Phosphorylation of Thyroid Hormone Receptors by Protein Kinase A Regulates DNA Recognition by Specific Inhibition of Receptor Monomer Binding*

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Thyroid hormone receptor (T3R) α-1 and its oncogenic derivative, the v-ERB A protein, are phosphorylated by cAMP-dependent protein kinase A. Although this phosphorylation appears to be necessary for the oncogenic properties of v-ERB A, the mechanism by which phosphorylation influences the functions of v-ERB A and of the normal T3R has not been established. The protein kinase A phosphorylation site in T3Rα-1 is within a domain that is known to contribute to the DNA recognition properties of these receptors. We therefore analyzed the effects of protein kinase A phosphorylation on DNA recognition by the normal T3Rα and by the v-ERB A oncoprotein. We report here that phosphorylation of these receptor derivatives does not significantly alter the overall affinity of receptor dimers for DNA. However, phosphorylation does notably alter DNA recognition by preventing, or greatly inhibiting, the ability of these receptors to bind to DNA as protein monomers. These studies suggest that the phosphorylation of T3Rα-1 and v-ERB A by protein kinase A may provide a means of altering promotor recognition through a post-translational modification.

The nuclear hormone receptors are a family of interrelated proteins that regulate the transcription of specific target genes in response to binding of cognate hormone ligand (1–8). They include the steroid hormone receptors, retinoic acid receptors, vitamin D3 receptor, and the thyroid hormone receptors (T3Rα and T3Rβ). The nuclear hormone receptors play many critical roles in vertebrate homeostasis, morphogenesis, development, and reproduction; in addition, aberrant nuclear hormone receptors have been implicated as causal agents in oncogenesis and in endocrine disorders (1–8). For example, the v-ERB A oncprotein is a neoplastic retroviral derivative of T3Rα (9, 10). T3Rs generally repress transcription in the absence of hormone and activate transcription in the presence of hormone (1–8). However retaining the ability to bind to DNA, v-ERB A has sustained multiple mutations relative to the T3Rα progenitor and has lost the ability to activate transcription directly (Fig. 1 and Refs. 11–13). Instead, v-ERB A functions in most contexts as a constitutive repressor and can interfere with target gene activation by a variety of nuclear hormone receptors, including T3Rs, retinoic acid receptors, and estrogen receptors (11–17). Therefore, v-ERB A is viewed as a dominant negative oncoprotein that acts in the cancer cell by antagonizing the actions of normal cellular receptors.

Nuclear hormone receptors recognize their target genes by binding to DNA sequences referred to as hormone response elements or HREs (1–8). Although DNA binding by these receptors is principally mediated by a "zinc finger" motif near the center of each receptor (Fig. 1A), additional flanking domains, both N- and C-terminal of the zinc finger itself, contribute to the DNA recognition specificity of each receptor (18–26). Most nuclear hormone receptors can bind to DNA as protein dimers, with each receptor molecule recognizing a "half-site," a conserved 6–8-base DNA sequence (1–8, 27, 28). HREs have therefore been traditionally viewed as composed of two half-sites, with the sequence, spacing, and orientation of the individual half-sites contributing to the specificity of DNA recognition (1–8). However, recent work has demonstrated that receptor dimers are not the only paradigm for DNA recognition and that certain members of the nuclear receptor family can also bind to single DNA half-sites as protein monomers or can bind to highly reiterated DNA half-sites as receptor oligomers larger than dimer in size (29–35).

Nuclear hormone receptors are substrates for a variety of protein kinases, and phosphorylation can have profound effects on the subcellular localization, DNA binding, and transcriptional activity of these receptors (36–40). For example, both v-ERB A, and its progenitor, avian T3Rα-1, are phosphorylated by protein kinase A (PKA) in vitro and, apparently, in vivo (41, 42). The major site of PKA phosphorylation in v-ERB A has been mapped to serine 16/serine 17 (Fig. 1A); both amino acids represent consensus PKA sites, and prior studies did not determine which of the two, or if both, were phosphorylated (42). The same PKA site(s) also exist and are phosphorylated in the avian T3Rα-1 progenitor (denoted serine 28/serine 29 in the T3R-numbering system; Fig. 1A). Significantly, preventing the phosphorylation of serine 16/17 in v-ERB A, either by substitution of alanines or by use of kinase inhibitors, dramatically impairs oncogenic transformation (41). In contrast, conversion of serines 16/17 to threonines (which retain the ability to be phosphorylated by PKA) preserves v-ERB A oncogenic activity (41). Alterations in PKA activity have also been reported to modify T3Rα-1 activity in cells (38, 43).

Although it appears that the phosphorylation of the PKA sites of v-ERB A (and probably of avian T3Rα-1) is critical for full function, the molecular mechanism behind this phenomenon has not been established. The PKA phosphorylation sites in v-ERB A/T3Rα-1 are within a domain that we have previ-
DNA Binding by T3R Monomers Is Inhibited by PKA

EXPERIMENTAL PROCEDURES

Mutagenesis of T3R-a and v-ERB A Phosphorylation Sites—All mutants were created by a two-step polymerase chain reaction mutagenesis technique (44). The wild-type avian T3R-1 clone was used as the initial template for mutagenesis (9). The flanking primers, which hybridize to sequences in the parental pGEX2T vector, were 5'-GAATG GAATT CTCAT GGAAC AGAAG CCGCG CCGC C-3' and 5'-AATAA GGAAAT TCCCT ATGCC CCTCT TCCCT C-3'. The internal oligonucleotide primers used to create the S28A mutants were 5'-AGAAG GGCCCG CGCGC CGCGC AAGTTT GGTGA AGGTC ATGAC CTGAG ATC-3' and 5'-TGGAG TGAGG TCACT GTGAC TCGAG ATC-3'), and an inverted repeat element containing a single half-site (1S, 5'-TGCTG TGGAC TACCT AGTGG TATGC TATGG CTCAT A-3'). Each probe was synthesized as two complementary oligonucleotides with 4-base overlaps; the complementary sequences were annealed, and the double-stranded DNAs were radiolabeled by extension using [α-32P]dGTP and the Klenow fragment of DNA polymerase.

Electrophoretic Mobility Shift Assays—Wild-type or mutant receptors (estimated as 1 ng per reaction, unless otherwise noted) were incubated with the purified catalytic subunit of PKA (either purchased from Promega or the gift of Dr. D. Walsh, University of California, Davis) in 6 μl of protein kinase buffer (40 mM Tris-HCl, pH 7.4, 20 mM magnesium acetate) in the presence or absence of rATP (200 μM) for 20 min at 30 °C. Non-recombinant GST or baculovirus extracts were employed in parallel as negative controls. Binding buffer (10 mM Tris-HCl, pH 7.5, 5% glycerol, 13.3 μg/ml bovine serum albumin, 66.7 mM KCl, 2 mM MgCl2, and 133 μg/ml poly(adenosine) dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)) followed by autoradiography and/or phosphorimager analysis.

RESULTS

Phosphorylation of v-ERB A and T3R-a 1 Alters the Proportion of Receptor Monomer to Receptor Dimer Complexes Formed on DNA—We first wished to establish that the recombinant avian T3R-1 and v-ERB A proteins synthesized in a baculovirus/Sf9 cell system could be phosphorylated in vitro by PKA. The wild-type T3R-1 protein, the v-ERB A protein, or equivalent nuclear extracts of S9 cells infected by a non-recombinant baculovirus were incubated with purified PKA and analyzed by SDS-PAGE and autoradiography (Fig. 2). The T3R and v-ERB A proteins, detected by Coomassie Blue stain (Fig. 2A), were both efficiently radiolabeled by this procedure (Fig. 2B), whereas no equivalent [32P]labeled bands were detected in the non-recombinant extract (Fig. 2B) or in the absence of added PKA (data not shown). The radiolabeled [32P] radiolabel incorporated was proportional to the T3R and v-ERB A protein concentration up to a maximum stoichiometry, calculated from specific activity criteria, of approximately 2.2 (± 0.2) mol of phosphate per mol of receptor. Similarly, PKA was able to radiolabel purified GST fusion proteins of T3R-a and v-ERB A but not non-recombinant GST (data not shown).

We next used electrophoretic mobility shift assays to determine if PKA phosphorylation altered the DNA binding properties of T3R-1 and v-ERB A. We first tested a DNA probe containing a prototypic HRE composed of a direct repeat of two AGGTCA half-sites, separated by 4 base pairs (denoted a HRE palindrome, as shown).

Molecular Cloning and Preparation of Recombinant Proteins—The wild type T3R-1, (S28A/T3R-a, (S29A/T3R-a, (S28A/S29A/T3R-a, and T3R-a, and wild type v-ERB A sequences were cloned as EcoRI to EcoRI fragments into the baculovirus transfer vector pVL1393 (17). Appropriate baculovirus clones, expressing the protein of interest, were obtained by in vitro recombination and plaque purification and were used to infect Sf9 cells to generate recombinant baculoviruses expressing wild-type or mutant T3R-a and v-ERB A proteins (17, 22, 26). Alternatively, the wild type, single point mutants, and double mutants of T3R-a and v-ERB A were also cloned as EcoRI to EcoRI fragments into pGEX-2T, and the resulting glutathione S-transferase (GST) fusion proteins were isolated from transformed Escherichia coli as described previously (45).

Preparation of Oligonucleotide Probes for the DNA Binding Assay—Three different DNA sequences were used as DNA binding probes as follows: a direct repeat element with a 4-base spacer (DR-4, 5'-TGAGTCAGAG TCACG GGAAG TCAGA G-3') and a inverted repeat element (TREpal, 5'-TGCGAG ATCTC AGTGG TATGC TATGG CTCAT A-3'). Each probe was synthesized as two complementary oligonucleotides with 4-base overlaps; the complementary sequences were annealed, and the double-stranded DNAs were radiolabeled by extension using [α-32P]dGTP and the Klenow fragment of DNA polymerase.
4). Untreated T3Ra or v-ERB A both efficiently bound to the DR-4 element, each forming two distinct protein-DNA complexes (denoted 1R and 2R in Fig. 2C); this property has been widely noted previously and reflects the ability of the T3R and v-ERB A proteins to bind to DNA either as protein monomers (the faster migrating 1R complexes) or, alternatively, as protein "dimers" (the slower migrating 2R complexes) (e.g. Refs. 29–32, 43, 46, and 47). This interpretation of the electrophoretic mobility shift is fully supported by our own data as follows. (a) Only the faster migrating monomer complex was detected when using a single half-site element as the DNA probe, whereas response elements containing two half-sites in a variety of orientations were bound as a mix of monomer and dimer complexes (Fig. 3). (b) Thyroid hormone selectively destabilized the slower migrating species (data not shown), a property specific for the homodimeric form of T3R (29–32, 46, 47). (c) RXRs form heterodimers with T3R, and addition of RXR to our binding reactions converted both monomer and homodimer T3R species to a distinct complex migrating at a position characteristic of heterodimers (Fig. 5).

Significantly, although untreated (or mock-treated) T3Ra and v-ERB A proteins bound to the DR-4 element as a mixture of monomers and dimers, PKA treatment of otherwise identical preparations of T3Ra or v-ERB A leads to the almost exclusive formation of dimeric complexes (Fig. 2A and B; compare the PKA-treated receptor preparations, lanes 1 and 2, to the mock-treated receptor preparations, lane 3). This effect was proportional to PKA concentration, was observed with a variety of purified PKA preparations, and was not detected if rATP was omitted from the kinase buffer, suggesting that it is indeed the phosphorylation of the receptors that is responsible for this phenomenon (Fig. 3, A and B; compare lanes 4–6 with lanes 1–3; and data not shown). Similarly, binding of T3Ra and v-ERB A to a palindromic DNA element (TREpal) also showed a reproducible decrease in monomer binding on PKA treatment (although the reduction on the palindromic element was not as dramatic as that observed with the DR-4 element). We conclude that PKA phosphorylation of either T3Ra-1 or v-ERB A significantly alters the ratio of receptors binding to DNA as protein monomers versus dimers. Phosphorylation by PKA appears to specifically destabilize the ability of receptor monomers to bind DNA, rather than alter the absolute affinity of the receptor for DNA or modify the
dimerization properties of the phosphorylated receptor. Several possible mechanisms could account for the observed reduction of monomer binding by PKA treatment of T3Rα and v-ERB A. Phosphorylation could enhance the protein-protein interaction between receptor molecules, resulting in an increased cooperativity on binding DNA. Alternatively, phosphorylation might conceivably increase the overall affinity of receptor for DNA. In either of these scenarios, the effect of phosphorylation would be to selectively enhance the formation of protein dimer-DNA complexes at the expense of protein monomer-DNA complexes. However, this effect was not, in fact, that which was observed (Fig. 3). Alternatively, phosphorylation could decrease the absolute affinity of receptor for the DNA half-site, causing a parallel inhibition of binding of the DNA probe by both receptor monomers and receptor dimers; however, again this was not observed (Fig. 3). Instead, phosphorylation by PKA selectively inhibited monomer complex formation with a significant change in the amount of dimer complex (Fig. 3, A–C; and quantified for the DR-4 element over a range of receptor concentrations in Fig. 4). This selective inhibition of monomer binding by PKA, and its independence from observable effects on dimer binding, was particularly striking on a DNA element containing only a single half-site, which is bound exclusively by receptor monomers (Fig. 5D). We suggest that there are differences in the precise mechanisms by which receptor monomers recognize DNA versus receptor dimers and that phosphorylation selectively inhibits DNA recognition by receptor monomers (see “Discussion”).

The above results were obtained using T3Rα as the sole receptor molecule. Notably, RXRs can dramatically enhance the ability of T3Rs to bind to certain DNA response elements by forming receptor heterodimers; these heterodimers display both an increased affinity for DNA, and enhanced transcriptional regulation properties, over those observed with the corresponding homodimers (1–8, 28). We therefore next tested the effects of PKA on RXR/T3Rα heterodimer formation. As previously reported (29–31), addition of RXR to the T3Rα preparations resulted in the formation of a novel complex migrating at a position consistent with that of an RXR/T3R heterodimer (Fig. 5, compare lanes 1–6 with lanes 7–12). This RXR/T3R heterodimeric complex was formed in preference to formation of the T3R monomer or T3R homodimer complexes, whereas RXR itself failed to detectably bind to the DR-4 DNA element (Fig. 5, lane 13). Prior incubation of the T3Rα with PKA resulted in no detectable alteration in the binding of the DNA by these RXR/T3Rα heterodimers (Fig. 5; compare PKA-treated lanes 1 and 2 to mock-treated lane 3). We conclude that PKA treatment specifically and strongly inhibits the ability of T3Rα monomers to complex with DNA, without detectably altering DNA recognition by either T3Rα homodimers or RXR/T3Rα heterodimers.

Thyroid hormone destabilizes the ability of T3R homodimers, but not monomers or RXR heterodimers, to bind to certain DNA response elements. Notably, however, cognate T3 hormone neither enhanced nor inhibited the effect of PKA phosphorylation when assayed on the DR-4, TREP1, or single half-site elements employed here (data not shown).

Serine 28 and Serine 29 Mediate the Effects of PKA on Receptor DNA Recognition—We next altered either serine 28 or serine 29 in T3Rα individually to alanine and examined these mutant receptors for the ability to be phosphorylated by PKA. Alanine substitution of either individual serine resulted in a partial decrease, but not a complete loss, of phosphorylation of the mutant receptor by PKA (quantified in Fig. 6A). These
results suggested that serines 28 and 29 might both be substrates for PKA. Supporting this hypothesis, substitution of both serine 28 and 29 to alanines, producing a S28A/S29A double mutant, resulted in a near-total abrogation of phosphorylation of the mutant receptor by PKA (Fig. 6A). We next examined the DNA binding properties of these different T3Ra mutants by gel mobility shift assay (Fig. 6B, and quantified in Fig. 6C). In the absence of PKA, the S28A/S29A mutant T3Ra bound to the DR-4 DNA element as a mixed monomer/dimer population indistinguishable from that produced by the wild-type T3Ra (Fig. 6B). After treatment with PKA, the wild-type T3Ra exhibited the expected loss in monomer complex formation, whereas the S28A/S29A double mutant fully retained the ability to bind to the DR-4 element as both dimeric and monomeric protein complexes (Fig. 6, B and C). A similar resistance to the effects of PKA were obtained with the S28A/S29A double mutant when using a monomer-specific DNA probe in the binding assay (Fig. 6D). In contrast to the double mutant, treatment of either the S28A or the S29A single mutant with PKA produced only a partial inhibition of monomer binding (40% and 39% inhibition respectively, on a DR-4 element). These results suggest that the two consecutive serine residues in avian T3Ra-1 are both phosphorylated by PKA and that both serines are likely to play a contributory role in mediating the effects of PKA on the receptor-DNA interaction.

We also tested the phosphorylation and DNA binding properties of a T3Ra mutant bearing a complete deletion of the receptor N-terminal domain (removing amino acids 1–32). This ΔN-terminal deletion mutant was not detectably phosphorylated by PKA, providing an independent confirmation that the PKA sites lie primarily within the N-terminal T3Ra domain that encompasses serines 28 and 29 (Fig. 6A). Intriguingly, the ΔN-terminal T3Ra mutant bound to DNA exclusively as a protein dimer in either the absence or the presence of PKA treatment (Fig. 6, B and C). Our mutational analysis therefore suggests that the N-terminal domain of avian T3Ra can play an important role in DNA recognition by determining if a given DNA sequence is bound by receptor monomers or by receptor dimers, and that either deletion of the N-terminal domain or phosphorylation of specific serines in this N-terminal domain by PKA can inhibit monomer binding without precluding dimer binding. Similar results were obtained with v-ERB A (data not shown).

**DISCUSSION**

*DNA Binding by T3Ra-1 Protein Monomers, but Not by Dimers, Is Specifically Inhibited by Receptor Phosphorylation by PKA—*The actions of a wide range of transcription factors, including the nuclear hormone receptors, are regulated by phosphorylation (36–40, 48). We sought to determine the effects of one particular protein kinase, PKA, on the actions of avian T3Ra-1 and its oncogenic derivative, v-ERB A. PKA phosphorylates two vicinal serines (codons 28 and 29) in the N-terminus of the T3Ra receptor, and these PKA phosphorylation sites have been previously demonstrated to be essential for the oncogenic abilities of the v-ERB A oncoprotein and, possibly, for full transcriptional activity by T3Ra-1 (41, 42). Alterations in PKA activity have also been reported to modify T3Ra-1 activity in cells (38, 43). We report here that PKA treatment manifests *in vitro* as a selective inhibition of the ability of T3Ra and v-ERB A to bind to DNA as receptor monomers. We suggest, therefore, that phosphorylation may also provide a means by which promoter recognition is modulated *in vivo* by altering the nature of the receptor-DNA complex. Our results are consistent with previous studies demonstrating that the N-terminal domain, in which serines 28/29 reside, can exert profound effects on DNA recognition by the nuclear hormone receptors and further emphasize that important elements of DNA recognition are controlled by determinants outside the zinc finger motif domain itself (22, 23, 25, 26).

Our basis for these conclusions is that treatment of T3Ra or v-ERB A with purified preparations of PKA resulted in a strong and selective inhibition of receptor monomer binding to DNA, without significantly inhibiting or enhancing the ability of the receptor to bind as a homodimer or as a heterodimer with RXR. These effects of PKA were observed with a variety of response elements, including a direct repeat element, a palindromic element, and an element containing only a single half-site. In all cases, and over a range of receptor concentrations, only the
binding of the monomer form of receptor was destabilized by PKA treatment. No evidence was obtained that PKA phosphorylation enhanced the intrinsic affinity of receptor for DNA; such a scenario would predict a PKA-mediated increase in overall DNA binding by receptor that was not observed. Nor did PKA appear to operate by enhancing the protein-protein interactions that lead to dimer formation; such a mechanism would require that the observed loss of monomer binding be paralleled by an equivalent gain in dimer formation, a phenomenon that was not observed. Instead, we consistently observed a PKA-mediated loss of monomer binding with little or no effect on homo- or heterodimer binding, suggesting that DNA recognition by monomers and dimers can be independently regulated.

Our results, therefore, suggest that the mechanism of DNA recognition by receptor dimers must be in some respects distinct from that by receptor monomers. It is notable in this regard that codons 28 and 29 reside in a region of the receptor that participates both in the DNA/protein contacts involved in DNA recognition and in the protein-protein contacts involved in receptor dimerization (24). Perhaps certain of the DNA contacts made by the T3R monomer are pre-empted by the protein-protein contacts involved in dimerization. Phosphorylation of serine 28/29 by PKA could disrupt these monomer-specific DNA contacts, thereby destabilizing monomer binding, but would have little or no effect on DNA binding by the dimer. The DNA/protein contacts made by the receptor molecules in a dimer and in a monomer appear to be non-identical (e.g. Refs. 47 and 49), thereby supporting our hypothesis. Our hypothesis is also consistent with the observed lack of monomer binding by an N-terminal deletion mutant of T3R; presumably in the absence of the N terminus of the receptor, the additional protein/DNA contacts necessary to stabilize monomer binding cannot occur. We are currently further testing aspects of this proposal.

Both Serine 28 and Serine 29 Play a Role in PKA Phosphorylation and in Inhibition of Monomer Binding—Previous studies localized the primary site of PKA phosphorylation in avian T3Rα-1 to a polypeptide containing serines 28 and 29 (42). Intriguingly, due to the presence of nested consensus motifs, both serine 28 and serine 29 represent potential PKA substrates, and the precise site of modification was not previously determined (42). Our own results strongly suggest that serines 28 and 29 are, in fact, both targets of PKA phosphorylation in vitro and that both sites can be phosphorylated simultaneously. First, our measurements of stoichiometry are consistent with a maximum of two phosphorylation sites per receptor molecule. Furthermore, single substitutions of either serine 28 or serine 29 with alanine reduced, but did not eliminate, T3Rα phosphorylation. In contrast, simultaneous substitution of both serines with alanines abrogated phosphorylation. Paralleling the phosphorylation results, single substitution of either serine reduced, but did not eliminate, the inhibitory effects of PKA on receptor monomer binding to DNA, whereas a double substitution mutant virtually completely eliminated the inhibitory effects of PKA. We conclude that either serine can be phosphorylated by PKA and that both serines are involved in the inhibition of monomer binding to DNA observed in the wild-type receptor.

Phosphorylation by PKA May Operate to Alter Promoter Recognition or Utilization by Avian T3Rα-1 and by v-ERB A—Phosphorylation by PKA (or a PKA-like kinase) appears to be crucial for the ability of v-ERB A to participate in erythro-leukemogenesis (41). The wild-type v-ERB A protein blocks erythroid differentiation, blocks expression of erythroid-specific genes, and allows proliferation of erythroid cells in simple media (50, 51). In contrast, conversion of both serines 16 and 17 in v-ERB A to alanines (equivalent to serines 28 and 29 in T3Rα-1) abolished all of these v-ERB A activities, as did treatment of wild-type v-ERB A-infected cells with a kinase inhibitor, H7. Similarly, inducers of PKA activity such as forskolin enhance phosphorylation of avian T3Rα-1 in cells and enhance T3Rα transcriptional activity, whereas inhibitors of PKA activity can attenuate both T3Rα phosphorylation and T3Rα transcriptional activity (38, 42, 52). Although some of these effects of kinase activators or inhibitors may be indirect, the preponderance of evidence suggests that v-ERB A, and likely of T3Rα, is important for function.

Despite this evidence supporting a critical role for PKA-mediated phosphorylation in T3R and v-ERB A function, the mechanism by which the PKA effects are mediated has remained unclear. The double alanine mutant of v-ERB A exhibits the same apparent stability, subcellular localization, and DNA binding (as a dimer) as does the wild-type protein (41). Furthermore, although the double alanine substitution fails to repress differentiation-specific genes in erythroid cells, it nonetheless efficiently represses reporter gene transcription from a prototypic two half-site response element in transient transfections (41). The results we present in this article suggest a possible explanation for these apparently contradictory results. As observed here, PKA phosphorylation selectively inhibits DNA binding by receptor monomers; this modification therefore has the potential to alter the pattern of promoter recognition by receptor. This may be manifested as preventing recognition of promoters composed of single half-sites (single half-sites can, in fact, mediate T3R transcriptional regulation; e.g. Refs. 32 and 53) or perhaps by altering recognition of promoters that have odd numbers (three or more) of half-sites, such as the malic enzyme promoter or the rat growth hormone promoter (54, 55). Ultimately, however, more work will be required to determine if PKA has effects on receptor function in addition to the DNA recognition properties examined here.

Previous studies have shown that phosphorylation of T3Rs can be mediated by a variety of different kinases and that these different kinases can have distinct effects on T3R function (38, 41, 42, 56–58). Both positive and negative effects on DNA recognition have been reported. For example, a HeLa cell kinase has been identified that enhances DNA binding by T3R dimers (47, 58), whereas the purified PKA activity characterized here operates to destabilize DNA binding by receptor monomers. The synergistic effects of these two kinase activities may play a complementary role in modulating the response to thyroid hormone under physiological conditions. Phosphorylation inhibits DNA binding by T3Rα-2, an “orphan” receptor with strong repression properties, although the kinase involved, and the precise effects on monomers versus dimers, have not been fully determined (39). Furthermore, many other nuclear hormone receptors, such as the retinoid and steroid receptors, are also targets of protein kinase modifications and are likely to be subject to similar, multiple levels of regulation.

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