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Novel Regulation of Keratin Gene Expression by Thyroid Hormone and Retinoid Receptors*

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Expression of keratin proteins, markers of epidermal differentiation and pathology, is uniquely regulated by the nuclear receptors for retinoic acid (RAR) and thyroid hormone (T3R) and their ligands: it is constitutively activated by unliganded T3R, but it is suppressed by ligand-occupied T3R or RAR. This regulation was studied using gel mobility shift assays with purified receptors and transient transfection assays with vectors expressing various receptor mutants. Regulation of keratin gene expression by RAR and T3R occurs through direct binding of these receptors to receptor response elements of the keratin gene promoters. The DNA binding "C" domain of these receptors is essential for both ligand-dependent and -independent regulation. However, the NH_2-terminal "A/B" domain of T3R is not required for either mode of regulation of keratin gene expression. Furthermore, v-ErbA, an oncogenic derivative of cT3R, also activates keratin gene expression. In contrast to the previously described mechanism of gene regulation by T3R, heterodimerization with the retinoid X receptor is not essential for activation of keratin gene expression by unliganded T3R. These findings indicate that the mechanism of regulation of keratin genes by RAR and T3R differs significantly from the mechanisms described for other genes modulated by these receptors.

Hormones and vitamins, such as thyroid hormone (T3)[1] and all-*trans*-retinoic acid (RA), are important regulators of development and differentiation in general and of the epidermis in particular. The effects of vitamin A, a precursor of RA, on the skin were observed first in 1922 (1). Since that time, the skin has been a model tissue for the study of RA action. It has been shown that hypovitaminosis A causes epidermal hyperkeratinization, while non-keratinizing tissues, such as conjunctiva and cornea, become keratinized. Conversely, hypervitaminosis A causes inhibition of keratinization, hyperplasia, and a block of terminal differentiation (1–6). Similarly, thyroid hormone deficiency results in a number of skin changes, including hyperkeratosis (7–10), and the thyroid hormone excess causes increased epidermal cell division (11). Similar effects of RA and T3 were observed in keratinocytes *in vitro* (2, 9, 12).

Keratins are the intermediate filament network proteins in many epithelia. Their expression is precisely controlled in various physiological and pathological states of the epidermis. When the basal keratinocyte becomes detached from the basement membrane, its commitment to differentiation is announced by suppression of the basal cell-specific keratins K5/K14 and the induction of the differentiation-specific keratins K1/K10 (13, 14). In wound healing and other hyperproliferative processes, keratinocytes express the activation-specific keratin pair K6/K16 (15, 16). During inflammation, keratin K17 is expressed, whereas transformed keratinocytes express keratins K8/K18 (17, 18).

Because a fairly large number of keratin genes are suppressed by RA and T3, these genes provide a unique opportunity to study the mechanisms of negative regulation by T3R and RAR on native regulatory elements. We have reported previously that keratin gene expression is suppressed by RA or T3 (19–21). To examine this regulation in more detail, we studied the response of three different keratin promoter-CAT constructs (K5, K14, K17) to RAR or T3R, in the presence or absence of their cognate ligands using mutants of T3R in transfection and gel mobility shift experiments (22–24). These promoters were chosen because K5 and K14 keratins are specific for the basal layer of the epidermis, the layer most proximal to the source of RA *in vivo*, whereas K17, although not present in healthy skin, is a marker of various inflammatory processes. Furthermore, all three promoters are expressed at high levels when transfected into cells of epithelial origin.

Our results show that T3R regulates keratin genes in a unique manner: unliganded T3R leads to activation while the addition of T3 results in suppression. The NH_2-terminal "A/B" domain of cT3Rα is not required for keratin gene regulation while the ligand binding and the DNA binding domains are essential. In addition, we found that v-ErbA is a constitutive activator of keratin genes and that it blocks ligand-dependent suppression by T3R and RAR. Furthermore, we found that T3R does not form heterodimers with RXR when bound to K14RE, and that addition of T3 promotes monomer binding at the expense of the homodimer. Last, mutants which do not form heterodimers with RXR do mediate constitutive activation of keratin genes. Taken together these results suggest that the regulation of keratin genes may be mediated by monomers, or perhaps homodimers, of T3R.

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[1] The abbreviations used are: T3, thyroid hormone; T3R, tyroid hormone receptor; RA, all-*trans*-retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; CAT, chloramphenicol acetyltransferase; RSV, Rous sarcoma virus; LTR, long terminal repeat; DBD, DNA binding domain; K14RE, K14 recognition element.

MATERIALS AND METHODS

Plasmids and Their Growth and Purification—Plasmids pK#14CAT, pK#5CAT pK#17CAT, and pRSVZ have been described previously (13, 17). The plasmids containing human RARα, RARβ, and RARγ nuclear receptors were gifts from Dr. P. Chambon. Plasmids cT3Rα(51–408), NH_2-terminal deletion mutant of T3R, cT3Rα(120–408) DBD^- mutant, heptad mutants cT3Rα(L365R) and cT3Rα(L372R), and v-ErbA were also described previously (23, 24, 26).[2] Plasmids were grown in JM101 *Escherichia coli* host to saturation density in LB medium. DNA was extracted and purified using the Magic Mega Prep Kit from Promega.

Cell Growth—HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% calf serum at 37 °C in a 5% CO_2 atmosphere in media containing penicillin and streptomycin as described (20, 27). The day before transfection, cells were plated onto 60-mm dishes. Four hours before transfection the medium was changed to DMEM supplemented with 10% calf serum depleted of RA and T3 as described (20).

Transfection Using $Ca_3(PO_4)_2$—We have generally followed published procedure for cells that were at 80% confluence (27). At the time of transfection into each dish were added 3 μg of the CAT plasmid, 1 μg of the nuclear receptor expression vector plasmid, 1 μg of pRSVZ reference plasmid and a sufficient amount of carrier to bring the total to 10 μg of DNA. The cells were harvested 48 h after transfection by scraping into 5 ml of phosphate-buffered saline, washed once more in phosphate-buffered saline, and resuspended in 150 μl of 0.25 M Tris buffer, pH 7.8. All transfections were performed in duplicate plates, and each transfection experiment was repeated two to five times. CAT and β-galactosidase assay were performed as described (20).

Electrophoretic Gel Mobility Shift Assays—*E. coli*-expressed hRARα and cT3Rα were purified as described previously (28). Oligonucleotides were synthesized on a Pharmacia Gene Assembler Plus Synthesizer. The sequence of oligonucleotides flanked by *Hin*dIII overhangs (5′-AGCTT-3′) are as follows: TREp, AGGTCATGACCT; mTRE, ACGTCATGACGT; K14RE, GCTAGCCTGTGGGTGATGAAAGCCAAGGGGAATGT. Double-stranded oligonucleotides corresponding to the K14RE and TRE palindrome were labeled with [α-^{32}P]dATP, using the Klenow fragment of *E. coli* DNA polymerase I. 30,000 cpm of the resulting probe was mixed with 2.5 fmol of purified receptor proteins and incubated first for 30 min at room temperature then for 10 min at +4 °C. The incubation was done in a 30-μl volume in 25 mM Tris, pH 7.8, 500 μM EDTA, 88 mM KCl, 10 mM 2-β-mercaptoethanol, 0.1 μg of aprotinin, 0.1 μg of poly(dI-dC), 0.05% Triton X-100 (v/v), 10% glycerol (v/v). Samples were loaded on 4% polyacrylamide gel and separated by electrophoresis (20–25 mA) at +4 °C for 2 h with a buffer containing 10 mM Tris, 7.5 mM acetic acid, and 40 μM EDTA, pH 7.8. Gels were dried and analyzed by autoradiography.

Competition experiments were performed as follows: a 100 M excess of the competitor DNA was incubated with protein at room temperature for 15 min prior to addition of the radioactively labeled DNA probe. Binding reactions were further incubated at room temperature for 15 min and then at +4 °C for additional 10 min.

cT3Rα(L372R) was obtained by *in vitro* translation using TNT T7-coupled Reticulocyte Lysate System from Promega with 1.5 μg of purified DNA. The wild type cT3Rα receptor was used as a control. The quality of both synthesized proteins was analyzed by SDS-gel electrophoresis and autoradiography. The relative amount at cT3Rα(L372R) protein was compared with wild type cT3Rα and determined by quantitating the incorporated ^{35}S corrected for the number of methionine residues per protein. One μl of reticulocyte lysate translated receptor was used in the binding reaction, in the presence of RNase A (0.5 μg) and RNase T_1 (1.5 units) as described (23).

RESULTS

Novel Regulation of the Keratin Promoters by Retinoic Acid and Thyroid Hormone Receptors—To analyze regulation of keratin gene expression by RA and T3 we used the promoters of the K14, K5, and K17 keratin genes linked to a CAT reporter gene. HeLa cells were co-transfected with the keratin promoter-CAT constructs along with vectors expressing wild type and mutants of chicken T3Rα and human RARs in the presence or absence of the respective ligands. We chose HeLa cells because we have shown previously that transfected keratin gene promoters behave identically in HeLa cells and in human epidermal keratinocytes (20, 21). However, the endogenous receptors are expressed at much lower levels in HeLa cells, which facilitates the interpretation of results with transfected receptors.

In the absence of RA, the RARs are without effect (Fig. 1). In the presence of RA, all three retinoic acid receptors (hRARα, hRARβ, and hRARγ) suppress expression of each of the keratin gene promoters 5- to 6-fold (Fig. 1). In contrast, TREpCAT, containing an optimized thyroid hormone/retinoic acid response element, was stimulated approximately 30-fold by all three receptors in the presence of RA.

To test whether T3 also regulates keratin gene expression, we co-transfected HeLa cells with the keratin-promoter CAT constructs and a cT3Rα expression vector and then incubated the cells in the presence or absence of T3. As previously found, the control reporter TREpCAT is stimulated approximately 35-fold by T3 and suppressed by unliganded T3R approximately 8-fold (23, 29). In contrast, cT3Rα has the opposite effect on keratin gene expression: unliganded T3R stimulates keratin K5, K14, and K17 gene promoters approximately 3-fold, whereas with T3 the basal expression of the three keratin promoter constructs is inhibited about 5-fold (Fig. 1). Comparing the results in Fig. 1 we find that RAR and T3R mediate ligand-dependent inhibition of keratin gene activity with similar efficiency.

To analyze the combined effect of T3R and RAR on the regulation of keratin gene promoters, both receptors were expressed using 5-fold more cT3Rα expression vector. Unliganded T3R blocked both the ligand-dependent inhibition of keratin genes by hRARα and the ligand-dependent stimulation of TREpCAT (Fig. 2). Conversely, when hRARα was expressed in a 5-fold excess over cT3Rα, it did not block the constitutive activation of keratin gene expression by unliganded T3R. In the presence of its ligand, however, hRARα was epistatic and completely blocked the activation by cT3Rα (Fig. 2). The effects of RA without co-transfected hRARα are due to the low levels of endogenous RARα.

hRARα and cT3Rα Bind to a Functional Element in the K14 Gene Promoter—To study the interaction of T3R and RAR with receptor-responsive sequences, we focused on the −96/−51 region of the K14 gene promoter in which we previously identified a TRE/RARE using site-specific mutagenesis (21). Gel mobility shift DNA binding assays were performed using hRARα and cT3Rα expressed and purified from *E. coli* (Fig. 3).

cT3Rα formed two mobility complexes with the K14RE probe, the monomer and the homodimer (28). cT3Rα predominantly binds K14RE as a homodimer. Binding is specific because it can be efficiently competed with a 100 M excess of cold K14RE and consensus TREpal. A mutated TREpal that does not bind cT3Rα (28) does not compete for the binding of cT3Rα to K14RE (Fig. 3).

Similarly, hRARα predominantly forms a homodimer complex with K14RE (Fig. 3). Binding is specific because it can be competed with an excess of K14RE or TREpal but not with mTRE. These results confirm that the −95/−51 region of the K14 promoter contains a functional TRE/RARE that binds both cT3Rα and hRARα receptors.

Because the addition of T3 changes transcriptional regulation from stimulation to repression, we investigated the effects of ligand binding. Interestingly, the addition of T3 dramatically inhibits the formation of homodimers of cT3Rα while increasing the monomer binding to K14RE (Fig. 4*A*). In contrast, addition of RA did not change the binding pattern of hRARα. A small change in mobility is due to the conformational change caused by ligand binding to the receptor (28).

The presence of T3 or RA did not change the pattern of

[2] Helmer, E., Raaka, B. M., and Samuels, H. H. (1996) *Endocrinology* **137**, in press.

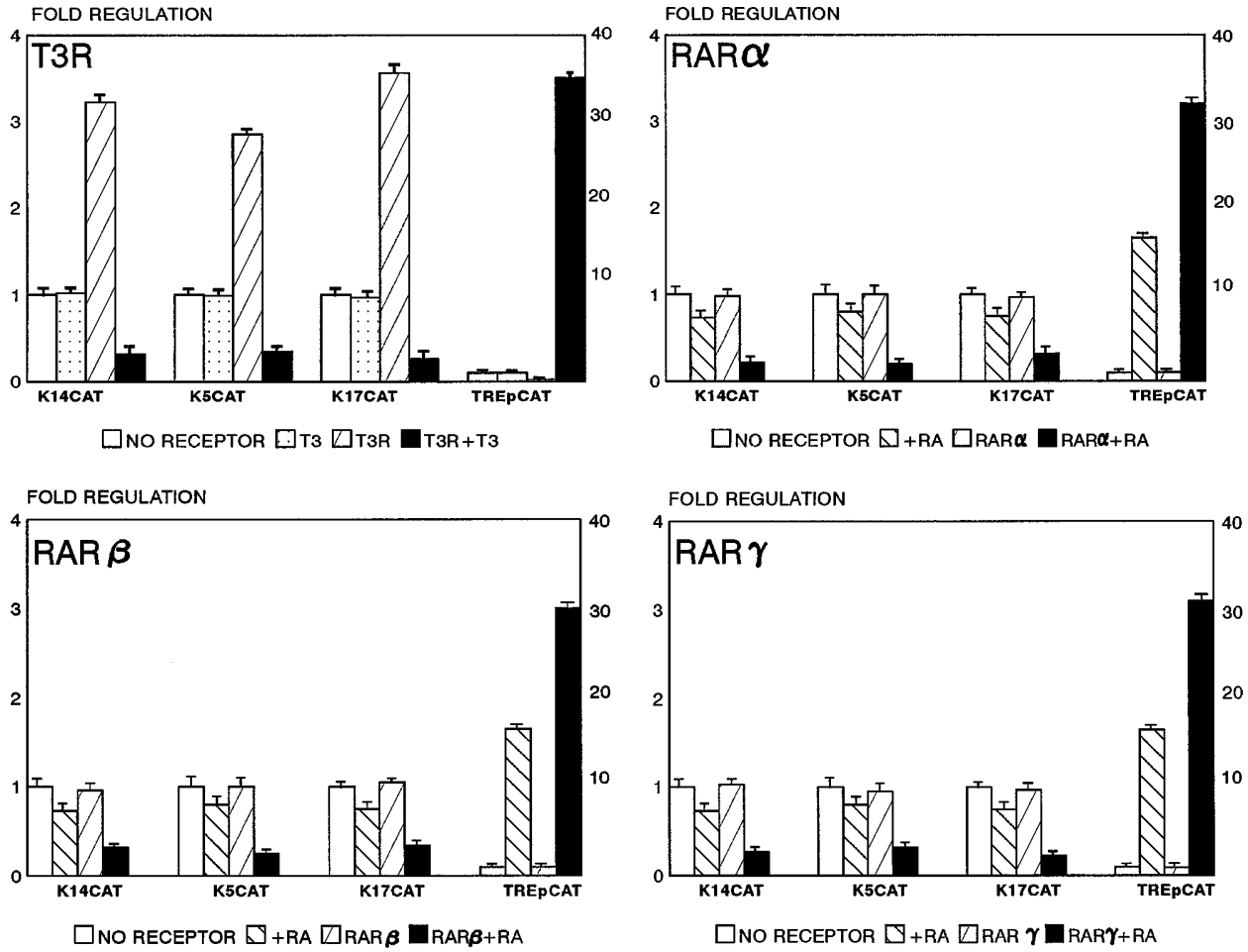

FIG. 1. **Regulation of keratin gene expression by RAR and T3R.** Regulation of K5, K14, and K17 keratin promoters by T3R and RARα, RARβ, and RARγ. The basic, unregulated activity of each CAT construct is designated as 1 to show -fold regulation by RAR and T3R. Numbers on the *left ordinate* represent regulation of keratin genes, and the numbers on the *right* represent -fold regulation of the TREpCAT.

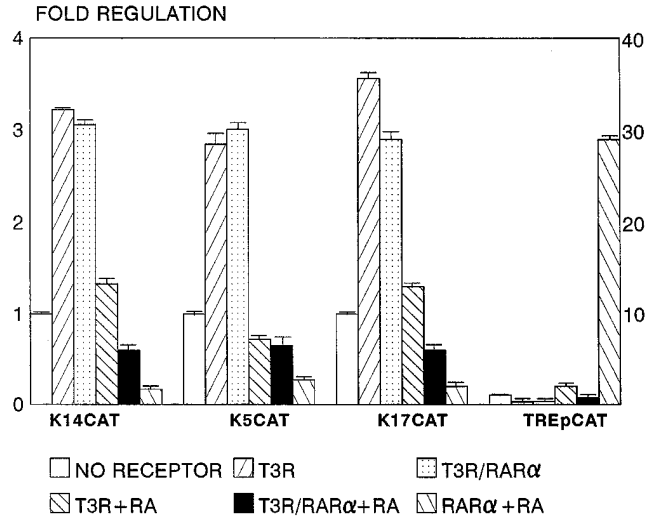

FIG. 2. **Unliganded T3R blocks suppression of keratin gene expression by RA and RARα.** Note, however, that the unliganded RARα does not block the induction by T3R.

binding of cT3Rα or hRARα to the TREpal as shown in Fig. 4*B*. Again there is small change in mobility of the complexes due to a conformational change. The K14RE has a lower binding affinity when compared with the optimized TREpal sequence, which is similar to other previously described native TRE/RAREs (30, 31).

We analyzed the combined effects of the receptors using gel mobility shift assays. In the absence of ligands three different complexes were detected: homodimers of cT3Rα, heterodimers of cT3Rα/RARα, and homodimers of hRARα (Fig. 4*A*, last four lanes). Addition of T3 inhibited the binding, whereas addition of RA did not affect it.

The Amino-terminal Region of the T3R Is Not Essential for Keratin Gene Regulation—To study the mechanism of keratin regulation by cT3Rα, we used variants of the receptor that have specific deletions and mutations in the NH_2-terminal A/B region, the DNA binding domain, or the ligand and heterodimerization domains, as well as v-ErbA (22–24). We first analyzed the role of the 50-amino acid NH_2-terminal region of cT3Rα, because this region has been reported to be important for hormone-independent activation of a sequence in the Rous sarcoma virus LTR (RSV-LTR) (22, 32). The receptor mutant cT3Rα(51–408) has a complete deletion of the 50-amino acid NH_2-terminal A/B domain but has normal DNA binding and ligand binding properties (22). Both in the absence and in the presence of T3, cT3Rα(51–408) functions essentially identical to the wild type cT3Rα (compare Fig. 5*A* with Fig. 1). Thus, the NH_2-terminal A/B region of cT3Rα is not essential for either constitutive activation or ligand-dependent inhibition of keratin promoter activity.

The DNA Binding Domain (DBD) of cT3Rα Is Essential for Keratin Gene Regulation—In contrast with the cT3Rα(51–408), a mutant lacking both the DNA binding domain and the NH_2-terminal A/B region (cT3Rα(120–408); also referred to as

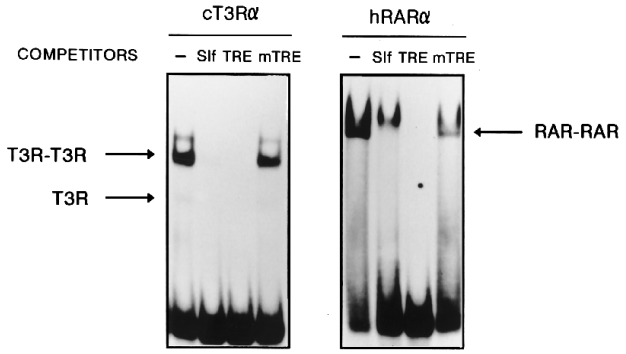

FIG. 3. **cT3Rα and hRARα specifically bind K14RE.** Autoradiograms of the gel mobility shift assay with K14RE probe are presented with cT3Rα (shown on the *left*) and hRARα (shown on the *right*). Binding of both receptors is efficiently competed with 100 M excesses of cold K14RE (*Slf*) and TREpal but not with mTRE DNA. Note significant increase in the amount of free probe in lanes competed with K14RE and TREpal.

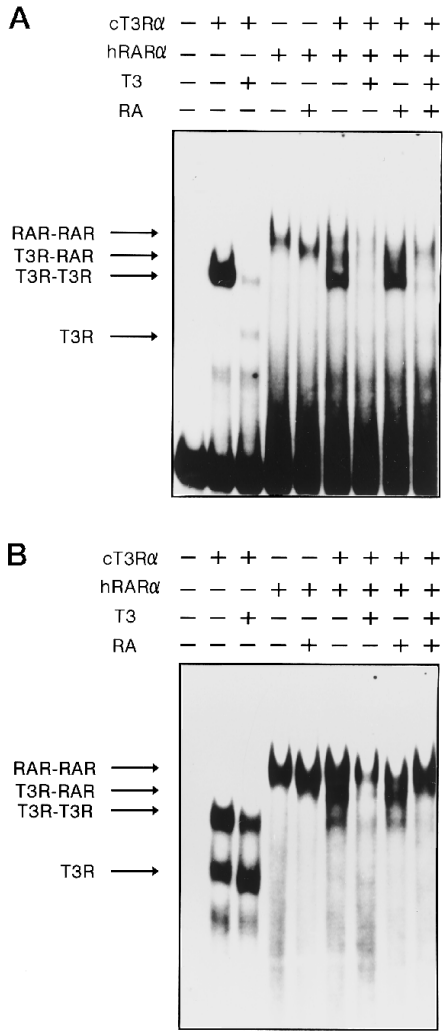

FIG. 4. **Effects of ligands on binding and dimerization of cT3Rα and hRARα.** Autoradiograms of the gel mobility shift assays are presented with K14RE (A) and TREpal probe (*B*).

DBD⁻) (24), did not influence keratin promoter activity (Fig. 5*B*). cT3Rα(120–408) has been shown to act as a dominant negative inhibitor of wild type T3Rs and RARs (26, 28), indeed it blocked the RA-dependent stimulation of TREpCAT (Fig. 6*A*). The inhibitory effect was enhanced by the addition of T3 to the medium. Unexpectedly, the suppression of keratin

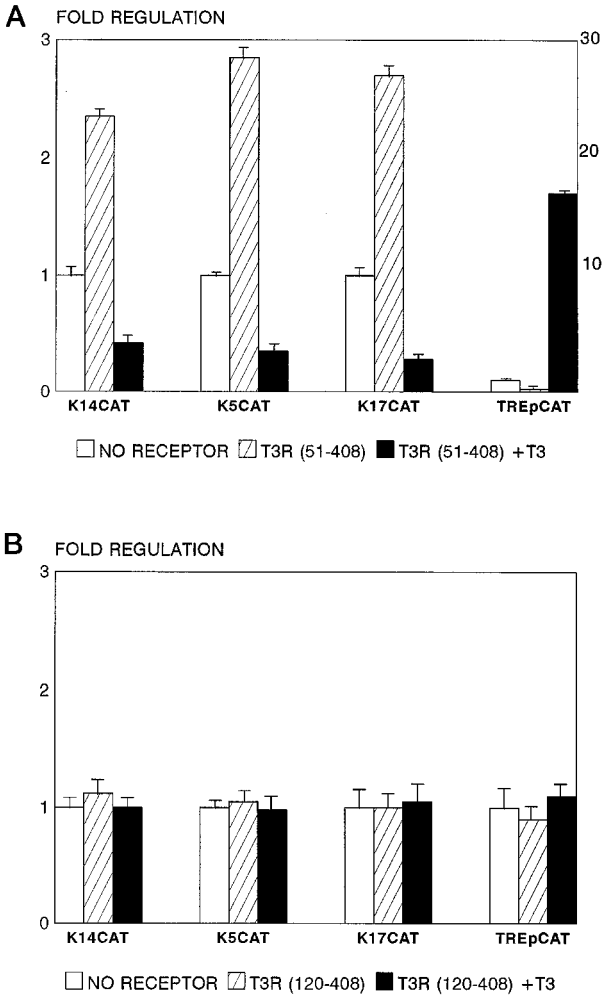

FIG. 5. **The DNA binding domain is essential for the regulation by T3R.** *A*, the NH_2-terminal mutant cT3Rα(51–408), which contains the DBD, regulates expression of keratin gene promoters the same as the wild type T3R (compare with Fig. 1). *B*, the cT3Rα(120–408) mutant of the T3R, which lacks the DBD, does not regulate expression of keratin gene promoters.

genes by hRARα was not affected by addition of the cT3Rα(120–408) in the presence or absence of T3 (Fig. 5*A*).

In view of the fact that cT3Rα(120–408) has no effect on regulation by hRARα, we were surprised to find that it blocks the effects of cT3Rα (Fig. 6*B*). The cT3Rα(120–408) mutant efficiently blocked both effects of wild type cT3Rα on the keratin K14 gene promoter: constitutive activation by unliganded receptor and the inhibition found in the presence T3. The blocking effect is not mediated through direct competition for the DNA binding, because cT3Rα(120–408) is not a DNA-binding protein. The inhibition most likely result from the dimeric interactions with cT3Rα (23, 24).

v-ErbA Constitutively Activates Keratin Gene Promoters—v-ErbA is an oncogenic variant of cT3Rα that binds T3 with very low affinity and constitutively represses promoters that contain a number of positive regulatory elements, including the TREp in TREpCAT (Fig. 7*A*) (24, 33, 34). In contrast with the repression seen with other elements, we find that v-ErbA constitutively activates the K5, K14, and K17 promoters about 2–3-fold, which is similar to the activation found for unliganded wild type cT3Rα (Fig. 7*A*). v-ErbA has also been found to act as a weak dominant negative inhibitor of wild type T3Rs and RARs (24). This effect is thought to result from direct competition for the DNA binding site rather than from interference

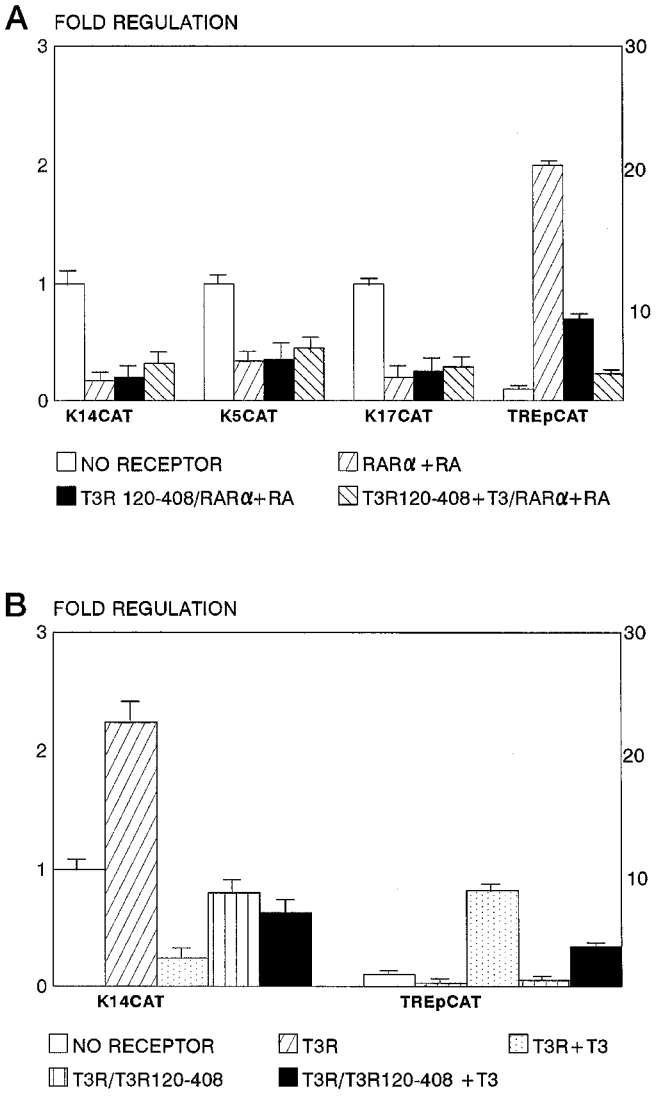

FIG. 6. **The DBD mutant specifically interferes with the regulation by T3R and not by RAR.** *A*, the cT3Rα(120–408) (DBD$^-$) mutant of the T3R does not block the suppression of keratin gene expression by RARα. Note that it blocks the induction of the TREpCAT. *B*, the cT3Rα(120–408) (DBD$^-$) mutant efficiently blocks regulation of K14 keratin gene expression by T3R, both the suppression and the induction in the presence and absence of T3, respectively.

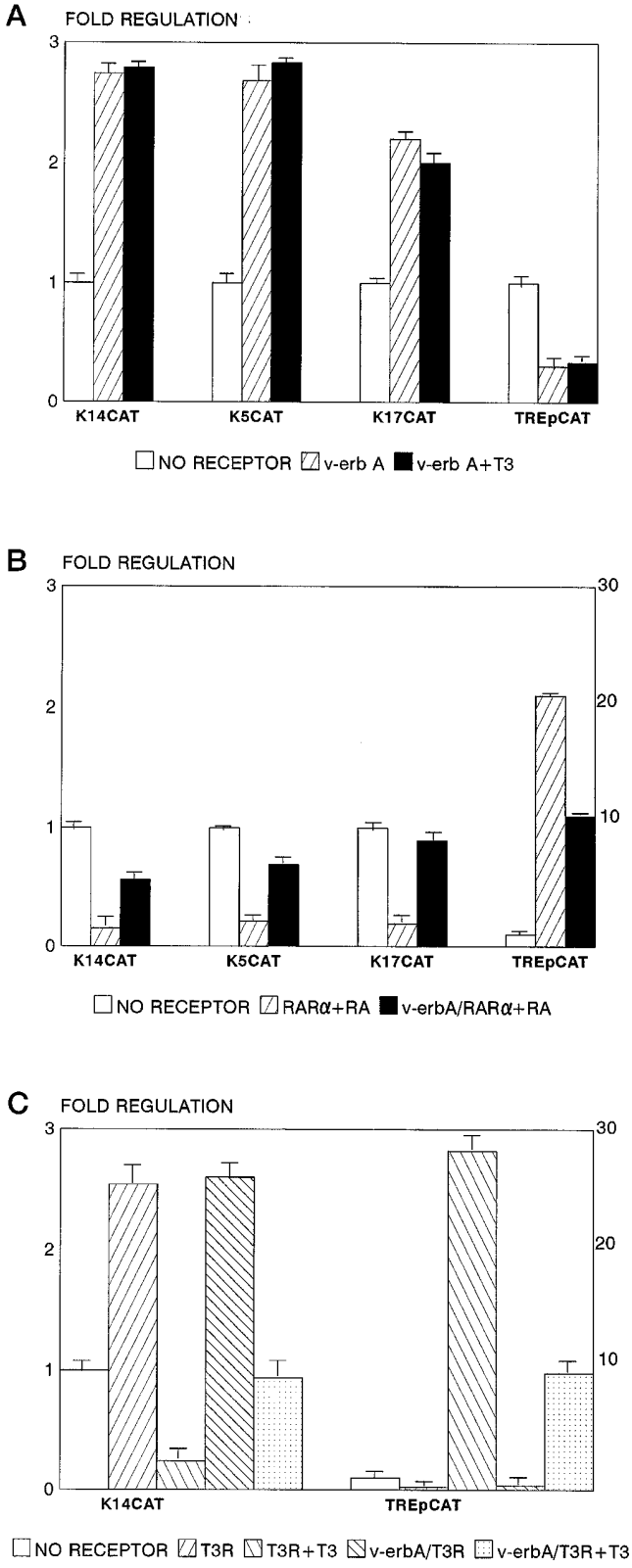

FIG. 7. **Regulation by v-ErbA.** *A*, by itself, v-ErbA constitutively stimulates keratin gene expression. *B*, v-ErbA blocks suppression of the keratin gene expression by RARα. *C*, v-ErbA blocks T3-dependent suppression of the K14 gene expression and induction of TREpCAT by T3R.

with heterodimerization with RXR (24). In contrast to cT3Rα(120–408), which did not affect inhibition by hRARα-RA, we found that v-ErbA efficiently blocked the effect of hRARα-RA (Fig. 7*B*). v-ErbA was equally efficient in blocking T3-dependent suppression of keratin promoter activity by cT3Rα (Fig. 7*C*). Thus, v-ErbA is not only a constitutive activator of keratin gene expression, but also an inhibitor of the suppression of keratin genes mediated by RA and T3.

Constitutive Activation of Keratin Gene Expression by T3R Does Not Require Heterodimerization with RXR—To study the role of homo- and heterodimerization in the constitutive activation of keratin genes by cT3Rα, we used mRXRβ and cT3Rα receptors in our gel mobility shift experiments (Fig. 8*A*). cT3Rα can bind as three complexes with TREpal in the presence of mRXRβ. These can be identified by size as T3R monomer, T3R homodimer, and RXR-T3R heterodimer. Addition of T3 did not change the binding pattern. In contrast RXR-T3R heterodimers are not formed with K14RE (Fig. 8*A*). The two complexes identified are the monomer and the homodimer of T3R. The addition of hormone promotes monomer binding at the expense of the homodimer. These results suggest that the regulation of keratin gene expression does not require heterodimer formation with RXR. To investigate this possibility further, we used two cT3Rα mutants in the ninth heptad of the ligand binding domain, cT3Rα(L365R) and cT3Rα(L372R), which have been

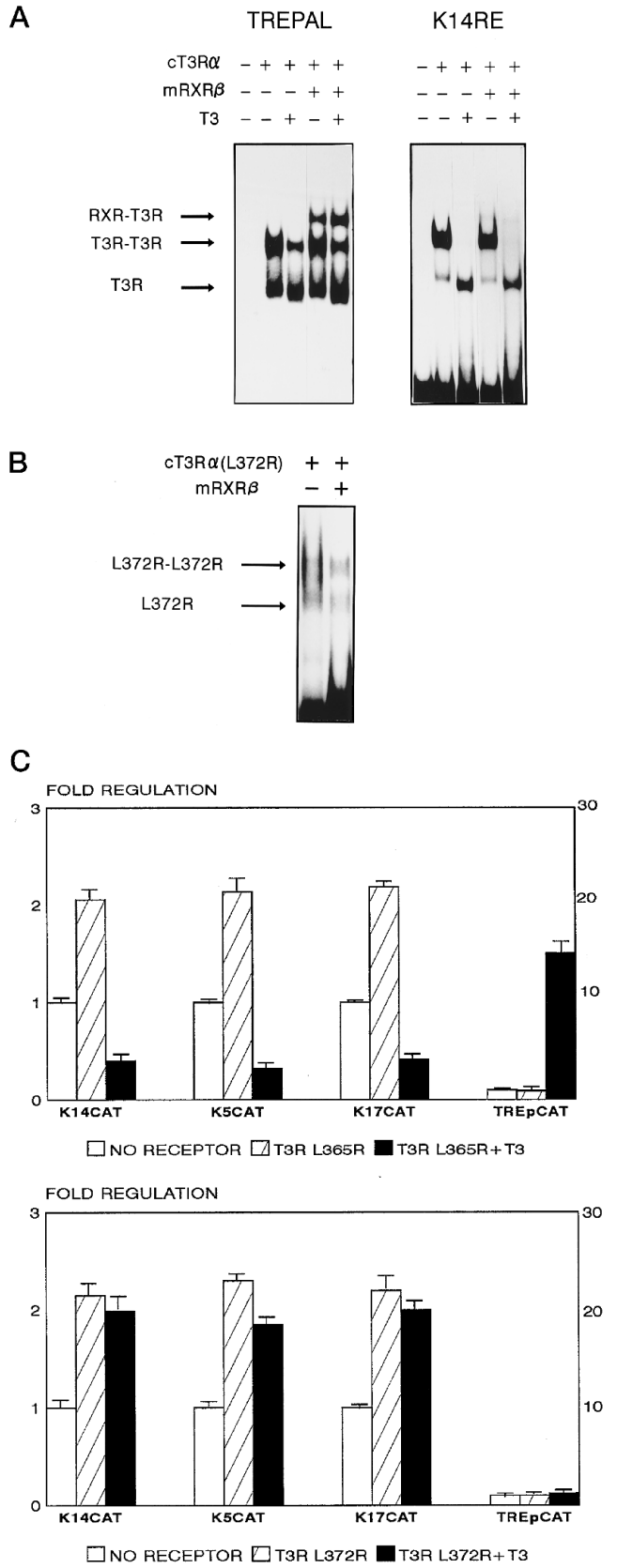

FIG. 8. **Regulation of keratin gene expression by cT3Rα does not involve heterodimerization with RXR.** *A*, gel mobility shift assays using TREpal (*left panel*) and K14RE (*right panel*) as probes with purified cT3Rα and mRXRβ receptors. *B*, autoradiogram of gel mobility shift assays using K14RE probe with mutant cT3Rα(L372R) receptor expressed in reticulocyte lysate system and purified mRXRβ. *C*, regulation by the two ninth heptad mutants of T3R. Mutant cT3Rα(L365R) regulates keratin genes as does the wild type T3R (compare with Fig. 1), while cT3Rα(L372R) constitutively stimulates keratin gene expression, similar to v-ErbA (Fig. 7*A*). Note the difference in regulation of TREpCAT.

shown to be critical for heterodimerization with RXR (23). These mutants bind to response elements as homodimers as efficiently as the wild type cT3Rα, but do not bind as heterodimers with RXR in the absence of T3 (23). With cT3Rα(L365R), but not with cT3Rα(L372R), T3 mediates a conformational change that results in the formation of cT3Rα/RXR heterodimers (23). The binding pattern at cT3Rα(L372R) mutant was identical to that of the wild type receptor; it formed two complexes with K14RE: the monomer and the homodimer (Fig. 8*B*). The addition of mRXRβ did not change the binding pattern, as expected, because this mutant is not capable of forming heterodimers with RXR receptors.

cT3Rα(L365R) stimulates the expression of TREpCAT in the presence of T3, but does not suppress basal expression in the absence of T3 (Fig. 8*C*). In contrast, cT3Rα(L365R) regulates keratin promoters similarly to wild type cT3Rα: it activates without T3, while it suppresses keratin expression in the presence of T3 (Fig. 8*C*). The mutant cT3Rα(L372R), which does not form heterodimers with or without T3, does not stimulate or repress TREpCAT, but can constitutively activate keratin gene promoters (Fig. 8*C*). cT3Rα(L372R) does not mediate negative regulation by T3 because it has a very low affinity for ligand (23). Constitutive activation of keratin promoters by the two mutants with the altered ninth heptad, cT3Rα(L365R) and cT3Rα(L372R), together with the results from gel mobility shift experiments support the notion that T3-independent stimulation of keratin gene expression by T3R occurs by a mechanism that is independent of heterodimerization with RXR.

DISCUSSION

The regulation of keratin gene expression by T3R and RAR described in this study is the inverse of the more commonly studied positive regulation of transcription. First, T3R without T3 constitutively activates keratin gene expression instead of silencing or suppressing the level of basal expression. Second, in the presence of T3, the constitutive activation of T3R is not only reversed, but the extent of transcriptional activity is further inhibited approximately 5-fold below the level of basal expression. Although RAR does not mediate constitutive activation, incubation with RA also leads to negative regulation. A number of natural promoters have been reported to be negatively regulated by either RAR or T3R and their ligands, but not by both receptors (35, 36). However, the large family of keratin genes is negatively regulated by both T3 and RA via their cognate receptors. Furthermore, keratin genes are the first group of genes reported which are not only suppressed by T3R in the presence of its ligand, but are also activated by unliganded T3R.

We provide three new lines of evidence for a direct effect of RAR and T3R on keratin gene promoters. Previously we have identified an RARE/T3RE in the K14 promoter using site-specific mutagenesis (21). In this paper we have shown that the identified responsive element physically binds nuclear receptors. We also show that the oncogenic derivative v-ErbA is an efficient competitor of the ligand-dependent regulation of keratin gene expression by RAR and T3R. Since it appears that v-ErbA acts by competing for DNA binding rather than by formation of nonfunctional heterodimers (24), our data with v-ErbA receptor support a direct regulatory mechanism. Furthermore, deletion of the DBD from the T3R aborts keratin gene regulation. Taken together, our results suggest that reg-

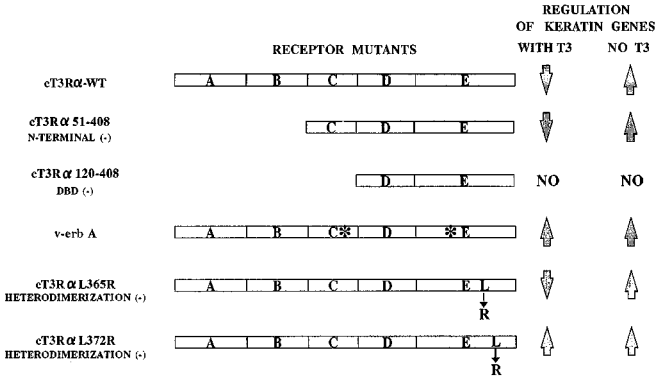

FIG. 9. **Summary of keratin gene regulation by various mutants of T3R.** *Asterisks* represent mutations and differences in sequence of v-ErbA *versus* cT3R, the wild type. Other mutations and deletions are indicated in the respective amino acid numbers.

ulation occurs via a direct interaction between RAR or T3R and keratin gene promoters.

Several different mechanisms of negative regulation by RAR and T3R have been described in literature. One mechanism involves blocking a positive transcription factor, such as AP1 (35–40). This mechanism is difficult to reconcile with the stimulation of keratin gene expression by the unliganded T3R. Furthermore, the negative regulation does not occur through blocking an AP1 binding site, because the K14 promoter does not appear to have an AP1 site and the apparent AP1 site in the K5 promoter is not required for inhibition by RAR and T3R (41, 42). A second mechanism of negative regulation by T3R was observed on the RSV-LTR in which T3 inhibits stimulation mediated by unliganded T3R (32). Negative regulation of the keratin genes differs from negative regulation of the RSV-LTR because ligand not only blocks induction by the unliganded receptor, but also suppresses basal expression by 5-fold. Furthermore, regulation of the RSV-LTR (32), but not of the keratin genes requires the A/B domains. Therefore, we conclude that regulation of keratin gene expression by RAR and T3R occurs through a distinct molecular mechanism.

To examine the molecular mechanisms through which regulation of keratin gene regulation occurs, we used several mutated T3Rs and tested their effects on regulation of keratin gene expression. These mutant T3Rs include complete deletions of the A and B domains, the DBD domain described above, as well as v-ErbA, a native variant of T3R, and point mutations in the ligand and heterodimerization domains. The results with these mutants are summarized in Fig. 9.

We were particularly interested in the constitutive activation mediated by unliganded T3R. In this paper, we have identified several characteristics that make this regulation novel and distinct. First, the constitutive activation elicited by unliganded T3R does not appear to be mediated through the binding of T3R/RXR heterodimers. Evidence from studies with ninth heptad mutants suggests that the activation of keratin genes is mediated by T3R homodimers. However, our gel mobility shift results suggest that the ligand-dependent inhibition may be mediated by T3R monomers.

Second, constitutive activation by cT3Rα appears to involve only a subset of the transactivation domains thought to be important for ligand-dependent transcriptional activation by T3R. Unlike the reduction in ligand-dependent transactivation by T3R on several other native response elements (22), activation of keratin genes by unliganded cT3Rα does not require the NH_2-terminal A/B domain of the receptor. This also further distinguishes activation of keratin gene expression from the regulation of RSV-LTR by unliganded cT3Rα, which requires the NH_2-terminal region for constitutive activation (32). In addition, v-ErbA also acts as a constitutive activator of keratin gene expression. This indicates that the putative transactivation domain that is deleted in v-ErbA at the COOH-terminal end of cT3Rα does not mediate constitutive activation (25). Thus constitutive activation may be mediated by another, so far unidentified, region of the receptor. This finding is consistent with the previous observation that cT3Rα(1–392), which lacks this putative activation domain, can constitutively activate the growth hormone or prolactin gene promoters in GH4C1 cells (26).

This novel mechanism of gene regulation may be particularly important in those tissues in which both T3 and RA play important roles determine the cell phenotype. While in some cells regulation that involves RXR integrates the response to hormones and vitamins, in the epidermis the response to each signal may need to be clearly distinct from responses to all other signals. If so, the RXR-independent regulation described here may provide the appropriate discrimination of signals reaching the epidermis. We expect, however, that this novel regulation operates in other systems as well.

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