to them. The interactions between RARs and AP1-binding factors are particularly interesting: they may be competitive, as in the case of the osteocalcin promoter, or direct, as in the regulation of the collagenase promoter (see above). These interactions link two separate signal transduction systems. It is not difficult to see how this link is necessary for the orchestration of cell growth and differentiation during the highly complex process of development.

SUMMARY

Over the past five years, a wealth of information has accumulated concerning the molecular mechanisms mediating RA effects on gene expression. The molecular cloning of the 3 retinoic acid receptors (RARs), of their 16 or so different isoforms, and of the 3 retinoid X receptors (RXRs) as well as the identification of at least 2 different active isomers of RA (all *trans*- and 9-*cis*-RA) and of several different CRABPs and CRBPs now provide the essential tools to explain the pleiotropy that has become associated with RA effects. In the years to come, a concentrated effort to delineate the complex interactions between the various components of the retinoid signal transduction system should shed light on the mechanisms underlying pattern formation during vertebrate development and point to new ways in which retinoids can be exploited therapeutically.

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Literature Cited

1. Alcalay, M., Zangrilli, D., Pandolfi, P. P., Longo, L., Mencarelli, A., et al. 1991. Translocation breakpoint of acute promyelocytic leukemia lies within the retinoic acid receptor alpha locus. *Proc. Natl. Acad. Sci. USA* 88:1977–81
1a. Altaba, A. R. I. 1991. Vertebrate development: an emerging synthesis. *Trends Genet.* 7:276–80
2. Altaba, A. R., Melton, D. A. 1989. Interaction between peptide growth factors and homeobox genes in the establishment of antero-posterior polarity in frog embryos. *Nature* 341:33–38
3. Astrom, A., Pettersson, U., Krust, A., Chambon, P., Voorhees, J. J. 1990. Retinoic acid and synthetic analogs differentially activate retinoic acid receptor dependent transcription. *Biochem. Biophys. Res. Commun.* 173:339–45
4. Bagchi, M. K., Tsai, S. Y., Tsai, M., O'Malley, B. W. 1991. Progesterone enhances target gene transcription by receptor free of heat shock proteins hsp90, hsp56 and hsp70. *Mol. Cell. Biol.* 11:4998–5004
5. Bailey, J. S., Siu, C. H. 1990. Purification of cellular retinoic acid-binding proteins types I and II from neonatal rat pups. *Methods Enzymol.* 189:356–63
6. Balling, R. 1991. CRABP and the teratogenic effects of retinoids. *Trends Genet.* 7:35–36
7. Blomhoff, R., Green, M. H., Berg, T., Norum, K. R. 1990. Transport and storage of vitamin A. *Science* 250:399–403
8. Borrow, J., Goddard, A. D., Sheer, D., Solomon, E. 1990. Molecular analysis of acute promyelocytic leukemia breakpoint cluster region of chromosome 17. *Science* 249:1577–80
9. Boylan, J. F., Gudas, L. J. 1991. Overexpression of the cellular retinoic acid binding protein-I (CRABP-1) results in a reduction in differentiation-specific gene expression in F9 teratocarcinoma cells. *J. Cell Biol.* 112:965–79
10. Brand, N. J., Petkovich, M., Chambon, P. 1990. Characterization of a functional promoter for the human retinoic acid receptor-alpha (hRAR-alpha). *Nucleic Acids Res.* 18:6799–6806
11. Brand, N., Petkovich, M., Krust, A., Chambon, P., de The, H., et al. 1988. Identification of a second human retinoic acid receptor. *Nature* 332:850–53
12. Brockes, J. 1991. We may not have a morphogen. *Nature* 350:15
13. Brockes, J. P. 1989. Retinoids, homeobox genes, and limb morphogenesis. *Neuron* 2:1285–94
14. Brockes, J. P. 1990. Retinoic acid and limb regeneration. *J. Cell Sci. Suppl.* 13:191–98
15. Busch, C., Sakena, P., Funa, K., Nordlinder, H., Eriksson, U. 1990. Tissue distribution of cellular retinol-binding protein and cellular retinoic acid-binding protein: Use of monospecific anti-bodies for immunohistochemical and cRNA for in situ localization. *Method Enzymol.* 189:315–24
16. Cavey, M. T., Martin, B., Carlavan, I., Shroot, B. 1990. In vitro binding of retinoids to the nuclear retinoic acid receptor alpha. *Anal. Biochem.* 186:19–23
17. Chambon, P., Zelent, A., Petkovich, M., Mendelsohn, C., Leroy, P., et al. 1991. The family of retinoic acid nuclear receptors. In *Retinoids: 10 Years On*, ed. J-H. Saurat, pp. 10–27. Basel, Switzerland: Karger
18. Chang, K. S., Trujillo, J. M., Ogura, T., Castiglione, C. M., Kidd, K. K., et al. 1991. Rearrangement of the retinoic acid receptor gene in acute promyelocytic leukemia. *Leukemia* 5:200–4
19. Chen, Z., Chen, S. J., Tong, J. H., Zhu, Y. J., Huang, M. E., et al. 1991. The retinoic acid alpha receptor gene is frequently disrupted in its 5' part in Chinese patients with acute promyelocytic leukemia. *Leukemia* 4:288–92
20. Cho, K. W. Y., De Robertis, E. M. 1990. Differential activation of *Xenopus* homeobox genes by mesoderm-inducing growth factors and retinoic acid. *Genes Dev.* 4:1910–16

21. Clarke, C. L., Graham, J., Roman, S. D., Sutherland, R. D. 1991. Direct transcriptional regulation of the progesterone receptor by retinoic acid diminishes progestin responsiveness in the breast cancer cell line T-47D. *J. Biol. Chem.* 266:18969–75
22. Cleary, M. L. 1991. Oncogenic conversion of transcription factors by chromosomal translocations. *Cell* 66:619–22
23. Clifford, J. L., Petkovich, M., Chambon, P., Lotan, R. 1990. Modulation by retinoids of mRNA levels for nuclear retinoic acid receptors in murine melanoma cells. *Mol. Endocrinol.* 4: 1546–55
24. Crettaz, M., Baron, A., Siegenthaler, G., Hunziker, W. 1990. Ligand specificities of recombinant retinoic acid receptors RAR alpha and RAR beta. *Biochem. J.* 272:391–97
25. Dalman, F. C., Sturzenbecker, L. J., Levin, A. A., Lucas, D. A., Perdew, G. H., et al. 1991. Retinoic acid receptor belongs to a subclass of nuclear receptors that do not form "docking" complexes with hsp90. *Biochemistry* 30:5605–8
26. Danielsen, M., Hinck, L., Ringold, G. M. 1989. Two amino acids within the knuckle of the first zinc finger specify DNA response element activation by the glucocorticoid receptor. *Cell* 57:1131–38
27. Darling, D. S., Beebe, J. S., Burnside, J., Winslowk, E. R., Chin, W. W. 1991. 3,5,3'-Triiodothyronine (T3) receptor-auxiliary protein (TRAP) binds DNA and forms heterodimers with the T3 receptor. *Mol. Endocrinol.* 5:73–84
28. Davidson, E. H. 1990. How embryos work: a comparative view of diverse modes of cell fate specification. *Development* 108:365–89
29. de Groot, R. P., Pals, C., Kruijer, W. 1991. Transcriptional control of c-jun by retinoic acid. *Nucleic Acids Res.* 19:1585–91
30. Dejean, A., de The, H. 1990. Hepatitis B virus as an insertional mutagene in a human hepatocellular carcinoma. *Mol. Biol. Med.* 7:213–22
31. Denner, L. A., Weigel, N. L., Maxwell, B. L., Schrader, W. T., O'Malley, B. W. 1990. Regulation of progesterone receptor-mediated transcription by phosphorylation. *Science* 250:1740–43
32. de Pablo, F., Roth, J. 1990. Endocrinization of the early embryo: an emerging role for hormones and hormone-like factors. *Trends Biochem. Sci.* 15:339–42
33. Desbois, C., Aubert, D., Legrand, C., Pain, B., Samarut, J. 1991. A novel mechanism of action for v-ErbA: Abrogation of the inactivation of transcription factor AP-1 by retinoic acid and thyroid hormone receptors. *Cell* 67:731–40
34. de The, H., Chomienne, C., Lanotte, M., Degos, L., Dejean, A. 1990. The t(15;17) translocation of acute promyelocytic leukemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. *Nature* 347:558–61
35. de The, H., del Mar Vivanco-Ruiz, M., Tiollais, P., Stunnenberg, H., Dejean, A. 1990. Identification of a retinoic acid responsive element in the retinoic acid receptor beta gene. *Nature* 343:177–80
36. de The, H., Lavau, C., Marchio, A., Chomienne, C., Degos, L., et al. 1991. The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell* 66:675–84
37. Diamond, M. I., Miner, J. I., Yoshinga, S. K., Yamamoto, K. R. 1990. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. *Science* 249:1266–72
38. Dolle, P., Ruberte, E., Kastner, P., Petkovich, M., Stoner, C. M., et al. 1989. Differential expression of gene encoding alpha, beta, and gamma retinoic acid receptors and CRABP in the developing limbs of the mouse. *Nature* 342: 702–5
39. Dolle, P., Ruberte, E., Leroy, P., Morriss-Kay, G., 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* 110:1133–51
40. Durston, A. J., Timmermans, J. P. M., Hage, W. J., Hendriks, H. F. J., de Vries, N. J., et al. 1989. Retinoic acid causes an anteroposterior transformation in the developing central nervous system. *Nature* 340:140–44
41. Eichele, G. 1989. Retinoids and vertebrate pattern formation. *Trends Genet.* 5:246–51
42. Ellinger-Ziegelbauer, H., Dreyer, C. 1991. A retinoic acid receptor expressed in the early development of *Xenopus laevis*. *Genes Dev.* 5:94–104
43. Evans, R. M. 1988. The steroid and thyroid hormone receptor superfamily. *Science* 240:889–95
44. Fawell, S. E., Lees, J. A., White, R., Parker, M. G. 1990. Characterization and colocalization of steroid binding and dimerization activities in the mouse estrogen receptor. *Cell* 60:953–62

45. Forman, B. M., Herbert, H. S. 1991. Interactions among a subfamily of nuclear hormone receptors: The regulatory zipper model. *Mol. Endocrinol.* 4:1293–1301
46. Forman, B. M., Yang, C. R., Au, M., Casanova, J., Ghysdael, J., et al. 1989. A domain containing leucine-zipper-like motifs mediate novel in vivo interactions between the thyroid hormone and retinoic acid receptors. *Mol. Endocrinol.* 3:1610–26
47. Frankel, A. D., Kim, P. S. 1991. Modular structure of transcription factors: Implications for gene regulation. *Cell* 65:717–19
48. Gerhart, J. 1989. The primacy of cell interactions in development. *Trends Genet.* 5:233–36
49. Giguere, V., Lyn, S., Yip, P., Siu, C-H., Amin, S. 1990. Molecular cloning of cDNA encoding a second cellular retinoic acid-binding protein. *Proc. Natl. Acad. Sci. USA* 87:6233–37
50. Giguere, V., Ong, E. S., Segui, P., Evans, R. M. 1987. Identification of a receptor for the morphogen retinoic acid. *Nature* 330:624–29
51. Giguere, V., Shago, M., Zirngibl, R., Tate, P., Rossant, J., et al. 1990. Identification of a novel isoform of the retinoic acid receptor gamma expressed in the mouse embryo. *Mol. Cell. Biol.* 10:2335–40
52. Giguere, V., Yang, N., Segui, P., Evans, R. M. 1988. Identification of a new class of steroid hormone receptors. *Nature* 331:91–94
53. Glass, C. K., Devary, O. V., Rosenfeld, M. G. 1990. Multiple cell type-specific proteins differentially regulate target sequence recognition by the alpha retinoic acid receptor. *Cell* 63:729–38
54. Glass, C. K., Lipkin, S. K., Devary, O. V., Rosenfeld, M. G. 1989. Positive and negative regulation of gene transcription by a retinoic acid-thyroid hormone receptor heterodimer. *Cell* 59:697–708
55. Graupner, G., Willia, K. N., Tzukerman, M., Zhang, X., Pfahl, M. 1989. Dual regulatory role for thyroid-hormone receptors allows control of retinoic-acid receptor activity. *Nature* 340:653–56
56. Green, S., Chambon, P. 1988. Nuclear receptors enhance our understanding of transcriptional regulation. *Trends Genet.* 4:309–14
57. Gudas, L. J. 1990. Assays for expression of genes regulated by retinoic acid in murine teratocarcinoma cell lines. *Methods Enzymol.* 190:131–40
58. Guiochon-Mantel, A., Lescop, P., Christin-Maitre, S., Loosfelt, H., Perrot-Applanat, M., et al. 1991. Nucleocytoplasmic shuttling of the progesterone receptor. *EMBO J.* 10:3851–59
59. Hamada, K., Gleason, S. L., Levi, B. Z., Hirschfeld, S., Appella, E., et al. 1991. H-2RIIBP, a member of the nuclear hormone receptor superfamily that binds both the regulatory element of major histocompatibility class I genes and the estrogen response element. *Proc. Natl. Acad. Sci. USA* 86:8289–93
60. Hard, T., Kellenbach, E., Boelens, R., Maler, B. A., Dahlman, K., et al. 1990. Solution structure of the glucocorticoid receptor DNA-binding domain. *Science* 249:157–60
61. Hashimoto, Y., Kagechika, H., Shudo, K. 1990. Expression of retinoic acid receptor genes and the ligand-binding selectivity of retinoic acid receptors (RAR's). *Biochem. Biophys. Res. Commun.* 166:1300–7
62. He, X., Rosenfeld, M. G. 1991. Mechanisms of complex transcriptional regulation: Implications for brain development. *Neuron* 7:183–96
62a. Heyman, R., Mangelsdort, P. J., Dyck, J. A., Stein, R. B., Eichele, G. et al. 1992. 9-cis retinoic acid is a high affinity ligand for the retinoid x receptor. *Cell* 68:397–406
63. Hoffman, M. 1990. The embryo takes its vitamins. *Science* 250:372–73
64. Hoffmann, B., Lehmann, J. M., Zhang, X-K., Hermann, T., Husmann, M., et al. 1990. A retinoic acid receptor-specific element controls the retinoic acid receptor beta promoter. *Mol. Endocrinol.* 4:1727–36
65. Hudson, L. G., Santon, J. B., Glass, C. K., Gill, G. N. 1990. Ligand-activated thyroid hormone and retinoic acid receptors inhibit growth factor receptor promoter expression. *Cell* 62:1165–75
66. Husmann, M., Lehmann, J., Hoffmann, B., Hermann, T., Tzukerman, M., et al. 1991. Antagonism between retinoic acid receptors. *Mol. Cell. Biol.* 11:4097–4103
67. Ishikawa, T., Umesono, K., Mangelsdorf, D. J., Aburatani, H., Stanger, B. Z., et al. 1990. A functional retinoic acid receptor encoded by the gene on human chromosome 12. *Mol. Endocrinol.* 4:837–44
68. Izpisua-Belmonte, J-C., Tickle, C., Dolle, P., Wolpert, L., Duboule, D. 1991. Expression of the homeobox Hox-4 genes and the specification of position in chick wing development. *Nature* 350:585–89

69. Jones, B., Jurutka, P., Haussler, C., Haussler, M., Whitfield, K. 1991. Vitamin D receptor phosphorylation in transfected ROS 17/2.8 cells is localized to the N-terminal region of the hormone-binding domain. *Mol. Endocrinol.* 5:1137–46
70. Kakizuka, A., Miller, W. H. Jr., Umesono, K., Warrell, R. P., et al. 1991. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. *Cell* 66:663–74
71. Kastner, P., Krust, A., Mendelsohn, C., Garnier, J. M., Zelent, A., et al. 1990. Murine isoforms of retinoic acid receptor gamma with specific patterns of expression. *Proc. Natl. Acad. Sci. USA* 87:2700–4
72. Kim, K. H., Griswold, M. D. 1990. The regulation of retinoic acid receptor mRNA levels during spermatogenesis. *Mol. Endocrinol.* 4:1679–88
73. Kimmel, C. B., Warga, R. M., Schilling, T. F. 1990. Origin and organization of the Zebrafish fate map. *Development* 108:581–94
73a. Kliewer, S. A., Umesono, K., Mangelsdorf, D., Evans, R. M. 1992. Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D_3 signalling. *Nature* 355:446–49
74. Kozak, M. 1991. An analysis of vertebrate mRNA sequences: Intimations of translational control. *J. Cell Biol.* 115:887–903
75. Krust, A., Kastner, P., Petkovich, M., Zelent, A., Chambon, P. 1989. A third human retinoic acid receptor, hRAR-alpha. *Proc. Natl. Acad. Sci. USA* 86:5210–14
76. Kumar, V., Chambon, P. 1988. The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* 55:145–56
77. Kumar, V., Green, S., Stack, G., Berry, M., Jin, J. R., Chambon, P. 1987. Functional domains of the human estrogen receptor. *Cell* 51:941–51
78. Kuo, C. J., Mendel, D. B., Hansen, L. P., Crabtree, G. R. 1991. Independent regulation of HNF-1 alpha and HNF-1 beta by retinoic acid in F9 teratocarcinoma cells. *EMBO J.* 10:2231–36
79. Landschultz, W. H., Johnson, P. F., McKnight, S. L. 1988. The Leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science* 240:1759–64
80. Lazar, M. A., Berrodin, T. J., Harding, H. P. 1991. Differential DNA binding by monomeric, homodimeric and potentially heteromeric forms of the thyroid hormone receptor. *Mol. Cell. Biol.* 11:5005–15
80a. Leid, M., Kautner, P., Lyons, R., Nakshatri, H., Saunders, M., et al. 1992. Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell* 68:377–95
81. Leroy, P., Krust, A., Zelent, A., Mendelsohn, C., Garnier, J-M., et al. 1991. Multiple isoforms of the mouse retinoic acid receptor alpha are generated by alternative splicing and differential induction by retinoic acid. *EMBO J.* 10:59–69
81a. Levin, A., Sturzenbecker, L., Kazmer, S., Bosakowski, T., Huselton, C., et al. 1992. 9-cis Retinoic acid stereoisomer binds and activates the nuclear receptor RXRα. *Nature* 355:359–61
82. Longo, L., Donti, E., Mencarelli, A., Avanti, G., Pegoraro, L., et al. 1990. Mapping of chromosome 17 breakpoints in acute myeloid leukemias. *Oncogene* 5:1557–63
83. Longo, L., Pandolfi, P. P., Biondi, A., Rambaldi, A., Mencarelli, A., et al. 1990. Rearrangements and aberrant expression of the retinoic acid receptor alpha gene in acute promyelocytic leukemias. *J. Exp. Med.* 172:1571–75
84. Lucas, P. C., Forman, B. M., Samuels, H. H., Granner, D. K. 1991. Specificity of a retinoic acid response element in the phosphoenolpyruvate carboxykinase gene promoter: Consequences of both retinoic acid and thyroid hormone receptor binding. *Mol. Cell. Biol.* 11:5164–70
85. Lucas, P. C., O'Brien, R. M., Mitchell, J. A., Davis, C. M., Imai, E., et al. 1991. A retinoic acid response element is a part of a pleiotropic domain in the phosphoenolpyruvate carboxykinase gene. *Proc. Natl. Acad. Sci. USA* 88:2184–88
86. Luisi, B. F., Xu, W. X., Otwinowski, Z., Freedman, L. P., Yamamoto, K. R., et al. 1991. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature* 352:497–505
87. Mader, S., Kumar, V., de Verneuil, H., Chambon, P. 1989. Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen from a glucocorticoid responsive element. *Nature* 338:271–74
88. Mangelsdorf, D. J., Ong, E. S., Dyck, J. A., Evans, R. M. 1990. Nuclear receptor that identifies a novel retinoic

acid response pathway. *Nature* 345:224–29

89. Mangelsdorf, D. J., Umesono, K., Kliewer, S. A., Borgmeyer, U., Ong, E. S., et al. 1991. A direct repeat in the cellular retinol-binding protein type II gene confers differential regulation by RXR and RAR. *Cell* 66:555–61
90. Martinez, E., Givel, F., Wahli, W. 1991. A common ancestor DNA motif for invertebrate and vertebrate hormone response elements. *EMBO J.* 10:263–68
91. Matareses, V., Buelt, M. K., Chinander, L. L., Bernlohr, D. A. 1990. Purification of adipocyte lipid-binding protein from human and murine cells. *Methods Enzymol.* 189:363–69
92. McDonnell, D. P., Nawaz, Z., O'Malley, B. W. 1991. In situ distinction between steroid receptor binding and transactivation at a target gene. *Mol. Cell. Biol.* 11:4350–55
93. Melton, D. A. 1991. Pattern formation during animal development. *Science* 252:234–41
94. Mendelsohn, C., Ruberte, E., LeMeur, M., Morriss-Kay, G., Chambon, P. 1991. Developmental analysis of the retinoic acid-inducible RAR-b2 promotor in transgenic animals. *Development* 113:723–34
95. Miller, W. H. Jr., Warrell, R. P. Jr., Frankel, S. R. A., Jakubowski, J. L., Gabrilove, J. M., et al. 1990. Novel retinoic acid receptor-a transcripts in acute promyelocytic leukemia responsive to all-trans-retinoic acid. *J. Natl. Cancer Inst.* 82:1932–33
96. Mornon, J. P., Bissery, V., Gaboriaud, C., Thomas, A., Ojasoo, T., et al. 1989. Hydrophobic cluster analysis (HCA) of the hormone-binding domain of receptor proteins. *J. Steroid Biochem.* 34:355–61
97. Munoz-Canoves, P., Vik, D. P., Tack, B. F. 1990. Mapping of a retinoic acid-responsive element in the promoter region of the complement factor H gene. *J. Biol. Chem.* 265:20065–68
98. Naar, A. M., Boutin, J., Lipkin, S. M., Yu, V. C., Holloway, J. M., et al. 1991. The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. *Cell* 65:1267–79
99. Nicholson, R. C., Mader, S., Nagpal, S., Leid, M., Rochette-Egly, C., et al. 1990. Negative regulation of the rat stromelysin gene promoter by retinoic acid is mediated by an AP1 binding site. *EMBO J.* 9:4443–54
100. Nohno, T., Muto, K., Noji, S., Saito, T., Taniguchi, S. 1991. Isoforms of retinoic acid receptor beta expressed in the chick embro. *Biochim. Biophys. Acta* 1089:273–75
101. Noji, S., Nohno, T., Koyama, E., Muto, K., Ohyama, K., et al. 1991. Retinoic acid induces polarizing activity but is unlikely to be a morphogen in the chick limb bud. *Nature* 350:83–86
102. Noji, S., Yamaai, T., Koyama, E., Nohno, T., Fujimoto, W., et al. 1989. Expression of retinoic acid receptor gene in keratinizing front of skin. *FEBS Lett.* 259:86–90
103. O'Donnell, A. L., Rosen, E. D., Darling, D. S., Koenig, R. J. 1991. Thyroid hormone receptor mutations that interfere with transcriptional activation also interfere with receptor interaction with a nuclear protein. *Mol. Endocrinol.* 5:94–99
104. Oro, A. E., McKeown, M., Evans, R. M. 1990. Relationship between the product of the ultraspiracle locus and the vertebrate retinoid X receptor. *Nature* 347:298–301
105. Pandolfi, P. P., Grignani, F., Alcalay, M., Mencarelli, A., Biondi, A., et al. 1991. Structure and origin of the acute promyelocytic leukemia myl/RAR alpha cDNA and characterization of its retinoid-binding and transactivation properties. *Oncogene* 6:1285–92
106. Petkovich, M., Brand, N. J., Krust, A., Chambon, P. 1987. A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature* 330:444–50
107. Petkovich, P. M., Heersche, J. N. M., Tinker, D. O., Jones, G. 1984. Retinoic acid stimulates 1,25-dihydroxyvitamin D3 binding in rat osteosarcoma cells. *J. Biol. Chem.* 259:8274–80
108. Picard, D., Khursheed, B., Garabedian, M. J., Fortin, M. G., Lindquist, S., et al. 1990. Reduced levels of hsp90 compromise steroid receptor action in vivo. *Nature* 348:166–68
109. Picard, D., Salser, S. J., Yamamoto, K. R. 1988. A movable and regulable inactivation function within the steroid binding domain of the gucocorticoid receptor. *Cell* 54:1073–80
110. Pratt, W. B., Jolly, D. J., Pratt, D. V., Hollenberg, S. M., Giguere, V., et al. 1988. A region in the steroid binding domain determines formation of the non-DNA-binding, 9 S glucocorticoid receptor complex. *J. Biol. Chem.* 263:267–73
111. Ragsdale, C. W., Petkovich, M., Gates, P. B., Chambon, P., Brockes, J. P. 1989. Identification of a novel retinoic acid receptor in regenerative tissues of the newt. *Nature* 341:654–57

112. Redfern, C. P. F., Daly, A. K. 1990. Cellular retinoic acid-binding protein from neonatal rat skin: purification and analysis. *Methods Enzymol.* 189:307–14
113. Redfern, C. P. F., Daly, A. K., Latham, J. A. E., Todd, C. 1990. The biological activity of retinoids in melanoma cells: Induction of expression of retinoic acid receptor beta by retinoic acid in S91 melanoma cells. *FEBS Lett.* 273:19–22
114. Rochette-Egly, C., Lutz, Y., Saunders, M., Scheuer, I., Gaub, M-P., et al. 1991. Retinoic acid receptor gamma: Specific immunodetection and phosphorylation. *J. Cell Biol.* 115:535–45
115. Rossant, J., Joyner, A. L. 1989. Towards a molecular genetic analysis of mammalian development. *Trends Genet.* 5:277–83
116. Rossant, J., Zirngibl, R., Cado, D., Shago, M., Giguere, V. 1991. Expression of a retinoic acid response element-hsplacZ transgene defines specific domains of transcriptional activity during mouse embryogenesis. *Genes Dev.* 5:1333–44
117. Rottman, J. N., Widom, R. L., Madal-Ginard, B., Mahdavi, V., Karathanasis, S. K. 1991. A retinoic acid-responsive element in the apolipoprotein Al gene distinguishes between two different retinoic acid response pathways. *Mol. Cell. Biol.* 11:3814–20
118. Rowe, A., Eager, N. S. C., Brickell, P. M. 1991. A member of the RXR nuclear receptor family is expressed in neural-crest-derived cells of the developing chick peripheral nervous system. *Development* 111:771–78
119. Ruberte, E., Dolle, P., Chambon, P., 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
120. Ruberte, E., Dolle, P., Krust, A., Zelent, A., Morriss-Kay, G., et al. 1990. Specific spatial and temporal distribution of retinoic acid receptor gamma transcripts during mouse embryogenesis. *Development* 108:213–22
121. Deleted in proof.
122. Ruiz, M. d. M. V., Bugge, T. H., Hirschmann, P., Stunnenberg, H. G. 1991. Functional characterization of a natural retinoic acid responsive element. *EMBO J.* 10:3829–38
123. Sani, B. P., Singh, R. K., Reddy, L. G., Gaub, M-P. 1990. Isolation, partial purification and characterization of nuclear retinoic acid receptors from chick skin. *Arch. Biochem. Biophys.* 283:107–13
124. Schule, R., Rangarajan, P., Kliewer, S., Ransone, L. J., Bolado, J., et al. 1990. Functional antagonism between oncoprotein c-jun and the glucocorticoid receptor. *Cell* 62:1217–26
125. Schule, R., Umesono, K., Mangelsdorf, D. J., Bolado, J., Pike, J. W., et al. 1991. Jun-fos and receptors for vitamin A and D recognize a common response element in the human osteocalcin gene. *Cell* 61:497–504
126. Selmi, S., Samuels, H. H. 1991. Thyroid hormone receptor/and v-erbA. *J. Biol. Chem.* 266:11589–93
127. Shubeita, H. E., Sambrook, J. F., McCormick, A. M. 1987. Molecular cloning and analysis of functional cDNA and genomic clones encoding bovine cellular retinoic acid-binding protein. *Proc. Natl. Acad. Sci. USA* 84:5645–49
128. Siegenthaler, G. 1990. Gel electrophoresis of cellular retinoic acid-binding protein, cellular retinol-binding protein and serum retinol-binding protein. *Methods Enzymol.* 189:299–307
129. Sive, H. L., Cheng, P. F. 1991. Retinoic acid perturbs the expression of Xhox lab genes and alters mesodermal determination in *Xenopus laevis*. *Genes Dev.* 5:1321–32
130. Smith, J. C. 1989. Induction and early amphibian development. *Curr. Opin. Cell Biol.* 1:1061–70
131. Smith, S. M., Eichele, G. 1991. Temporal and regional differences in the expression pattern of distinct retinoic acid receptor-beta transcripts in the chick embryo. *Development* 111:245–52
132. Smith, W. C., Nakshatri, H., Leroy, P., Rees, J., Chambon, P. 1991. A retinoic acid response element is present in the mouse cellular retinol binding protein I (mCRBP1) promoter. *EMBO J.* 10:2223–30
133. Stellmach, V., Leask, A., Fuchs, E. 1991. Retinoid-mediated transcriptional regulation of keratin genes in human epidermal and squamous cell carcinoma cells. *Proc. Natl. Acad. Sci. USA* 88:4582–86
134. Stoner, C. M., Gudas, L. J. 1989. Mouse cellular retinoic acid binding protein: Cloning, complementary DNA sequence, and messenger RNA expression during the retinoic acid-induced differentiation of F9 wild type and RA-3-10 mutant teratocarcinoma cells. *Cancer Res.* 49:1497–1504

135. Struhl, K. 1991. Mechanisms for diversity in gene expression patterns. *Neuron* 7:177–81
136. Sucov, H. M., Murakami, K. K., Evans, R. M. 1990. Characterization of an autoregulated response element in the mouse retinoic receptor type beta gene. *Proc. Natl. Acad. Sci. USA* 87:5392–96
137. Suva, L. J., Ernst, M., Rodan, G. A. 1991. Retinoic acid increases zif268 early gene expression in rat preosteoblastic cells. *Mol. Cell. Biol.* 11:2503–10
138. Tabin, C. J. 1991. Retinoids, homeoboxes, and growth factors: Toward molecular models for limb development. *Cell* 66:199–217
139. Tamara, K., Kagechika, H., Hashimoto, Y., Shudo, K., Ohsugi, K., et al. 1990. Synthetic retinoids, retinobenzoic acids, Am80, Am580 and Ch55 regulate morphogenesis in chick limb bud. *Cell Differ. Dev.* 32:17–26
140. Tasset, D., Tora, L., Fromental, C., Scheer, E., Chambon, P. 1990. Distinct classes of transcriptional activating domains function by different mechanisms. *Cell* 62:117–87
141. Thaller, C., Eichele, G. 1990. Isolation of 3,4-didehydroretinoic acid, a novel morphogenic signal in the chick wing bud. *Nature* 345:815–19
142. Tomomura, M., Kodomatsu, K., Nakamoto, M., Muramatsu, H., Kondoh, H., et al. 1990. A retinoic acid responsive gene, MK, produces a secreted protein with heparin binding activity. *Biochem. Biophys. Res. Commun.* 171:603–9
143. Tora, L., Gronemeyer, H., Turcott, E. B., Gaub, M. P., Chambon, P. 1988. The N-terminal region of the chicken progesterone receptor specifies target gene activation. *Nature* 333:185–88
144. Umesono, K., Evans, R. M. 1989. Determinants of target gene specificity for steroid/thyroid hormone receptors. *Cell* 57:1139–46
145. Umesono, K., Murakami, K. K., Thompson, C. C., Evans, R. M. 1991. Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors. *Cell* 65:1255–66
146. Urios, P., Duprez, D., Le Caer, J-P., Courtois, Y., Vigny, M., et al. 1991. Molecular cloning of RI-HB, A heparin binding protein regulated by retinoic acid. *Biochem. Biophys. Res. Commun.* 175:617–24
147. Vasios, G., Mader, S., S., Gold, J. D., Leid, M., Lutz, Y., et al. 1991. The late retinoic acid induction of laminin B1 gene transcription involves RAR binding to the responsive element. *EMBO J.* 10:1149–58
148. Vasios, G. W., Gold, J. D., Petkovich, M., Chambon, P., Gudas, L. J. 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
149. Wagner, M., Thaller, C., Jessell, T., Eichele, G. 1990. Polarizing activity and retinoid synthesis in the floor plate of the neural tube. *Nature* 345:819–22
150. Wanek, N., Gardiner, D. M., Muneoka, K., Bryant, S. V. 1991. Conversion by retinoic acid of anterior cells into ZPA cells in the chick wing bud. *Nature* 350:81–83
151. Webster, N. J. G., Green, S., Jin, J. R., Chambon, P. 1988. The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. *Cell* 54:199–207
152. Webster, N. J. G., Green, S., Jin, J. R., Hollis, M., Chambon, P. 1988. The yeast UAS is a transcriptional enhancer in human HeLa cells. *Cell* 52:169–78
153. Wei, L-N., Tsao, J-L., Chu, Y-S., Jeannotte, L., Nguyen-Huu, M. C. 1990. Molecular cloning and transcriptional mapping of the mouse cellular retinoic acid-binding protein gene. *DNA Cell Biol.* 9:471–78
154. White, J. H., Brou, C., Wu, J., Burton, N., Egly, J-M., et al. 1991. Evidence for a factor required for transcriptional stimulation by the chimeric acidic activator GAL-VP16 in HeLa cell extracts. *Proc. Natl. Acad. Sci. USA* 88:7674–78
155. Wood, W. M., Ocran, K. W., Gordon, D. F., Ridgway, E. C. 1991. Isolation and characterization of mouse complementary DNA's encoding alpha and beta thyroid hormone receptors from thyrotrope cells: The mouse pituitary-specific beta-2 isoform differs at the amino terminus from the corresponding species from rat pituitary tumor cells. *Mol. Endocrinol.* 5:1049–61
156. Yang, N., Schule, R., Mangelsdorf, D. J., Evans, R. M. 1991. Characterization of DNA binding and retinoic acid binding properties of retinoic acid receptor. *Proc. Natl. Acad. Sci. USA* 88:3559–63
157. Yang-Yen, H-F., Chambard, J-C., Sun, Y-L., Smeal, T., Schmidt, T. J., et al. 1990. Transcriptional interference between c-jun and the glucocorticoid receptor: Mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell* 62:1205–15
157a. Yu, V. C., Delsert, C., Andersen, B.,

Holloway, J. M., Derary, O., et al. 1991. RXRβ: A coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. *Cell* 67:1251–66

158. Zelent, A., Krust, A., Petkovich, M., Kastner, P., Chambon, P. 1989. Cloning of murine alpha and beta retinoic acid receptors and a novel receptor gamma predominantly expressed in skin. *Nature* 339:714–17

159. Zelent, A., Mendelsohn, C., Kastner, P., Krust, A., Garnier, J-M., et al. 1991. Differentially expressed isoforms of the mouse retinoic acid receptor beta are generated by usage of two promoters and alternate splicing. *EMBO J.* 10:71–81

160. Zhang, X., Hoffman, B., Tran, P., Graupner, G., Pfahl, M. 1992. Retinoid X receptor is an auxiliary protein for thyroid hormone receptors and retinoic acid receptors. *Nature* 355:441–46