Tissue-specific Stabilization of the Thyroid Hormone β1 Nuclear Receptor by Phosphorylation*

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Yuan-Tsang Ting, Manoj Kumar Bhat, Rosemary Wong‡, and Sheue-yann Cheng§

From the Laboratory of Molecular Biology, National Cancer Institute, and the ‡Molecular and Cellular Endocrinology Branch, NIDDK, National Institutes of Health, Bethesda, Maryland 20892-4255

The present study evaluated the expression and regulation of endogenous thyroid hormone receptors (TRs) in cultured cells. In COS-1 cells, the endogenous TR, subtype β1 (TRβ1), but not subtype β2 or α1, was induced to express by okadaic acid (OA) in a concentration-dependent manner. The induced TRβ1 had immunoreactivity and partial V8 proteolytic maps similar to those of the transfected and in vitro translated human TRβ1 (h-TRβ1). The OA-induced expression of endogenous TRβ1 was, however, not observed in a variety of other cultured cell lines tested, indicating that the induction was cell type-dependent. TRβ1 induced by OA was a multisite phosphorylated protein, in which serine and threonine in a ratio of 10:1 were phosphorylated. The induced TRβ1 was functional as it could mediate the thyroid hormone-dependent transcriptional activity via several thyroid hormone response elements. The induction of endogenous TRβ1 expression by OA was not accompanied by an increase in mRNA levels but was the result of an increase in the stability of the TRβ1 protein. This is the first report to indicate that one of the mechanisms by which the TR isoforms are differentially expressed is via the tissue-specific stabilization of the TR isoform proteins. Furthermore, this selective stability of TRβ1 could be conferred by phosphorylation.

Thyroid hormone nuclear receptors (TRs) are members of the steroid hormone/retinoic acid receptor superfamily. Two TR genes, TRα and TRβ, have been identified which are located on chromosome 17 and chromosome 3, respectively. Four isoforms, β1, β2, α1, and α2, are generated from each of two TR genes, α and β, by alternative splicing (1, 2). They are transcription factors that regulate the expression of target genes by interacting with specific DNA sequences known as thyroid hormone response elements (TREs) in the promoter region of target genes (1, 2). The transcriptional activity of TRs is not only dependent on thyroid hormone 3,3',5-triiodo-L-thyronine (T3) but also on the type of TRE. Recent studies have indicated that the transcriptional activity of TRs is further modulated via interaction with four groups of cellular proteins: (a) members of the nuclear receptor superfamily, notably the retinoid X receptors (1, 2); (b) corepressors including p270N-CoR (3), SMRT (4), TRIP (5), SHP (6), and TRACs (7); (c) coactivator SRC-1 (8); and (d) the tumor suppressor p53 (9). It is clear that regulation of the transcriptional activity of TRs is more complex than envisioned previously.

One important but less well studied mode of modulation of TR activity is the regulation of TR expression at the protein level. TRa1 and TRβ1 are differentially expressed at different developmental stages and at different levels in various tissues (10–12). Moreover, using the specific T3 binding activity and immunoreactivity as measures of the expressed receptor proteins, it was found that there are marked variations in the TR protein: mRNA ratios in different tissues (11, 12), suggesting isoform- and tissue-dependent posttranslational modification of TRs. However, it is unknown how TRs could be modulated at the protein level. We studied this problem by first using the cultured rat growth hormone-producing cell line GH3, which has long been used as a model cell line for studying thyroid hormone action (13). Unfortunately, preliminary experiments indicate that the anti-TR antibodies currently available are not sensitive enough to detect the endogenous TRs in GH3 cells either by immunoprecipitation or Western blotting. However, we subsequently discovered that in COS-1 cells, endogenous TRβ1 could be induced to express by okadaic acid (OA), a potent and specific inhibitor of serine/threonine phosphoprotein phosphatases 1 and 2A (14). COS-1 cells are known to be functionally deficient in TRs and have been used widely as recipient cells for gene transfer to study TR action. We therefore characterized the process of induction of TRβ1 by OA as we hoped to use COS-1 cells as a model cell line to understand the regulation of expression of endogenous TRs. Thus, in the present paper, we studied the molecular basis of the induction of endogenous TRβ1 expression and found that the OA-induced TRβ1 was a phosphoprotein possessing the same characteristics and functions as transfected human (h)-TRβ1 in mediating thyroid hormone-dependent transactivation. Moreover, the induction of endogenous TRβ1 protein expression was cell type-dependent in that it did not occur in GH3 (13), neuro-2A (16), human choriocarcinoma JEG-3, human epidermal carcinoma A431, mouse fibroblast NIH3T3, and the human cervical carcinoma HeLa cells. The increase in TRβ1 protein expression by OA in COS-1 cells was not accompanied by increases in mRNA level or in the rate of synthesis of TRβ1 protein. Rather, our results showed that the increase in TRβ1 protein expression by OA was the result of increased stability of the TRβ1 protein most probably via phosphorylation and defined a novel role of phosphorylation in regulating the levels of TRβ1 in different cell types.

Experimental Procedures

Immunoprecipitation—Monkey kidney COS-1 cells (8.2 × 10^6 cells/100-mm dish) were plated. Forty-eight h later, cells were preincubated in methionine or phosphate-free medium for 90 min and incubated with...
The lysed cell suspension was made isotonic by adding 1 ml of 0.5 M and for (27 times) through a 1-ml syringe with a 25 G needle. After labeling with either [35S]methionine or [32P]orthophosphate, labeled TRβ1 was immunoprecipitated with 5 μg of monoclonal antibody (mAb) C4 (lane 17) or J52 (18) as described by Lin et al. (16). Identical conditions were used for detection of endogenous TRβ1 in GH3, neuro-2a, JEG-3, A431, NIH3T3, and HeLa cell lines. Analysis of [35S]-labeled peptides by V8 Protease Digestion—Gel pre-electrophoresis (Fig. 1A) was performed, and the dried gel was autoradiographed.

Two-Dimensional Analysis of Phosphoamino Acids and Tryptic Mapping—Two-dimensional analysis of phosphoamino acids and tryptic mapping was carried out according to Ref. 20.

Binding of [125I]T3 to Isolated Nuclei—COS-1 cells (2 × 10^6 cells/15-cm dish) were cultured in the presence or absence of 100 nM OA at 37 °C for 3 h. Nuclei were isolated according to Samuels et al. (21) and Zhao and Padmanabha (22). [125I]T3 binding was carried out as described by Samuels et al. (21) and Koerner et al. (23). Briefly, cells were scraped in buffer H (20 mM Hepes, pH 7.5, 5 mM KCl, 0.5 mM MgCl2, and 0.5 mM dithiothreitol) with or without 100 nM OA, and cells were pelleted at 800 × g for 10 min at 4 °C. Cells were lysed by passing back and forth (27 times) through a 1-mm syringe with a 25 × 1.56-cm needle. The lysed cell suspension was made isotonic by adding 1 ml of 0.5 M sucrose, and the nuclei were pelleted at 1,200 × g for 10 min. The nuclei were washed twice in 2 ml of resuspending buffer B (0.32 M sucrose, 3 mM MgCl2, and 20 mM Tris, pH 7.8). The washed nuclei were incubated with 0.5 nM [125I]T3 in 200 μl of binding buffer B for 30 min at 37 °C. The binding was also carried out in the presence of 1 μM unlabeled T3 to determine the nonspecific binding. At the end of the incubation, an equal volume of 1% Triton X-100 was added, and the nuclei were pelleted. The nuclei were washed further with 1 ml of binding buffer B containing 0.5% Triton X-100, and the radioactivity bound to nuclei was determined in a γ-counter. The nuclear protein concentrations were determined by Coomasie Plus Protein Assay Reagent (Pierce Chemical Co.) using aliquots of nuclear suspension.

Determination of OA-induced Expression of TRβ1 on Transcription—COS-1 cells (3 × 10^6/100-mm dish) were plated 1 day before transfaction. The cells were transfected with 1.5 μg of a reporter gene containing two copies of the palindromic TRE in tandem (pTK28m) or the chicken lysozyme TRE (pTKLy) upstream of the chloramphenicol acetyltransferase gene. pBluescript plasmid (Stratagene, La Jolla, CA) was used to adjust the total DNA to 3 μg/dish. Various concentrations of OA were added to the cells 3 h before the cells were lysed for analysis of chloramphenicol acetyltransferase activity, which was normalized to protein concentration.

Northern Analysis—Total RNA was isolated from COS-1 cells cultured in the presence or absence of 100 nM OA (RNasea, Qiagen, Chatsworth, CA). Fifteen μg of RNA was electrophoresed on a 1% formaldehyde gel and blotted onto a nylon membrane, which was pre-hybridized at 68 °C with QuikHyb solution (Stratagene) for 1 h, followed by hybridization at 65 °C for 6 h with a radioactive rat TRβ1 cDNA probe prepared from a rat-TRβ1 expression plasmid (gift of Dr. R. Koenig, University of Michigan Medical Center). A human β-actin cDNA probe was also prepared as an internal control. The membrane was washed under stringent conditions with 0.2 × SSPE at 60 °C for 10 min. The labeled filters were analyzed by exposure to x-ray film, stripped, and reprobed with the β-actin cDNA probe.

Reverse Transcriptase-Polymerase Chain Reaction (PCR)—2 μg of total RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Clontech, Palo Alto, CA) after treatment with RNase-free DNase I (Boehringer Mannheim). 5–20 μl of the resultant 100-μl cDNA reaction was used for PCR to ensure linearity of reaction. Primers identical to sequences common to human and mouse TRβ1 were used to amplify a 378-base pair cDNA fragment. h-TRβ1 was also used as a template for PCR to serve as a control. PCR products were assessed by Southern analysis using the above rat TRβ1 cDNA probe.

Determination of the Stability of TRβ1 Protein—COS-1 cells were transfected with h-TRβ1 expression vector (pCLC51) and incubated in methionine-free medium in the presence or absence of OA. Cells were pulse labeled with 50 μCi of [35S]methionine for 30 min and chased for various time periods. Cell lysates were prepared, and immunoprecipitation was performed as described above.

RESULTS

Cell Type- and TR Isoform-dependent Induction of Expression of Endogenous TRβ1 Protein by OA—When COS-1 cells were treated with 250 nM OA, we found that a protein band with a molecular weight identical to that of transfected h-TRβ1 was indeed OA-stimulated in GH3, neuro-2a, JEG-3, A431, NIH3T3, and HeLa cell lines. Pulse-chase experiments with [35S]methionine (ICN Biomedicals, Inc., Costa Mesa, CA) or [32P]orthophosphate, labeled TRβ1 was immunoprecipitated with 5 μg of monoclonal antibody (mAb) C4 (lanes 3, 6, 9, 12, 15) or empty vector (lanes 2, 5, 8, 11, and 14) were labeled with 100 μCi [35S]methionine in the absence of OA (lanes 1–6) or in the presence of 250 nM OA (lanes 7–15). After lysis of cells, immunoprecipitation was carried out with mAb 52 (lanes 1–3, 7–9), mAb C4 (lanes 4–6, 10–12), a control antibody MOPC (lanes 13–15). Panel B, partial peptide maps of TRβ1. The gel slices containing immunoprecipitated endogenous TRβ1 (lane 3), transfected h-TRβ1 (lane 2), in vitro translated h-TRβ1 (lane 4), and the cytosolic thyroid hormone-binding protein, p58 protein (lane 1) were digested with S. aureus V8 protease according to "Experimental Procedures."
Regulation of the Expression of TRβ1

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Fig. 2. Panel A, effect of OA on the expression of endogenous TRβ1 in different cell types. Cells were labeled in the absence (odd numbered lanes) or presence of 100 nM OA (even numbered lanes), and the lysates were immunoprecipitated using mAb C4. Panel B, dose dependence of OA-induced TRβ1 expression. COS-1 cells were labeled as described in Fig. 1A either in the absence of OA (lane 1) or in the presence of 25, 50, 100, and 250 nM OA for lanes 2, 3, 4, and 5, respectively. Immunoprecipitation using mAb C4 was carried out as described in Fig. 1A.

V8 protease digestion. The peptide map of the immunoprecipitated [35S]methionine-labeled TRβ1 derived from OA-treated cells (Fig. 1A, lane 3) was compared with the maps obtained from either transfected h-TRβ1 (lane 2) or in vitro translated h-TRβ1 (lane 4). An identical pattern of four peptides, a, b, c and d, with molecular masses of 14, 12.5, 11, and 9 kDa, respectively, was seen in lanes 2–4. The weaker band (peptide a) and the stronger band (peptide d) seen in lane 3 compared with those in lanes 2 and 4 probably were due to more extensive proteolysis of peptide a to peptide d in TRβ1 derived from COS-1 cells. The differences in the protease susceptibility of peptide a could reflect the differences in local aggregated charge or structure surrounding the V8 cleavage sites due to minor differences in the primary sequences of human and monkey TRβ1. However, a completely different protein (p58, a cytosolic thyroid hormone-binding protein; 24) yielded a completely different peptide map, as shown in lane 1. Therefore, based on the immunoreactivity and peptide mapping, we have clearly established that in COS-1 cells, OA induced the expression of TRβ1.

mAb C4 recognizes both TRβ2 and TRα1 because of the common conserved epitope at the COOH terminus. However, examination of lanes 10 and 11 of Fig. 1A further shows that no protein band larger or smaller than TRβ1 (molecular mass ~55,000) was detected, indicating that neither TRβ2 (molecular mass ~58,000) nor TRα1 (molecular mass ~47,000) was induced to express by OA. To assess whether OA could also affect the expression of TRβ1 in other cell types, we similarly treated the cells derived from different tissues with OA. Fig. 2A shows that no OA-induced expression of TRβ1 was detected in GH3, neuro-2a, JEG-3, A431, NIH3T3, and HeLa cells. These results indicate that the induction of the expression of TRβ1 was TR isoform- and cell-type-dependent.

We further examined the effect of the concentration of OA, ranging from 25 to 250 nM, on the induction of TRβ1 in COS-1 cells. Lane 2 of Fig. 2B shows that at 25 nM, no TRβ1 was induced. At 50 nM (lane 3), a low level of TRβ1 was expressed, and a maximal level of TRβ1 was expressed at 100 nM of OA (lane 4). A further increase in the OA concentration (250 nM) led to a fall in the expression of TRβ1.

TRβ1 Induced by OA Was a Phosphoprotein—Previously we have shown that transfected h-TRβ1 is a phosphoprotein and that phosphorylation is enhanced by OA (16, 25). Therefore we asked the question of whether the induced TRβ1 was also a phosphoprotein. Fig. 3 shows that endogenous TRβ1 induced by OA was a phosphoprotein. The extent of phosphorylation was similar at 100 nM (lane 2 of Fig. 3) and 250 nM of OA (lane 3 of Fig. 3). Lane 4 is a negative control to indicate that when an irrelevant antibody was used, no phosphorylated TRβ1 was immunoprecipitated. However, it is important to point out that the TRβ1 induced at 250 nM OA was about 1/25 of that at 100 nM (see Fig. 2B). Therefore, the extent of phosphorylation of TRβ1 at 250 nM would be expected to be higher than that seen for TRβ1 induced at 100 nM OA (lane 3 versus lane 2 of Fig. 3). These results are consistent with earlier observations that phosphorylation of transfected h-TRβ1 was enhanced when the OA concentration was increased from 100 to 250 nM (16). Similar to the transfected h-TRβ1 (16), phosphorylated serine and threonine in a ratio of 10:1 were detected for the induced endogenous TRβ1 (data not shown). Two-dimensional tryptic mapping indicates one major phosphopeptide, corresponding to 60% of total counts, and five other minor phosphopeptides (data not shown), suggesting that multiple phosphorylation sites were present in phosphorylated TRβ1 induced by OA.

OA Increased T3 Binding and Enhanced Endogenous TRβ1-Mediated Transcription—Whether the TRβ1 induced by OA was functional was first assessed by T3 binding. Nuclei were isolated from COS-1 cells cultured in the presence or absence of OA and incubated with [125I]T3. As shown in Table I, the nuclei isolated from cells treated with OA had nearly 2-fold more T3 binding than those not treated with OA, indicating that OA-induced TRβ1 was functional as a T3 binder.

We further examined the transcriptional activity of OA-induced endogenous TRβ1. Two reporter genes, one containing palindromic TRE and the other containing chicken lysozyme TRE, were transfected into cells. As shown in Fig. 4, the T3-dependent transcriptional activity of TRβ1 was OA-dependent. An approximately 1.8- and 2.5-fold increase in the transcriptional activity on chicken lysozyme TRE was observed at 100 and 250 nM OA, respectively. On palindromic TRE, the increase was slightly lower (~1.5- and 1.8-fold increase at 100 and 250 nM, respectively). These findings are similar to that observed
Previously of a 2-fold increase in the transcriptional activity of the transfected TRβ1 by OA (17). Thus, the induced TRβ1 was functional, and its transcriptional activity was enhanced by phosphorylation.

**mRNA Level of TRβ1 Was Decreased by OA**—The induction of expression of TRβ1 protein by OA may occur at either the level of transcription or translation. To understand the molecular basis of the induction, we first determined the TRβ1 mRNA levels in cells treated with or without OA by Northern analysis. Although it was possible to detect TRβ1 mRNA from total RNA (Fig. 5A, lane 1) and mRNA (lane 2) prepared from control cells (GH3), no TRβ1 mRNA was detectable from total RNA (lanes 3 and 4) or mRNA (lanes 5 and 6) prepared from COS-1 cells in the presence (lanes 4 and 6) or absence (lanes 3 and 5) of OA. β-Actin mRNA was detectable in GH3 cells (lane 2 of Fig. 5B) and also in COS-1 cells either from total RNA (lanes 3 and 4 of Fig. 5B) or at a much greater abundance using the mRNA (lanes 5 and 6 of Fig. 5B), confirming the presence of intact RNA. Comparing the intensities of the β-actin bands in lanes 3 and 4 or 5 and 6, it can be seen that OA had no effect on the expression of β-actin mRNA.

In light of the inability to detect TRβ1 mRNA in COS-1 cells by Northern analysis, we performed reverse transcriptase-PCR, and the resultant cDNA was amplified by PCR and identified by Southern analysis. In preliminary experiments, we had first titrated the amounts of RNA so that RNA used in reverse transcriptase-PCR was in the linear range, and the resultant cDNA was capable of being compared quantitatively. Lane 1 of Fig. 5C shows that at 25 nM OA, TRβ1 mRNA was similar to that without OA treatment. Further increases in OA concentration led to a dose-dependent reduction of mRNA up to 100 nM, which then leveled off at 250 nM. Lanes 6–10 are controls to indicate that when no reverse transcription was carried out, no band

![Fig. 5. Panel A, detection of TRβ1 RNA in GH3 and COS-1 cells by Northern analysis. Total and poly(A)⁺ RNA from the following samples were loaded onto a 1.0% formaldehyde gel and probed with a rat TRβ1 cDNA probe: lane 1, GH3 total RNA (20 μg); lane 2, GH3 poly(A)⁺ RNA (10 μg); lane 3, COS-1 total RNA without OA (20 μg); lane 4, COS-1 total RNA with OA (20 μg); lane 5, COS-1 poly(A)⁺ without OA (20 μg); lane 6, COS-1 poly(A)⁺ with OA (20 μg). Hybridization was carried out to a rat TRβ1 cDNA probe, and autoradiography was performed for 9 h. Panel B, the blot from above was then stripped and reprobed with a β-actin cDNA probe. Panel C, effect of OA treatment on TRβ1 mRNA from COS-1 cells. 2 μg of total RNA prepared from COS-1 cells not treated with OA or treated with increasing concentrations of OA (25–250 nM) was reverse transcribed (RT). 15 μl of the resultant 100 μl of synthesized cDNA was taken for PCR after establishing that this volume was within the linear range for PCR. Lanes 1–5 show RNA samples treated with reverse transcriptase; lanes 6–10, without reverse transcriptase. Lane 11 shows the position of the amplified fragment from h-TR1 plasmid which was used for PCR. Southern analysis was then performed to detect the amplified cDNA.

![Table I: Comparison of [125I]T3 binding to isolated nuclei of COS-1 cells cultured in medium with or without OA](image)

| OA    | Specific [125I]T3 bound | fmol/50 μg nuclear proteins |
|-------|-------------------------|-----------------------------|
| −     | 0.34 ± 0.06             |                             |
| +     | 0.58 ± 0.07             |                             |

![Fig. 4. Regulation of endogenous TRβ1-mediated transcription by OA. COS-1 cells (3 × 10⁶ cells/60-mm dish) were transfected with pTK28m (open bars; palindromic TRE or Pal) or pTKLys (shaded bars, chicken lysozyme TRE, or Lys). 24 h later, cells were treated with medium containing thyroid hormone-depleted serum with or without 100 nM T3 for an additional 24 h. In the last 3 h, various concentrations of OA as indicated were added to the medium. Cells were lysed, and chloramphenicol acetyltransferase (CAT) activity was measured. Data are the average of three experiments each with duplicates (mean ± S.D., n = 3).](image)

![Fig. 3. A, TRβ1 mRNA was detectable from total RNA (lanes 3 and 4) or mRNA (lanes 5 and 6) prepared from COS-1 cells in the presence (lanes 4 and 6) or absence (lanes 3 and 5) of OA. β-Actin mRNA was detectable in GH3 cells (lane 2 of Fig. 5B) and also in COS-1 cells either from total RNA (lanes 3 and 4 of Fig. 5B) or at a much greater abundance using the mRNA (lanes 5 and 6 of Fig. 5B), confirming the presence of intact RNA. Comparing the intensities of the β-actin bands in lanes 3 and 4 or 5 and 6, it can be seen that OA had no effect on the expression of β-actin mRNA.](image)
was detected. These results indicate that OA affected the expression of endogenous TRβ1 at the mRNA level.

OA Increased the Stability of TRβ1 Protein—Because the expression level of endogenous TRβ1 was virtually nondetectable under the present experimental conditions (Fig. 1A and lane 1 of Fig. 2A), it was not possible to compare the effect of OA on the expression of endogenous TRβ1 at the translation level. However, since we have shown that the characteristics and functions of endogenous TRβ1 were indistinguishable from those of transfected TRβ1 (see above), we used transfected TRβ1 to study the effect of OA on the stability of TRβ1. The stability of TRβ1 was examined by a brief pulse with [35S]methionine followed by a chase for different lengths of time in the presence (Fig. 6A, lanes 8–14) or absence of 100 nM OA (lanes 1–7). Comparison of the intensities of labeled TRβ1 at each time point indicates that TRβ1 from cultures in the presence of OA was more stable than without OA treatment. Quantitation analyses indicate that $t_{1/2}$ of TRβ1 was $-1.88 \pm 0.07$ and $0.96 \pm 0.02$ h (Fig. 6B) in the presence of OA or absence of OA, respectively, indicating that treatment of cells with OA led to a 2-fold increase in the stability of TRβ1.

We further examined the effect of OA on the rate of synthesis of transfected TRβ1 by labeling cells with [35S]methionine for various lengths of time in the presence (Fig. 6C, lanes 7–12) or absence (lanes 1–6) of OA. The intensities of bands were quantified, and the rates of synthesis were calculated to be $3,546 \pm 607$ and $3,402 \pm 250$ counts/min for TRβ1 synthesized in the absence or presence of OA, respectively. These data indicate that OA had no effect on the rate of synthesis of TRβ1, suggesting that the increased stability of the TRβ1 protein was the major factor responsible for the OA induction of the TRβ1 protein.

DISCUSSION

The mRNAs of the TR isoforms and their encoded receptor proteins are known to be expressed differentially in a tissue-specific manner. The ratios of the mRNA versus the protein levels vary widely from tissue to tissue (11, 12). Moreover, the timing of the appearance of the various TR isoforms and the ratios of these isoforms are regulated tightly during development (10, 11). However, the mechanisms underlying this tissue-specific and developmental regulation of TRs are largely unknown. In the present study we discovered that the expression of endogenous TRβ1 protein, but not TRβ2 or TRα1, could be induced by OA in COS-1 cells, which are believed to be functionally deficient in response to T₃. However, this induction is unique among the cell lines examined including T₃ functionally competent GH3 cells (13) and neuro-2a cells (15) and T₃ functionally deficient cells (JEG-3, NIH3T3, HeLa, and A431 cells). Therefore, our findings highlight a potentially novel mechanism explaining the tissue-specific expression of TR isoforms.

Our data indicate that the molecular mechanisms of the induction of TRβ1 expression by OA were complex. The induction was mediated at both the transcriptional and translational levels. The amounts of mRNA of TRβ1 were reduced as the concentration of OA was increased. However, paradoxically, although OA treatment led to a reduction of mRNA, the TRβ1 proteins were increased by OA except at a high concentration of 250 nM. The sharp increase in the level of TRβ1 protein from 50 to 100 nM (Fig. 2B) was not proportional to the corresponding decrease in mRNA (Fig. 5C), reminiscent of what has been observed in the in vivo studies reported by Schwartz et al. (12) in that there are large variations in the mRNA:TR protein ratios, suggesting additional regulation at the posttranslational level. Indeed, we found that although OA had no effect on the synthesis of TRβ1, OA increased its stability by nearly 2-fold. Therefore, the increase in the stability of TRβ1 plays a major role in the function of TRs. This is the first clear report demonstrating that one of the mechanisms of the regulation of TR expression is at the posttranslational level.

The reasons for the increased stability of TRβ1 protein by OA are not entirely clear. OA is an inhibitor of phosphoprotein phosphatase 1 and 2A and has been used widely as a tool to study the functions of phosphorylation in cellular processes (14). We have demonstrated that OA not only induced TRβ1 expression but also increased dramatically the extent of phosphorylation. The induced TRβ1 was a phosphoprotein that was phosphorylated at multiple Ser or/and Thr residues by the treatment of cells with OA could change the conformation of TRβ1, thereby decreasing its susceptibility to proteolytic degradation. Phosphorylation-induced structural modification is not without precedent. Structural changes in glycogen phosphorylase have been studied by x-ray crystallography (26). As a result of phosphorylation of Ser-14, the interaction between subunits is strengthened, and the binding sites for allosteric effectors and substrates are altered (26). However, conformational changes...
are not obligatory for enhanced susceptibility of TRβ1 to proteolytic degradation. The increased phosphorylation of Ser or Thr sites could alter the interaction of TRβ1 with cellular proteases, thus altering the kinetics of degradation. Alteration of the stability of proteins by phosphorylation has been demonstrated directly for human IκB-α (27), nicotinic acetylcholine receptors (28), and the Drosophila yan protein (29). Similar to the present studies of TRβ1, the changes in the stability of these proteins led to functional consequences (27–29).

Phosphorylation of TRs is not only limited to the α1 subtype. Chicken TRα1 (30, 31) and rat TRα2 (32) were also found to be phosphorylated. Therefore the different extents of phosphorylation at different sites in TR isoforms could affect their stability differently. The present study thus raises the interesting possibility that variations in the protein stability of TRβ1 may be one mechanism by which the tissue-specific and development-specific expression of the TR isoforms are regulated. Although it remains to be established that the stabilities of other TR isoforms are regulated by phosphorylation, what is clear from our study is that differential phosphorylation of TR isoforms in different cell types adds yet another level of fine tuning to the already multifaceted and complex mechanisms of regulation of TR-dependent gene transcription.

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