MINIREVIEW

Nuclear Receptors, Transcriptional Regulation, and Oncogenesis (43323A)

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The role of steroid hormones in hormonally dependent neoplasia has been well documented (1). In this Minireview, I will discuss the structure and function of members of the steroid/thyroid (nuclear) receptor superfamily, the mechanism by which they regulate gene transcription, and their potential role in oncogenesis. I will not extensively discuss mutant nuclear receptors that have been found to be associated with various tumors (2) or the regulation of proto-oncogene expression by nuclear receptor/hormone complexes (3), as these subjects have been recently reviewed elsewhere. Rather, I will concentrate on some recent results that have been obtained using molecular approaches with cloned receptor genes. These studies point to some potentially novel and complex mechanisms whereby steroid/thyroid hormones and their receptors may regulate gene expression and carcinogenesis. Some of what is discussed here will be of a speculative nature, and it is hoped that this will point toward future research approaches that will lead to a greater understanding of the roles for these receptors in neoplastic transformation.

Structure of Nuclear Receptors

Nuclear receptor proteins are ligand-dependent transcription factors and include proteins that bind glucocorticoids, estrogens, progestins, androgens, mineralocorticoids, thyroid hormone, vitamin D, and retinoic acid (4). Two other potential members may bind xenobiotic ligands that have been directly implicated in carcinogenesis (5, 6). In addition, molecular cloning of cDNA from various cells based on DNA sequence homology has identified numerous other potential members of this superfamily, the so-called "orphan receptors" (7; see below). A diagrammatic representation of the structure of the known nuclear receptors is seen in Figure 1. A nonconserved amino terminus of varying length (the modulating domain) is followed by a highly conserved DNA-binding domain. This domain contains two cysteine "zinc fingers," which are involved in specific binding of the receptor to a DNA sequence called the "response element." This is followed by a nonconserved hinge region and, finally, by a conserved ligand-binding domain at the carboxyl terminus.

A number of these receptors exist as macromolecular complexes when the protein is in its unliganded, non-DNA-binding (untransformed) state. At least one component of the complex appears to be a dimer of the heat-shock protein, hsp90 (8, 9; Fig. 2). It is not clear whether hsp90 is associated with nuclear receptors that are tightly bound to the nucleus even in the absence of ligand, such as the thyroid hormone and retinoic acid receptors. Other low and high molecular weight components may also be present in the untransformed receptor. After binding the ligand, subunit dissociation occurs. This generates the monomeric, liganded, DNA-binding (transformed) receptor. Although dissociation of hsp90 is required for receptor transformation, it may not be sufficient to cause receptor-dependent gene regulation. An additional conformational change in receptor structure may also be necessary for full transcriptional activity. After transformation, the receptor protein then binds to its cognate, specific DNA response element to regulate transcription. Current evidence suggests that the nuclear receptors interact with DNA as homodimers (10, 11; see below).

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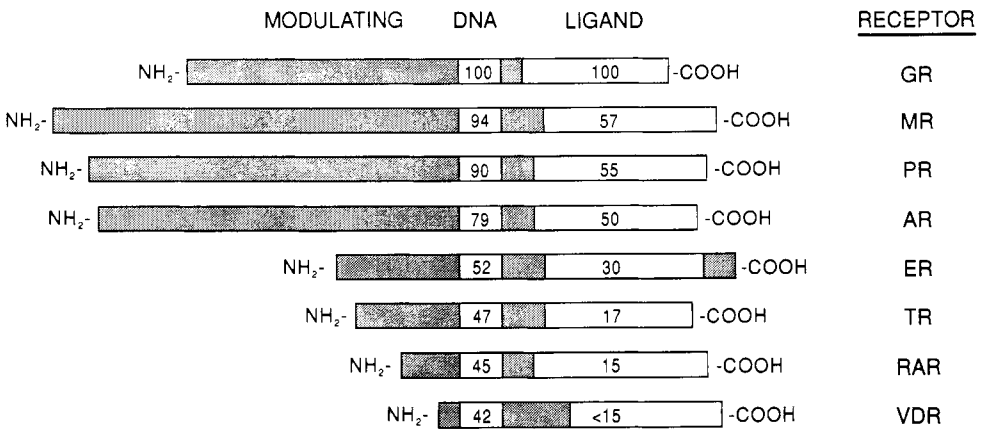

Figure 1. Structure of nuclear receptors. Nuclear receptors comprise three domains. The amino terminal region, called the "modulating domain," is not conserved among the superfamily of nuclear receptors, is of varying length, and may contain sequences that are involved in the transcriptional regulation of gene expression (transactivation). The second region is the "DNA-binding domain" that interacts specifically with DNA response elements (Fig. 3) via two cysteine zinc fingers. It is the most highly conserved region of the nuclear receptors. The carboxyl terminal region is the "ligand-binding domain" that binds the appropriate ligand with high affinity and specificity. It is also quite conserved among nuclear receptors, although less so than the DNA-binding domain. The numbers in the DNA-binding and ligand-binding domains represent the percentage of amino acid identity in these regions, compared to those in the glucocorticoid receptor (GR). Other receptors diagrammed are the mineralocorticoid (aldosterone) receptor (MR), progesterone receptor (PR), androgen receptor (AR), estrogen receptor (ER), thyroid hormone receptor (TR), retinoic acid receptor (RAR), and vitamin D receptor (VDR).

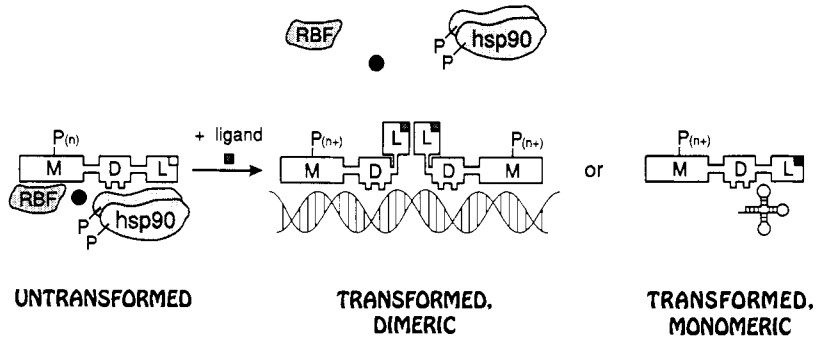

Figure 2. Mechanism of nuclear receptor transformation. In the unliganded state, the receptor monomer exists in an oligomeric, non-DNA-binding (untransformed) state. In addition to the ligand-binding subunit, this complex may contain a dimer of hsp90, a low molecular weight inhibitor of transformation (solid sphere), and macromolecular receptor binding factors (RBF). Ligand (small shaded square) binding destabilizes the subunit interactions, and an increase in the phosphorylation state of the proteins could occur. The receptor monomers dissociate, dimerize, and bind to the appropriate DNA response element with high affinity and specificity. This is mediated by two cysteine zinc fingers in the DNA-binding domain of the receptor protein. By some unknown mechanism, transcription of the adjacent gene is stimulated. Receptor-RNA complexes have been detected (right panel; transformed, monomeric receptor), and it has been suggested that such an interaction may be important in the posttranscriptional regulation of mRNA stability (discussed in Ref. 25). The oligomeric, untransformed receptor structure has not been convincingly demonstrated for receptors that are tightly bound to the nucleus even in the absence of bound ligand (TR; RAR). M, modulating domain; D, DNA-binding domain; L, ligand-binding domain.

Steroid Response Elements and the Regulation of Gene Expression

Consensus sequences have been generated for the DNA response elements to which the nuclear receptors bind (Fig. 3). Until very recently, it was thought that all nuclear receptors bound to palindromic DNA sequences, and that the primary nucleotide sequence of the DNA was the only significant determinant of receptor-binding specificity. One notable exception to the palindromic rule was the retinoic acid response element (RARE) present in the 5′ end of the retinoic acid receptor-β gene. This response element contains a *direct repeat* of the sequence (G/A)GTTCA separated by five nucleotides (12–14). The spacing in the various response elements suggests that the two interaction sites for the receptor are on the same face of the DNA, separated by about one turn of the DNA helix. This supports the concept that the receptor binds to the response element as a homodimer, with each receptor monomer binding to one half-site in the response element (11). Binding of the receptor to DNA in some way places the transactivating features of the receptor protein into a conformation which promotes the stimulation of gene transcription, although the precise molecular mechanism involved is not yet understood.

Two recent series of studies demonstrate that this model for receptor-response element interaction is far too simple. Umesono *et al.* (15) have shown that not

Figure 3. DNA response elements for nuclear receptors. The core consensus sequences for the various members of the nuclear receptor superfamily are presented. Significant variations in a number of nucleotides in the response element can occur, depending upon the specific promoter in which it is found. Although the core sequences for the GRE, MRE, PRE, and ARE are the same, additional flanking nucleotides may modify the affinity of the core sequence to render response elements that are selective for one of these receptors. All of the response elements are palindromes (inward-pointing arrows), except for the RARE found in the promoter of the RARβ gene, which contains a direct repeat of the sequence (A/G)GTTCA. The response elements shown are for the nuclear receptors that bind glucocorticoids (GRE), mineralocorticoids (MRE), progesterone (PRE), androgens (ARE), estrogen (ERE), thyroid hormone (TRE), vitamin D (VDRE), and retinoic acid (RARE). N, any nucleotide; dashes, there is no nucleotide at this position.

only is the primary nucleotide sequence important, but also that the orientation and spacing between the two half-sites of the response element are crucial for the specificity of the response to various ligands and their receptors. While the palindromic thyroid response element (TRE; see Fig. 3) was responsive to stimulation by both the thyroid and retinoic acid receptors, the *direct repeat* of the core sequence, AGGTCA, varied in its responsiveness. Thus, if three, four, or five nucleotides occur between the two half-sites in the direct repeat, the response element is positively regulated by the vitamin D, thyroid hormone, or retinoic acid receptors, respectively. In other words, this "3-4-5" rule states that the same nucleotide core sequence can be regulated by three different ligand-receptor complexes, and that the receptor-specific response depends solely upon the relative spacing between the two half-sites. In a related study, it was shown that both the orientation and spacing of core DNA sequences are important in determining the responsiveness of a gene to the estrogen, thyroid hormone, and retinoic acid ligand-receptor complexes (16). Thus, if a core sequence exists as a palindrome, an inverted palindrome, or a direct repeat, and depending upon the spacing of the two half-sites, not only is the ligand-receptor complex specificity altered, but (at least in the case of the thyroid hormone receptor) the direction of transcriptional regulation (stimulatory, inhibitory; see below) and the necessity for bound ligand also vary. These studies are too complex to describe in detail in this Minireview.

Some important general conclusions that can be drawn from these and other studies should be summarized. First, the nucleotide sequence of the response element is of great importance in determining whether a gene is regulated by a given nuclear receptor. Second, it is possible to have a gene containing a certain response element (e.g., the palindromic TRE) regulated by overlapping nuclear receptor systems (in this case, thyroid hormone and retinoic acid). An identical core sequence also appears to be involved in the regulation of gene transcription by the glucocorticoid, mineralocorticoid, progesterone, and androgen receptors (see Fig. 3). In this instance, the major determinant of which receptor is involved in regulating gene expression may be the tissue-specific expression of the respective receptor proteins. It is also possible that additional sequences flanking the core sequence may contribute somewhat to the particular nuclear receptor specificity. Third, the same core DNA sequence can be used to regulate gene expression by different ligand-receptor complexes if they exist as *direct repeats*. However, absolute ligand-receptor specificity can be obtained by varying the spacing between the two half-sites of the direct repeat (e.g., the 3-4-5 rule). Finally, the orientation of the response element half-site (palindrome, inverted palindrome, direct repeat) can strongly influence both the ligand-receptor specificity and the directionality of the transcriptional response. Thus, the nucleotide sequence, the half-site spacing, and the orientation of the half-sites all contribute to the effectiveness and specificities of DNA response elements. A major unanswered question is the way in which nuclear receptors could possibility interact as homodimers given this variety in response element architecture. It is easy to envisage how a receptor could form a tail-to-tail homodimer (Fig. 2) and bind to a palindromic response element in which the half-sites occur on the same face of the DNA double helix. It is much more difficult to imagine how a homodimer could interact with a direct repeat. Furthermore, while a direct repeat TRE with a four-nucleotide spacing would center the half-sites on the same plane of the DNA, the deletion (three-nucleotide spacing) or addition (five-nucleotide spacing) of one nucleotide to this sequence would cause a 3.4 Å alteration in distance and a 36° rotation between the center of the half-sites (15). How homodimers of nuclear receptors can accommodate such a large degree of variation in the geometry of the DNA, and still bind to the DNA in a transcriptionally productive complex, remains to be determined.

The major mechanism for nuclear receptor regulation of gene expression involves the stimulation of target gene transcription. However, steroid hormones can also specifically inhibit gene transcription, one

example being glucocorticoid-inhibition of prolactin gene transcription by binding of the glucocorticoid receptor to a negative glucocorticoid response element (17). Also, steroid hormones can alter the stability of hormonally regulated messenger RNA, both positively and negatively (18). Finally, translational and other nongenomic modes for regulating cellular physiology have also been postulated, but these have not been elucidated in great detail.

Autoregulation of Nuclear Receptor Levels

It is generally believed that the level of the circulating ligand is the major physiological regulator of gene expression via nuclear receptors, and this is almost certainly true. However, any condition that alters the intracellular *nuclear receptor level* will dramatically affect the responsiveness of the cell to the ligand. While a wide variety of circumstances can regulate intracellular receptor levels and activity, autoregulation of nuclear receptors by their cognate ligand and cross-regulation through other nuclear receptors are potentially the most interesting and important.

The autoregulation of glucocorticoid receptor (GR) levels by corticosteroids has been extensively studied. In many cell lines, chronic glucocorticoid treatment causes a down-regulation of hormone-binding activity (19, 20). Importantly. this is mirrored by a similar decrease in the GR mRNA level in the cell (21–24). A number of studies have been carried out to determine the mode of receptor mRNA regulation. Both transcriptional (23, 24) and posttranscriptional (25) modes of regulation have been postulated. In addition, glucocorticoid treatment of rat hepatoma tissue culture cells causes an increased rate of GR *protein* degradation (24). This complexity of regulation is even more dramatically evidenced by results obtained in immature T cell-derived lymphoid cells. The response of these cells to glucocorticoids is cell death. Corticosteroid treatment results in an *increase* in the GR mRNA levels in human CEM-C7 acute lymphoblastic leukemia (26) and mouse S49 lymphoma (E. Vig and W. V. Vedeckis, unpublished) cells. This may be important in ensuring that the proper physiological response (cell death) occurs when corticosteroid levels are elevated. Increasing the intracellular GR levels would, thus, promote lymphocytolysis. Therefore, not only is the mechanism of GR autoregulation complex, but the nature of the regulation appears to vary in different cell types.

The estrogen (27, 28), progesterone (29), and retinoic acid (30) receptors, as well as other members of the nuclear receptor superfamily, are also autoregulated by their cognate ligands. Furthermore, the liganded estrogen receptor can also transcriptionally up-regulate the level of the progesterone receptor, and this has been used diagnostically for the identification of hormonally responsive breast tumors (31). Thus, autoregulation and cross-regulation of the levels of nuclear receptor proteins are additional mechanisms for modulating a cell's responsiveness to a hormonal ligand, and this may have important implications for both normal cell physiology and neoplastic growth.

Nuclear Receptors and Proto-Oncogenes

The GR was the first member of the steroid/thyroid receptor superfamily of nuclear receptors for which a cDNA clone and sequence information were obtained (32). Even at this early stage, it was recognized that the GR has amino acid sequence similarity to a previously discovered, retrovirally derived oncogenic protein, v-*erbA* (33). Additional studies indicated that the corresponding cellular proto-oncogene, c-*erbA*, codes for the thyroid hormone receptor (TR). Two major subtypes of thyroid hormone receptor, TRα (corresponding to c-*erbA*α; 34) and TRβ (corresponding to c-*erbA*β; 35), have been identified. Thus, this is a clear instance in which a gene coding for a normal member of the nuclear receptor family (the thyroid hormone receptor) has been mutated into an active oncogene (v-*erbA*). It is of great interest to determine the molecular mechanism whereby this change has occurred. Most notably, the v-*erbA* protein is incapable of binding thyroid hormone. In studies with other nuclear receptors, such as the GR (36), it was shown that deletion of the ligand-binding domain results in a receptor that is constitutively active in stimulating gene expression, although the level of transcriptional activation is usually somewhat less than the level seen for the liganded, wild-type receptor. It has been postulated that the ligand-binding domain directly and/or indirectly (e.g., by binding hsp90) represses the transactivating function(s) of the receptor protein. The elimination of the ligand-binding domain would, therefore, permanently remove this inhibition. However, given the differentiation-promoting activity of thyroid hormone, it was difficult to envision why a constitutively active thyroid hormone receptor would promote dedifferentiation and neoplastic transformation.

Regarding this apparent paradox, it was subsequently shown that although the v-*erbA* protein can bind to the normal thyroid hormone response element, a transcriptionally active complex is not formed (37). Thus, it is possible that both the normal TR and the mutated version (v-*erbA*) can bind to the TRE in the absence of the hormone, but that neither forms a transcriptionally active complex. Upon binding the hormone, the wild-type TR is converted into an active transcription factor, while v-*erbA* cannot bind thyroid hormone, and thus remains transcriptionally inert. However, a derivative of v-*erbA* that can bind thyroid hormone (although to a lesser extent than the wild-type TR), but has a deletion at the extreme carboxyl terminus, is still inactive in regulating erythroid differentia-

tion (38). The extreme carboxyl terminus of the protein has the potential to form an amphipathic helix, and it lies adjacent to a putative "leucine-zipper" dimerization motif in the TR (see below). This suggests that the ligand-binding and transcriptional activation functions may be distinct in the TR protein structure, and raises the possibility that a defect in the ability of the protein to dimerize may also render it transcriptionally inactive. In any event, it seems likely that the mechanism by which v-*erbA* causes transformation is by binding to the TRE in a *nonproductive conformation* that is not regulatable by the ligand. This nonproductive interaction could inhibit the productive binding of liganded wild-type receptors via the physical occupation of the response element by either a v-*erbA* homodimer or an inactive TR/*v-erbA* heterodimer (Fig. 4a). In this way, the differentiation-promoting effects of the normal thyroid hormone-TR complex could be blocked. This was the first example of a mutant nuclear receptor acting as a "dominant negative oncogene" (37) or "dominant repressor" (38). A similar mutation (loss of the carboxyl-terminal 70 amino acids) has been identified in a naturally occurring mutant of the retinoic acid receptor(RAR)-α protein present in a retinoic acid-nonresponsive embryonal carcinoma cell line (39). Again, this mutant receptor acted as a dominant repressor of gene transcription when the expression of a transfected retinoic acid-responsive reporter gene was assayed. Presumably, the mutant RARα protein binds to the RARE in a nonproductive fashion and competes with the wild-type receptor for this response element. This could block binding of the wild-type receptor and suppress retinoic acid-mediated gene expression.

It would be of great interest to see whether similar mutant receptors act as dominant negative oncogenes, particularly in naturally occurring diseased states. In this regard, it has recently been demonstrated that a RARα gene (located on chromosome 17) translocation into chromosome 15 in acute promyelocytic leukemia gives rise to a transcript that could potentially code for an aberrant *myl*/RARα fusion protein (40). (The locus on chromosome 15 into which the RARα gene translocates has been designated *myl* [for myelocytic leukemia].) Interestingly, acute promyelocytic leukemia can be effectively treated by the administration of retinoic acid (41). Thus, it is possible that the aberrant *myl*/RARα fusion protein acts as a dominant repressor of retinoic acid action and that this can be overcome by treatment with retinoic acid. Perhaps when high intracellular retinoic acid levels are maintained, the liganded wild-type RARα protein can effectively compete with *myl*/RARα and reestablish a differentiated state in the leukemic cells. Alternatively, high concentrations of retinoic acid may allow sufficient binding to the *myl*/RARα fusion protein to promote its conversion into a transcriptionally active form. Future experiments will yield the answer to this clinically important question. Finally, recent studies have shown that a naturally occurring, mutant TRβ protein may act as a dominant repressor in a human disease, "generalized resistance to thyroid hormone" (42). Additional studies will reveal whether other endocrine disorders are due to dominant repressor effects of mutated receptor proteins.

Formation of Heterodimers

It is now widely accepted that the nuclear receptors interact with their response elements as homodimers, and good experimental evidence for this has been obtained for the glucocorticoid (11), progesterone (11), and estrogen (10) receptors. Heterodimer formation has also been suggested for the thyroid hormone and retinoic acid receptors (43, 44). *In vitro* mutagenesis and expression studies with cloned receptor genes have localized domains believed to be involved in the protein interactions necessary for dimerization. Comparisons of the amino acid sequences have revealed possible "heptad repeats" or "leucine zipper motifs" (45, 46). These structures were initially postulated to be involved in dimerization of the CCAAT/enhancer binding protein, which is involved in the regulation of gene expression (47). This was quickly extended to other proteins involved in nuclear DNA binding. Perhaps the most dramatic example of this involves the formation of a heterodimer between two proto-oncogene proteins, c-*fos* and c-*jun* (48, 49). In this case, the c-*jun* protein appears to bind directly to DNA, while the c-*fos* protein binds to c-*jun* (but not DNA) via the leucine zipper motif. Only when this heterodimer is formed and bound at the AP-1 site in DNA does efficient transcription of the adjacent gene occur.

A similar heptad repeat motif has been discovered in the nuclear receptor superfamily members (45, 46). This raises the potential for the formation of heterodimers between the various nuclear receptor proteins and may be the basis for heterodimer formation between the TR and RAR (43, 44). This is notable because the RAR can stimulate gene expression by binding to the palindromic TRE (Fig. 3; 50). Thus, consider the following possible scenario, using the TR solely as an example (the same could potentially be true for any nuclear receptor).

The TR can bind to its response element to regulate gene expression. This could involve homodimers of the TR (TRα/TRα, TRβ/TRβ) or heterodimeric thyroid receptors (TRα/TRβ; Fig. 4b). The dimers formed could potentially have quite different affinities for sequences that are true thyroid response elements, but which vary slightly from the consensus TRE sequence (e.g., TRE 1, TRE 2, TRE 3; Fig. 4b). Thus, the intracellular level and composition of the TR dimers could regulate which subsets of thyroid hormone-responsive genes are activated at a given time in a given

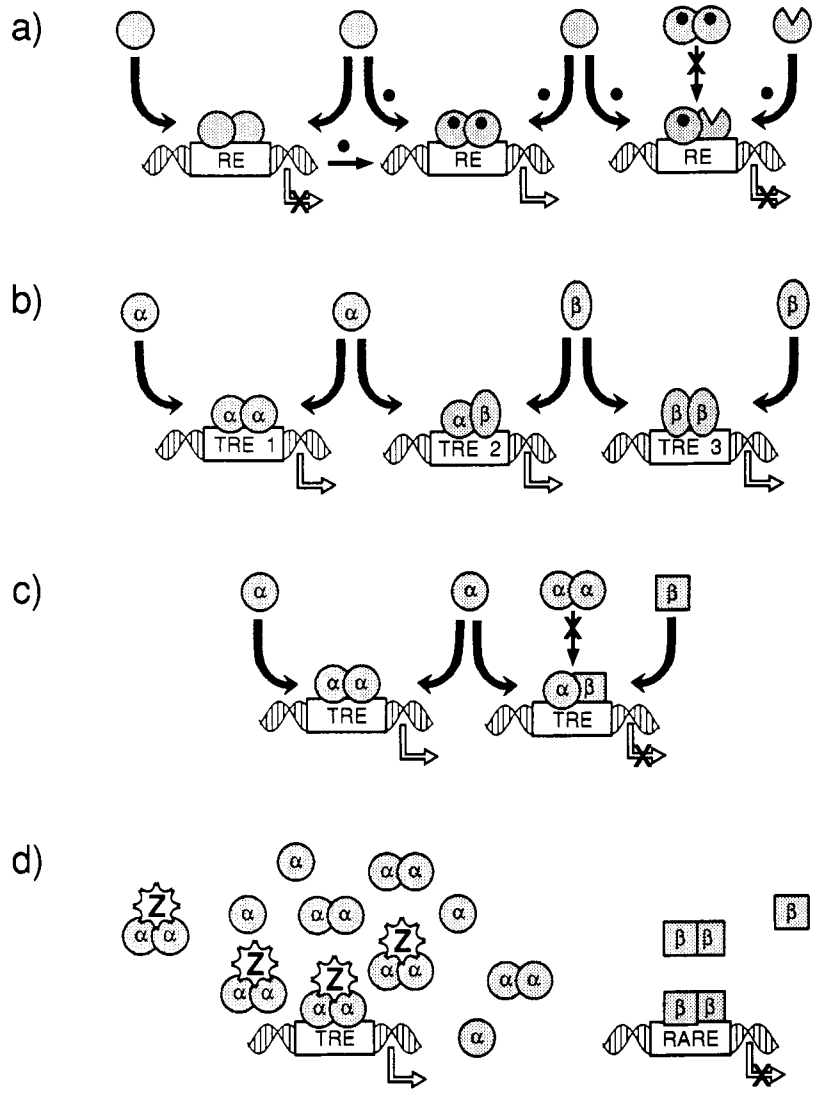

Figure 4. Complexity of transcriptional regulation by nuclear receptors. While data are available in support of a number of these mechanisms, some degree of speculation is also present. In all of the models shown, an active receptor complex is represented by both subunits being shown in front of the response element, while inactive complexes are designated by the rightmost subunit being behind the plane of the response element. (a) Dominant negative repression. The unliganded receptor (shaded circle) binds to its response element (RE) as a homodimer in a transcriptionally nonproductive complex (left panel). The inability of this complex to transactivate is represented by the "X" on the rightward open arrow, which designates gene transcription. The subsequent binding of the ligand (solid sphere) converts the dimer into a transcriptional activator (horizontal filled arrow). Alternatively, the receptor may first bind the ligand, followed by its productive binding to the response element (middle panel). A mutated receptor which, for example, is incapable of binding hormone and becoming transcriptionally active may form a homodimer (not shown) or a heterodimer with the wild-type receptor. Either of these complexes would interact with the response element nonproductively (right panel). This could block binding of the active wild-type receptor to the response element, thereby inhibiting normal ligand-mediated gene transcription. (b) Differential affinity of receptor subtypes for response elements. Three thyroid receptor response elements (TRE 1–3), each differing slightly in their nucleotide sequences from the consensus TRE, are depicted. Also shown are the various dimer combinations possible for two TR subtypes (TRα and TRβ). It is postulated that the various dimers bind with high affinity to one of the three noncanonical TRE, but with low affinity to the other two thyroid response elements. In this way, the same ligand (thyroid hormone) could differentially regulate gene expression (e.g., in different cell types or at different times), depending upon the presence and relative levels of the thyroid receptor subtypes. Alternatively, one of the subunits could be a subtype of one nuclear receptor (e.g., TRα), while the other could be a subtype of a different nuclear receptor superfamily member (e.g., RARβ). In this manner, identical genes could be cross-regulated by different nuclear receptors. Although not shown, all receptor subunits contain bound ligand. (c) Direct receptor interference. Depicted at the left is a TRα homodimer bound to a TRE in a transcriptionally active complex. If heterodimers are formed between TRα monomers and monomers of some other nuclear receptor superfamily member (e.g., RARβ), this could result in the formation of transcriptionally inactive heterodimers. Binding of this complex to the TRE would extinguish the stimulation of gene transcription by the active TR homodimer. This would be an example of a naturally occurring (and perhaps physiologically relevant) dominant negative repression, which might occur when the concentration of one nuclear receptor (in this case, RARβ) is elevated in the cell. Although not shown, all receptors are assumed to be liganded. (d) Receptor competition for a common transcription factor. In this model, it is proposed that maximum transcriptional activation by the nuclear receptor not only involves binding of the receptor dimer to the response element, but also requires the interaction of some other, general, transcription factor ("Z") with the receptor dimer. Depicted are subunits and homodimers of both the TRα and RARβ proteins and their respective response elements (TRE, RARE). All receptors are assumed to be liganded. If the concentration of the common transcription factor ("Z") was limiting, a much higher concentration of one nuclear receptor superfamily member (in this case, TRα) would effectively compete with the other nuclear

cell. In addition to this, the RARα (51, 52), -β (53, 54), and -γ (55, 56) forms could also potentially form RAR homodimers or heterodimers (RAR/RAR) and regulate the expression of thyroid hormone-specific genes under the appropriate conditions, since the RAR can bind to the palindromic TRE. This would depend upon the thyroid hormone, retinoic acid, TR, and RAR concentrations in each particular cell, and upon the relative affinities of the various receptor homodimers and heterodimers for the response element. In addition to this, the RAR could regulate the expression of its own panel of genes by binding to the authentic RARE. Furthermore, TR/RAR heterodimers could form, and these could regulate the expression of thyroid hormone- *and/or* retinoic acid-responsive genes, depending upon the relative ligand concentrations and the affinity of the heterodimers for the respective response elements. Finally, the formation of a TR/RAR heterodimer may result in the formation of a transcriptionally inactive heterodimeric complex, which could *inhibit* the expression of genes that are responsive to the respective homodimers (Fig. 4c). That is, these heterodimers could act as naturally occurring, physiologically relevant dominant repressors of gene transcription. Add to this the possibility of forming heterodimers with other members of the nuclear receptor superfamily, and one can generate a highly complex, and potentially very sophisticated, network of gene regulatory proteins that could activate or repress a wide spectrum of cellular genes. The potential for the formation of nuclear receptor heterodimers and their effects on gene transcription will be an intense area of research in the future.

Nuclear Receptors, Proto-Oncogenes, and Transcriptional Regulation

As if the scenario presented above were not complicated enough, recent studies point to yet another level of biological complexity for gene regulation by nuclear receptors. In a number of studies, it has been suggested that a nuclear receptor (the GR) can form heterodimers with other leucine-zipper proteins that are not nuclear receptor family members, that is, the c-*jun* and c-*fos* proto-oncogene proteins (57–60). Reporter gene expression studies have revealed fascinating results. Phorbol esters stimulate gene transcription through c-*fos*/c-*jun* binding to the AP-1 site in DNA, while glucocorticoids stimulate gene expression by binding to the GR, which then binds to the glucocorticoid response element in DNA. If high levels of the c-*jun* protein are expressed in cells containing the GR, glucocorticoid-regulated gene expression is extinguished. The reciprocal is also true: high levels of GR expression attenuate the responsiveness of AP-1 site-containing genes to the stimulatory effect of phorbol esters. Cross-linking experiments support the concept that c-*jun* and c-*fos* are capable of interacting with the GR protein. In a more general sense, high levels of a transcription factor A could bind to a heterologous transcription factor B to form a heterodimer (A/B), and this could deplete the cellular pool of transcription factor B such that it no longer would be available to form other transcriptionally active homodimeric (B/B) or heterodimeric (e.g., B/C) complexes. This "cross-talk" between two intracellular signaling systems via heterodimer formation yields a novel "dominant negative" mechanism for the inhibition of gene transcription, and opens up a whole new research avenue.

Recent studies using protein cross-linking have also suggested that the RARα protein may form heteromeric complexes with a number of as-yet-unidentified proteins, that the spectrum of proteins with which RARα interacts varies in a cell type-specific manner, and that the various heteromers have different binding affinities to slightly different retinoic acid response elements (61). These "co-regulators" of nuclear receptor activity may give rise to tissue-specific differences in response to the same ligand and the same receptor protein (by modifying the binding affinities of the nuclear receptors for DNA response elements). Which nuclear receptor-responsive genes are activated would depend upon which co-regulator is present, and at what amount, in a specific cell type. Similarly, it appears that the TR interacts with nuclear proteins, and this may modulate the responsiveness of thyroid hormone-responsive genes (62–65). Additional studies have also suggested that a variety of *known* transcription factors can act cooperatively with nuclear receptors to regulate gene expression (66). Finally, evidence exists that the estrogen, progesterone, and glucocorticoid receptors may compete for common transcription factors (67), so that high levels of one receptor may prevent efficient regulation of gene expression by a different nuclear receptor that is present at a lower concentration and requires the same transcription factor for maximal transcriptional activity (Fig. 4d).

It has already been mentioned that steroid/thyroid ligands can either up-regulate or down-regulate (or both) their cognate receptors, and that cross-regulation of steroid receptor levels can occur. In addition, different receptor subtypes that bind the *same ligand* can also be cross-regulated. For example, it has been shown that retinoic acid binding to the RARα subtype results

receptor (RARβ) for the common factor. Therefore, even though both nuclear receptors and their respective ligands are present, thyroid hormone-specific gene expression would be stimulated, whereas retinoic acid-responsive gene expression would be repressed, by virtue of the high intracellular TRα levels. It should be noted that numerous other scenarios for transcriptional regulation by nuclear receptors are not included in this Figure.

in the stimulation of RARβ gene transcription, presumably by the binding of the RARα protein to an RARE in the 5′ end of the RARβ gene (30). Thus, prior to retinoic acid treatment, or at early times of exposure, only RARα homodimers may be present in the cell. After a sufficient time of treatment, RARα/RARβ heterodimers may predominate, while RARβ homodimers may be the major species present after long periods of exposure to retinoic acid. If these various RAR dimers have slightly different affinities for retinoic acid response elements that diverge somewhat from the RARE consensus sequence, there could be a cascade of activation of different gene networks at various times after exposure to the ligand or at various levels of the ligand. Additionally, if one of these secondarily formed complexes (e.g., RARα/RARβ) binds nonproductively to a RARE that selectively interacts with the RAR species originally present (RARα/RARα), then this could extinguish the expression of genes regulated by the initial RAR dimer, while simultaneously stimulating the expression of retinoic acid-responsive genes that have a different RARE that selectively binds the secondary RAR dimer structure (RARα/RARβ).

Besides the ability for receptor proteins to form heterodimers with other members of the nuclear receptor superfamily, it is also possible for ligands to bind to heterologous receptors. For example, it is known that progesterone can bind to the glucocorticoid receptor, if the concentration of ligand is high enough, and that the mineralocorticoid receptor binds corticosteroids even better than the classical glucocorticoid receptor. Thus, it is feasible that the binding of heterologous ligands could cause "cross-regulation" of genes by various ligands (if a transcriptionally productive complex is formed). Alternatively, suppression of transcriptional activation by the homologous ligand would result if the heterologous ligand-receptor complex formed were not transcriptionally active. In this case, the heterologous ligand would be acting as an "antagonist," similar to a dominant suppressor effect. If one considers autoregulation of other members of the nuclear receptor family by homologous and heterologous ligands, with the concomitant formation of heterodimers between different members of the nuclear receptor family, the level of complexity in gene regulation becomes enormous.

Another potentially important mechanism for regulating the transcriptional activity of nuclear receptors may involve the orphan receptors mentioned previously (7). These were discovered by: screening cDNA libraries from various cell sources at low stringency with probes derived from authentic, characterized members of the nuclear receptor superfamily; isolating and sequencing the novel cDNA; and comparing the derived amino acid sequences to those of bona fide nuclear receptors. Putative functions for these orphan receptors have not yet been determined in all cases. However, functional homologs to a variety of vertebrate orphan receptors have been shown to have profound effects on development and differentiation in Drosophila. For example, the fly seven-up sequence (which controls photoreceptor cell fate) is homologous to the COUP transcription factor (68), and ultraspiracle (which is required maternally and zygotically for segmental pattern formation) is very similar to the retinoid X receptor (69). It is beyond the scope of this Minireview to discuss such relationships in detail. However, the fact that these orphan receptors are expressed at the mRNA level in vertebrate cells and the profound effects of the insect homologs on developmental processes suggest that the orphan receptor proteins may play important physiological roles in mammalian cells. Although speculative, there seem to be two obvious mechanisms by which these orphan receptors could regulate gene expression. First, there may be as-yet-unidentified normal, cellular ligands that bind to the orphan receptors, resulting in their transformation into DNA-binding, gene-regulatory proteins. Second, these proteins may not bind any endogenous ligands, but, rather, function as ligand-unregulated, constitutive, transcription factors. Of course, the expression, levels, and activities of these orphan receptor proteins could be regulated by mechanisms other than their binding of any ligands, for example, by some other intracellular signaling pathway. Phosphorylation of these proteins would be one such mechanism. In addition, these orphan receptors could potentially replace, synergize, or extinguish gene regulation by the ligand-dependent nuclear receptor superfamily members, depending upon the nature of the DNA response element to which they bind, the relative location of the response element in the promoter region of the gene, etc. Once again, the complexity and potential for "fine-tuning" of gene expression afforded by the existence of orphan receptors may be immense.

Finally, besides the level of circulating hormone and the intracellular level of the respective nuclear receptor protein, posttranslational modifications of the receptor present yet another level at which further regulation of transcription is possible. The best-studied and most likely posttranslational modifications involved in regulating nuclear receptor activity are phosphorylation and dephosphorylation. This has been the subject of a recent, comprehensive review (70), and the results obtained are too complex to be discussed in detail here. To summarize, it has been shown that nuclear receptors can be phosphorylated on serine, threonine, and tyrosine residues. Most significantly, there appears to be an increase in receptor phosphorylation upon binding of the ligand. Because ligand binding promotes the transactivating activities of nuclear receptors, it is tempting to speculate that ligand-dependent receptor phosphorylation is intimately in-

volved in this process. *In vitro* treatment of steroid receptors with phosphatases and phosphatase inhibitors can cause varying effects on ligand binding and DNA binding, but *no definitive* experiments showing that these are important *in vivo* have yet been presented. Undoubtedly, studies on the role of nuclear receptor phosphorylation in ligand binding, DNA binding, transactivation, and protein turnover will be an extremely active and important area of research in the future. Although unequivocal answers are still lacking, it is highly likely that this and other covalent modifications of nuclear receptors will be crucial in the molecular mechanism of nuclear receptor regulation of gene transcription.

Nuclear Receptors and Oncogenesis

The role of nuclear receptors in oncogenesis is a burgeoning research area. The role for the thyroid receptor (TRα; c-*erbA*) and its retroviral oncogene counterpart (v-*erbA*) in neoplastic transformation has been alluded to earlier. The putative role for the RARα fusion protein (*myl*/RARα) in the development of acute promyelocytic leukemia was also mentioned. Another example of a possible role for a nuclear receptor in oncogenesis may involve that of RARβ in hepatocarcinogenesis. Indeed, the initial identification of this RAR species was made because of a hepatitis B virus integration into the RARβ locus (53, 54, 71). Although no fusion protein or mRNA was demonstrated in the hepatocellular carcinoma (due to the unavailability of the original tumor tissue), it is possible that a viral/RARβ fusion protein was produced and was responsible for the neoplasm. Additional studies demonstrated the expression of a 2.5-kb RARβ transcript in hepatoma-derived cell lines, but not in normal adult or fetal livers (72). This may represent the transcription of the normal RARβ gene in an inappropriate tissue, although mutations in the gene were not analyzed in these studies. Thus, the expression of a normal nuclear receptor in an inappropriate tissue could theoretically give rise to the stimulation of aberrant gene expression in these tissues and result in oncogenesis. Alternatively, expression of RARβ in hepatic carcinomas may merely be a result, rather than a cause, of the neoplasm.

Although detailed information is still lacking, two potential, naturally occurring members of the nuclear receptor family that may be involved in carcinogenesis have been identified. One of these binds dioxin (5). Although the gene for this receptor has not yet been cloned, the physicochemical properties of the protein, and its mode of transcriptional regulation via the putative interaction with a DNA response element, make it extremely likely that it is a member of the nuclear receptor superfamily. The other nuclear receptor superfamily member has been identified by cDNA cloning experiments (6). Although a direct binding of ligand remains to be shown, the activity of this nuclear receptor member can be stimulated by a class of compounds called peroxisome proliferators, which give rise to hepatocarcinomas (6). It is, of course, highly unlikely that these man-made chemicals are the natural, physiological ligands for these two putative receptor proteins. Thus, it would be of great importance to determine what the natural ligand is, and what role it might play in normal cellular physiology. Presumably, the binding of these carcinogens to these nuclear receptors competes for binding of the endogenous ligand and results in the aberrant regulation of gene transcription. This could be analogous to the deleterious effects that occur when diethylstilbestrol binds to the estrogen receptor to promote breast and uterine cancers. It will be interesting to determine whether other man-made chemicals that cause neoplasms do so by binding to other members of the nuclear receptor superfamily (such as the TR and RAR) and prevent the differentiation-promoting effects of the natural ligands. Although not related to oncogenesis, one possible other example of this may be the teratogenic effects of thalidomide, whose ringed structure is similar to that of a steroid. Thalidomide has extremely deleterious effects on human limb development, and retinoic acid (through binding to the RAR) is a potent morphogen that *controls* normal vertebrate limb development (73). Might thalidomide bind to the RAR in limb buds of human fetuses and disrupt normal retinoic acid-mediated limb differentiation? What other teratogens may elicit their effects via binding to members of the nuclear receptor superfamily?

With the current rapid rate of progress, it is inevitable that the precise roles of nuclear receptors in neoplasia will be forthcoming. It should be evident that even at just the present level of complexity, a wide variety of possible mutations in nuclear receptors, proto-oncogene transcription factors, other transcription factors, and co-regulators are possible. For example, mutations in the receptor ligand-binding domain could result in constitutively active proteins or dominant negative repressors. Mutations in the DNA-binding domain could render the receptors inactive, more active, or modify their specificity for binding to certain response elements (either by weakening or strengthening the protein-DNA interaction). Mutations in the leucine-zipper motifs of either the nuclear receptors or the other nuclear proto-oncogene proteins could disrupt normal subunit interactions and greatly alter their transcriptional activity. Mutations in the promoter sequences controlling the transcription of nuclear receptors could give rise to receptors that are either over- or underexpressed. Chromosomal translocations of nuclear receptor genes into foreign loci could also result in the production of nuclear receptor-fusion proteins that have altered activity. Because nuclear receptors are powerful regulators of gene expression, any modifica-

tions in their structure or activity would have great potential for contributing to altered transcriptional activity and neoplastic transformation.

Conclusions and Future Directions

The observations presented above point to a dauntingly complex, yet marvelously versatile, regulatory system. The future for research into the mode of nuclear receptor action and its role in oncogenesis looks very exciting. We have only begun to scratch the surface regarding nuclear receptor effects on normal and abnormal cellular physiology. With the recent advent of the polymerase chain reaction, it will be possible to rapidly analyze tumor cells for mutated receptor genes. In addition, *in vitro* mutagenesis of nuclear receptors, the use of transgenic mouse technology, and the current developments in the generation of transgenic animals using homologous recombination gene replacement will allow us to study the effects of mutated nuclear receptors in intact animals. With the further elucidation of possible protein-protein interactions of nuclear receptors with proto-oncogene-derived (and other) transcription factors, it will be possible to analyze the physicochemical bases for nuclear receptor function. In addition, recent successes in developing *in vitro* transcription systems will allow precise studies on the mechanism of transcriptional regulation by these proteins. Cloning and sequencing of the 5′ promoters of the nuclear receptor genes will allow researchers to determine which sequences and transcription factors are involved in the transcriptional regulation of nuclear receptor levels. All of these approaches will result in an explosion in our knowledge of the molecular mechanism of nuclear receptor action and the effects of these important regulators on the control of cell growth and neoplastic transformation. Once these details are worked out, it should be possible to devise focused strategies for extending this knowledge into the realm of clinical treatment of certain neoplasms. At this point, our basic knowledge about nuclear receptor action will lead to tremendous applications important to human health.

Notes Added in Proof. The locus in chromosome 15 into which the RARα gene translocates, designated *myl* above, has been renamed PML. The cDNA for this gene has been cloned, and it may code for a novel transcription factor (Cell **66**:663–674, 1991; Cell **66**:675–684, 1991). It has also been suggested that the PML-RARα fusion protein could act as a dominant suppressor of normal PML expression, resulting in the onset of acute promyelocytic leukemia.

A recent study (Cell **66**:885–893, 1991) suggests that the oncogenic activity of v-*erbA* best correlates with its ability to act as a dominant suppressor of normal RAR action, rather than by blocking TR-mediated responses.

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