Chicken thyroid hormone receptor α requires the N-terminal amino acids for exclusive nuclear localization

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Abstract The subcellular localization of natural and engineered forms of the chicken thyroid hormone receptor (cTRα) is dependent on amino acids encoded in the N-terminal region. The full length receptor protein, cTRα-p46, was found to localize exclusively to the nucleus, whereas the N-terminally shorter variant, cTRα-p40, localizes to both the nucleus and the cytoplasm. The exclusive nuclear localization of cTRα-p46 is dependent on the presence of the first 11 N-terminal amino acids, but independent of the phosphorylation of the serine at position 12. Our data identify a novel role for an N-terminal domain of the full length thyroid hormone receptor.

Key words: Thyroid hormone receptor; Subcellular localization; P75$^{gag-v-erbA}$; Isoform; N-terminus; Chicken

1. Introduction

Steroid and thyroid hormone receptors function as ligand dependent transcription factors through binding to enhancer sequences that regulate transcription of target genes [1–4]. These receptors have functional domains in common: a ligand binding domain (LBD), a DNA binding domain (DBD), and activation function domain(s) (for review see [5]). The receptors for the steroids, i.e. glucocorticoid, progesterone and mineral corticoid, interact with cytosolic heat shock proteins which dissociate from the receptor in presence of hormone ligand [6]. Heat shock proteins are, however, not associated with the other subgroup of hormone receptors such as the thyroid hormone receptor (TR), the retinoid receptors and the vitamin D3 receptor [7]. Unlike the other steroid receptors, the estrogen receptor (ER) does not require hormone for nuclear transport [8,9]. The TR as well as the ER are constitutively localized to the nucleus and are thus independent of ligand for nuclear localization [10].

The nuclear localization of proteins is mediated through a conserved nuclear localization signal (NLS) that allows them to bind transporter molecules in the cytoplasm or at the nuclear pore [11,12]. Consensus NLS sequences have been defined, and consist of a short stretch of arginine and lysine residues [13,14]. The NLS sequences are sufficient for conferring a nuclear localization on most proteins; however, the glucocorticoid receptor (GR) needs additional signals, i.e. hormone binding and phosphorylation [15]. Thus, the mechanism by which nuclear transcription factors expose their NLS sequences may involve modifications of the receptors such as phosphorylation, dephosphorylation or binding of ligand [16,17].

The chicken TRα receptor mRNA produces two proteins due to two translational initiation sites in the 5′ untranslated region. A 46 kDa protein is made when the first AUG is used (cTRα-p46), whereas initiation at the second AUG yields a protein of 40 kDa (cTRα-p40), 36 amino acids shorter. Both proteins bind T_3, heterodimerize with the retinoic X receptor (RXR), and transactivate target genes. The N-terminal region (amino acids 1–36) of the cTRα-p46 contain an N-terminal activating function domain (AF-1) [18], and harbors two phosphorylation sites, one at serine residue 12 and another at serine 28/29. The serine residue 12 is a phosphorylation site for casein kinase II (CKII) [19] whereas the serine residues 28/29 are defined as a protein kinase A (PKA) phosphorylation site [20]. The aforementioned N-terminal features are absent in cTRα-p40. Both receptor forms have been found in vivo [21].

To elucidate the role of the sequences outside the NLS in subcellular localization we have studied the localization of naturally occurring and engineered forms of cTRα. Our data show that exclusive nuclear localization of TR is dependent on a short N-terminal domain consisting of 11 amino acids. The difference in partitioning of the two N-terminal variant receptors indicates that the two isoforms may operate in part through distinct mechanisms.

2. Materials and methods

2.1. Expression vectors

The pSG5 vector was used for expression of receptor proteins in transfected cells and in *Xenopus* oocytes. To express TRα-p46 and cTRα-p40 individually, we used two plasmids expressing one isoform only. The AUG for the smaller protein was replaced with a codon for leucine allowing expressing of TRα-p46, and to express the cTRα-p40 protein the first initiation codon of the cDNA was removed [22]. The receptor plasmids expressing cTRα, TRβ0, P75$^{gag-v-erbA}$ or pSG-V3 have been described [23–27]. A plasmid containing part of the retroviral gag region (nucleotides 1597–2243) of pSFCVneo [28] fused to the full length cDNA of cTRα (pAXΔgagTR) was subcloned into the *Eco*RI sites of pSG5 (gagTRα). The cTRα-A12 mutant [19] receptor fragment was taken from the plasmid pNEO-MAV-c-erbA A12 [29] and inserted into *Eco*RI sites of pSG5.

2.2. Cells and transfections

5×10^4 JEG-3 human choriocarcinoma cells [25] were seeded onto 12 mm coverslips in 24 well plates or into tissue culture chambers (Nunc) containing Dulbecco's medium with 8% fetal calf serum. The medium was changed the following day and transfections were performed as described [30]. Cells used for immunocytochemistry were transfected with 0.03–3 μg of receptor plasmid DNA. 7×10^4 QT6 cells [28] were seeded in 6 well plates and cells were transfected the next day with 500 ng of receptor plasmid per well. Twenty-four hours post transfection the cells were harvested and total extract was taken up in 20 μl 250 mM Tris pH 7.8. 5 μl of extract was loaded on a 10% SDS gel for Western blot analysis.

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2.3. Immunocytochemistry

Forty-eight hours after transfection cells were washed with PBS and treated with serial dilutions of methanol followed by fixation in 4% paraformaldehyde in PBS at +4°C for 2–16 h. After three washes with PBS, the coverslips were transferred to a rack with the cell side up. Cells were incubated with 0.3% normal goat serum (NGS), 0.2% bovine serum albumin (BSA) in PBS for 10 min; NGS in BSA/PBS+0.3% Triton X-100 for 5 min followed by 1% H_2O_2 in BSA/PBS for 5 min. After three washes the cells were incubated with primary antibody in a humidified chamber at +4°C overnight. A rabbit polyclonal cTRβ antiserum recognizing cTRα, cTRβ and $P75^{gag-v-erbA}$ [25] and a control antibody recognizing snRNP [31] were used at dilutions of 1:200 and 1:10 respectively. After three washes in PBS cells were incubated with biotin-coupled goat anti-rabbit IgG or anti-mouse IgM secondary antibody (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) for 45 min. Following washes, the cells were incubated with ABC reagent (avidin conjugated to horseradish peroxidase) for 45 min, and the immunoreaction was visualized by adding diaminobenzidine (DAB) chromogen substrate (Dako Corporation, Carpinteria, CA) in 0.1% H_2O_2. The coverslips were washed in H_2O and then dipped in ethanol followed by mounting onto glass slides for microscopy. Stained cells were photographed with 64 ASA Tungsten color film in microscope with a 100× objective. Slides were scanned into a computer and the picture panels were designed using Adobe Photoshop.

2.4. Sequence homologies and protein secondary structure prediction

Homology searches were done in the EMBL and SwissProt data banks. The secondary structure of the first 50 amino acids of cTRα-p46 was predicted using the Predict Protein program, EMBL.

2.5. Oocyte injection

50–250 ng receptor DNA in pSG5 was injected into *Xenopus* eggs. The next day the oocyte nucleus was extracted in 5 μl and applied in a 10% SDS gel in parallel with the extract from the cytoplasmic fraction taken up in 20 μl. Proteins were detected with anti-TRα [25] in Western blot.

2.6. Western blot

Total extracts from QT6 cells or from single oocytes were loaded onto 10% SDS-PAGE gels. Gels were run with 100 V for 2 h and proteins were then transferred to nitrocellulose filters (PVDF Transfer Membrane, DuPont) using semi-dry transfer. After 1 h in blocking buffer, the filter was incubated with the primary antibody on a rocking platform in +4°C overnight. A washing step was followed by adding the secondary antibody for 30 min at room temperature. After subsequent washing steps the filter was subjected to ECL treatment (Amersham) followed by exposure to X-ray films (Kodak).

3. Results

3.1. Cytoplasmic and nuclear localization of cTRα

As a result of utilizing different AUG codons in the 5′ untranslated region, the mRNA for cTRα yields two different

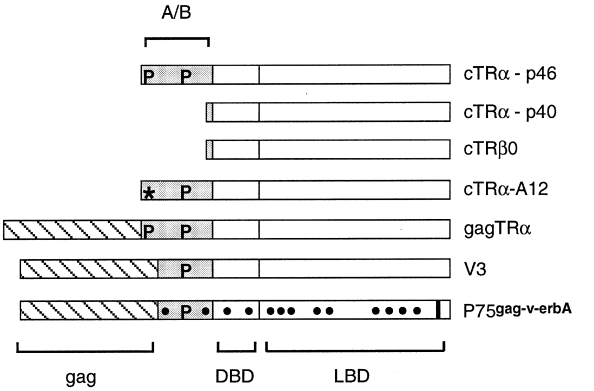

Fig. 1. Thyroid hormone receptors. The cTRα-p46 and cTRα-p40 isoforms are naturally occurring receptors. The shorter isoform lacks the first 36 amino acid residues of cTRα-p46. Variant cTRα receptors are: cTRα-A12 containing a serine to alanine mutation at residue 12 of cTRα-p46; gagTRα, containing a portion of the retroviral gag gene fused to cTRα-p46; $P75^{gag-v-erbA}$, the oncogenic homologue of cTRα, which lacks the 12 first amino acids of the N-terminus as well as the last nine amino acids in the C-terminus and also contains 13 amino acid substitutions; V3, containing the $P75^{gag-v-erbA}$ N-terminal gag domain fused to cTRα-p46 at residue 13. cTRβ has two N-terminal isoforms and shown here is the short isoform, cTRβ0. White colored boxes indicate the ligand binding domain (LBD) and the DNA binding domain (DBD). The N-terminus is indicated with light shading. The positions of point mutations (black dots), phosphorylation sites (P) and mutated phosphorylated site (*) are indicated.

receptor proteins, cTRα-p46 and cTRα-p40 (see Fig. 1). The latter protein is more abundant in vivo [21]. Our initial immunocytochemical analyses demonstrated that cells transfected with a cDNA allowing both receptors to be expressed contained receptor protein both in the nucleus and in the cytoplasm (not shown). To determine the identity of the cytoplasmic receptor, we transfected cells with plasmids expressing either the long or the short isoform [22]. Subsequent immunocytochemical analysis show that the cTRα-p46 isoform localized exclusively to the nucleus (Fig. 2a), whereas cTRα-p40 partitioned to both the nucleus and the cytoplasm (Fig. 2b). This localization pattern was fully reproduced in all cells studied (Table 1).

To verify the above results with another experimental approach, we injected 8–10 *Xenopus* oocytes with constructs expressing cTRα-p40 or cTRα-p46. A Western blot analysis of the nuclear and cytoplasmic fractions from single oocytes is shown in Fig. 3. The results demonstrate that close to 100% of

Table 1
cTRα and variant receptor subcellular distribution

Receptor	Cells with		Number of cells counted	% cells with exclusive nuclear localization
	exclusive nuclear localization	cytoplasmic and nuclear localization		
TRα-p46	29	1	30	97
TRα-p40	4	29	33	12
TRβ0	5	27	32	16
$P75^{gag-v-erbA}$	0	30	30	0
V3	3	24	27	11
gagTR	28	2	30	93
TRα-p46	98	2	100	98
TRα-A12	77	23	100	77

Cells were transfected with cTRα variant expression plasmids and stained cells were counted. Numbers are averages of three independent experiments.

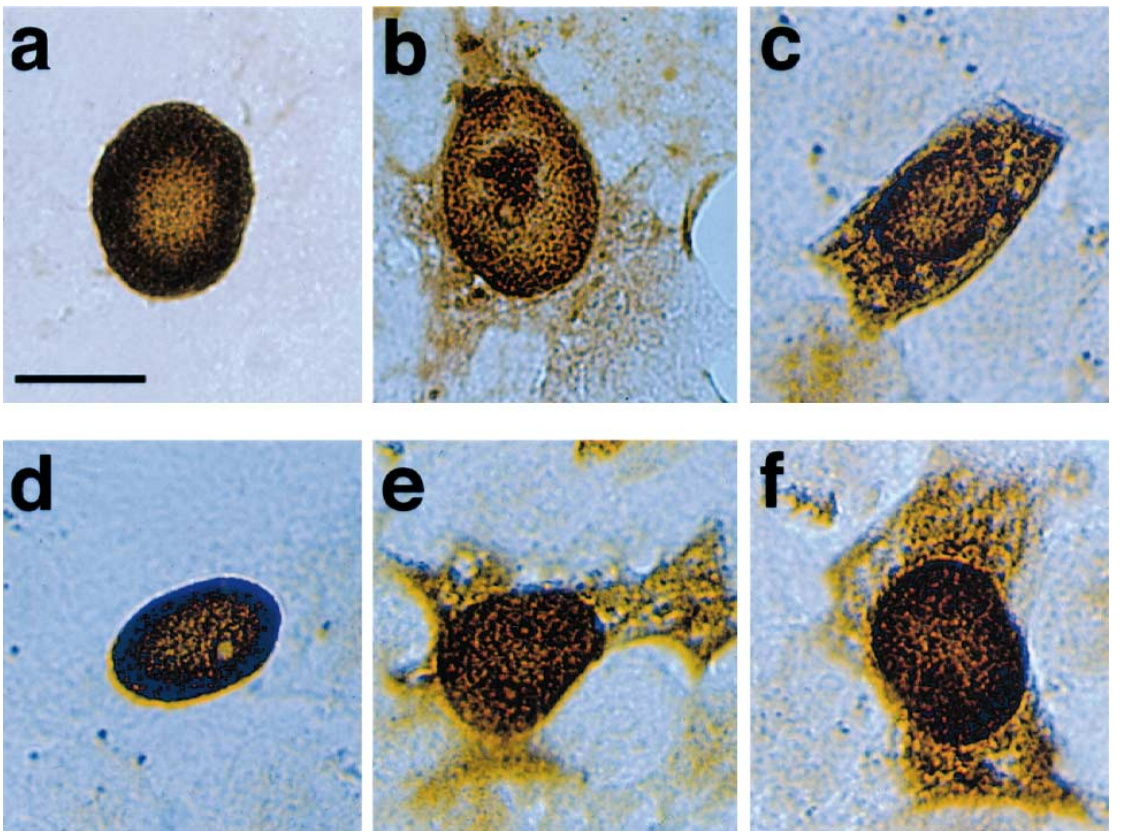

Fig. 2. Subcellular localization of cTR isoforms as shown by immunocytochemistry. Images of representative cells from the experiment quantified in Table 1 are shown. Transfected JEG cells were grown in the presence (a,b,c) or absence (d,e,f) of thyroid hormone. Cells were transfected with cTRα-p46 cDNA (a and d), with cTRα-p40 cDNA (b and e), or with cTRβ0 cDNA (c and f). Size bar: 10 μm.

cTRα-p46 was found in the nucleus, whereas the nuclear to cytoplasmic ratio in the oocyte expressing cTRα-p40 was approximately 50%. Similar results were obtained with the remaining oocytes, and no signal was detected in extracts from oocytes injected with an empty plasmid vector.

The cTRβ0 isoform (Fig. 1) is encoded by a distinct gene and has an overall high homology with cTRα-p40. However, their 14 amino acids preceding these DBDs are identical [32]. Our analyses show that cTRβ0 has nuclear translocation properties similar to those of cTRα-p40, since it partitioned to both cellular compartments (Fig. 2c). We conclude that amino acids in the N-terminal region of cTRα-p46 but absent in cTRα-p40 and cTRβ0 confer the exclusive nuclear localization.

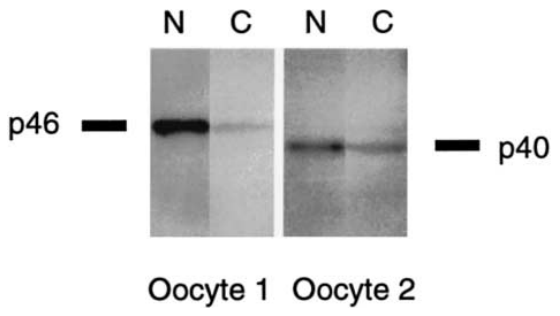

Fig. 3. Localization of cTRα isoforms in single microinjected *Xenopus* oocytes. 8–10 oocytes were injected with plasmid expressing the indicated cTRα isoform. The nuclear (N) and cytoplasmic (C) fractions from single, representative, oocytes were analyzed by Western blot.

To test if receptor protein localization was an effect of the amount of DNA added in transfection and if increased amounts of receptor plasmid could saturate the expression in the nucleus, transfections were done with increasing amounts of DNA, ranging from 30 ng to 3 μg per well. The results showed that increased amounts of plasmid did not alter the distribution of the receptor (not shown). This suggests that the cells were not saturated with receptor protein at the highest DNA plasmid concentrations used, and that leakage from the nuclear compartment to the cytosol was unlikely.

The above experiments were done in media with normal fetal calf serum. Experiments done in media depleted of thyroid hormone showed that absence of ligand does not affect the localization of TRs in the cell (Fig. 2d,e,f).

3.2. Subcellular localization of alternative forms of TRs

The viral homologue of cTRα, $P75^{gag-v-erbA}$, differs from cTRα-p46 in a number of ways [23]. As a consequence of an N-terminal fusion with retroviral gag sequences, P75 lacks the first 12 amino acids of cTRα-p46 (Fig. 1). In addition, the viral protein lacks 9 amino acids close to the C-terminus and has sustained 13 amino acids distributed along the molecule. $P75^{gag-v-erbA}$ has been shown to be localized both in the nucleus and in the cytoplasm [33]. To establish how the structural changes in the $P75^{gag-v-erbA}$ determine the localization of the receptor we first tested $P75^{gag-v-erbA}$ by immunocytochemistry. Fig. 4a shows that the viral protein localizes prom-

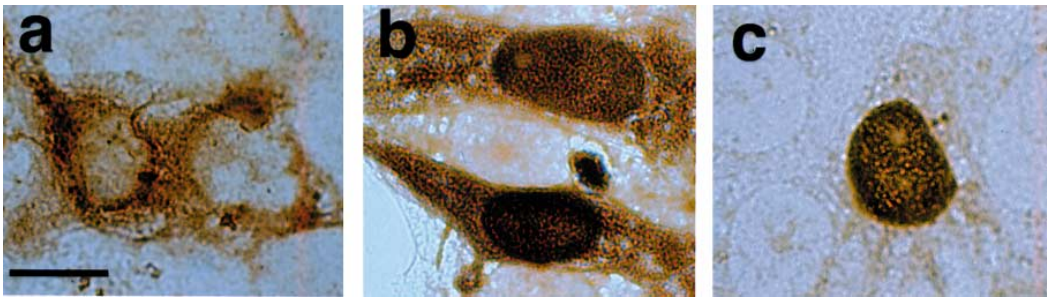

Fig. 4. Subcellular localization of the cTR variants $P75^{gag-v-erbA}$ (a), V3 (b) and gagTRα (c). Transfected cells were analyzed by immunocytochemistry. Size bar: 10 μm.

inently to the cytoplasm, although nuclear staining was evident as well. This observation is in agreement with the biochemical results obtained previously [33]. To further study the influence of the N-terminal deletion, we used a chimeric receptor V3 (Fig. 1) which contains the gag region of $P75^{gag-v-erbA}$ fused to cTRα-p46 at residue 13 as in $P75^{gag-v-erbA}$ [27]. Cells transfected with this construct contained immunoreactive protein in both the cytoplasm and the nucleus (Fig. 4b). The observation that $P75^{gag-v-erbA}$ and the V3 receptor partitioned to both compartments (Table 1) suggests that this property is due to the lack of the first 12 amino acid residues present in cTRα-p46, or to the presence of gag sequences. To exclude that the gag sequences did not influence the subcellular localization we transfected a construct expressing the full length cTRα-p46 fused to gag sequences. The resulting gagTRα protein was found exclusively in the nucleus (Fig. 4c), suggesting that a fusion with gag does not influence nuclear localization of cTRα-p46, and that the first 12 amino acids of TR are critical for exclusive nuclear localization.

3.3. Serine to alanine mutation of residue 12 in TRα-p46

The observation that all the receptor proteins that lack the first 12 amino acids (cTRα-p40, cTRβ0, $P75^{gag-v-erbA}$ and V3) localized to both cellular compartments led us to suspect that the serine CKII site at position 12 is necessary for complete nuclear transfer. To test this we used a plasmid expressing a mutant receptor that contains an alanine residue at the site of serine 12 (cTRα-A12). Subsequent analysis showed that most of the cells expressing cTRα-A12 localized to the nucleus and

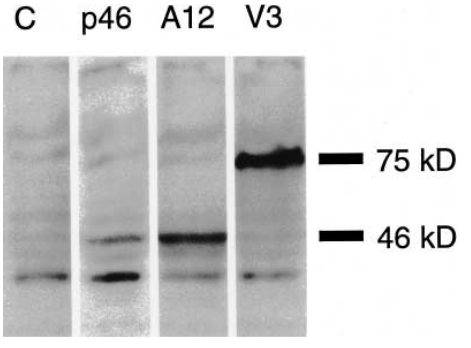

Fig. 5. Detection of intact receptors in transfected cells. Protein extracts were separated on SDS-PAGE, and analyzed by Western blot. The control lane (C) contains extract from cells transfected with an empty expression vector. cTRα-p46 and cTRα-A12 have molecular weights of 46 kDa and the weight of V3 is 75 kDa.

that a minor portion of the cells expressed this receptor in both compartments (Table 1). This demonstrates that a serine to alanine exchange at residue 12 in cTRα-p46 does not significantly alter the expression pattern in most cells, and that the serine in the CKII phosphorylation site is not essential for exclusive nuclear localization of the receptor.

To verify the integrity of the receptor proteins we performed Western blot analysis of the whole cell extracts of cells transfected with vectors expressing cTRα-p46, cTRα-A12 or V3. Fig. 5 shows that all three receptor proteins were intact.

4. Discussion

Previous reports have identified an important nuclear translocation sequence signal in the 'hinge' domain, C-terminal to the DNA binding region [34]. However, the data concerning the subcellular localization of cTRα and its oncogenic variant $P75^{gag-v-erbA}$ have been conflicting [23,33]. We therefore elucidated further the requirements for subcellular localization of thyroid hormone receptors and their variants. Our results show that the ultimate N-terminal domain contains a novel domain that confers exclusive nuclear localization (ENL) to cTRα-p46, and that it is absent in shorter TR isoforms and in the oncoprotein $P75^{gag-v-erbA}$.

Proteins that are transported to the nucleus usually contain a basic stretch of amino acids referred to as the nuclear localization signal, NLS. While the prototype NLS [14] consists of five basic amino acids, the glucocorticoid receptor has two such signals, one in the hinge region (region D) and one in the ligand binding domain [15]. In contrast, the hinge region of TR contains a single NLS that was reported to be sufficient for exclusive nuclear localization since it was transferable to an unrelated cytoplasmic protein [35]. Our data indicate that the ENL complements the function of NLS, since it confers exclusive nuclear localization to an intact, NLS containing receptor.

In cTRα the long and the short isoforms are identical in their amino acid sequence except for their N-terminal regions. Sequence alignment of N-terminal regions of TRα from different species shows that the region is considerably conserved (Fig. 6). Two characteristic domains are located within the first 36 amino acid residues. The first one, containing mainly acid acids at position 1–16, encompasses the first 11 amino acids required for exclusive nuclear localization and the phosphorylation site at position 12. This region may form a loop that is involved in forming binding sites, possibly to aid in interaction with other proteins [18], i.e. basal transcription factors or factors that enhance nuclear localization. The sec-

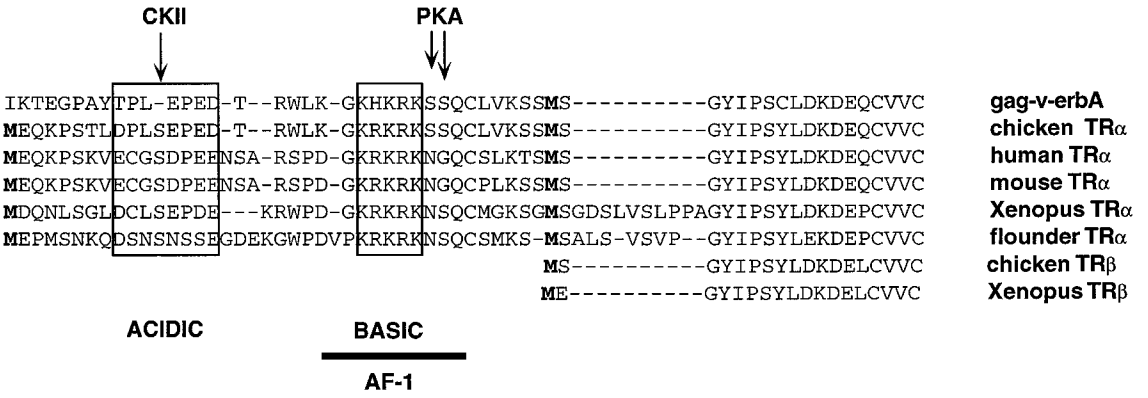

Fig. 6. Alignment of N-terminal sequences in TRα from different species. The acidic and basic regions are boxed. Residues 21–30 are essential for transcriptional activation function 1 [18], indicated by a solid bar. The PKA and CKII phosphorylation sites are indicated.

ond region, amino acids 21–30, is basic and constitutes the AF-1 domain [18]. The PKA site at position 28/29 is conserved only in receptors from birds and cold-blooded vertebrates. It is tempting to speculate that cTRα-p40 contains, in addition to the NLS, a nuclear export signal, whereas cTRα-p46 in addition contains the ENL that counteracts the export.

That TR isoforms, lacking N-terminal amino acid residues, localize to both the nucleus and the cytoplasm shows that nuclear import is independent of phosphorylation in the N-terminus, a conclusion supported by our observation that the serine 12 phosphorylation site is dispensable for subcellular localization. We cannot, however, easily determine the reason for the cytoplasmic localization, although several possibilities can be suggested. Other post-translational modifications could be a prerequisite for efficient nuclear import, or the lack of such modifications could result in poor nuclear retention [36]. Such a modification that localizes a variant receptor to the cytoplasm may cause an inefficient import through the nuclear pore leaving a portion of receptors in the cytoplasm; or may result in an accelerated export of the receptors. As some of the variant TRs that lack the first amino acid residues from cTRα-p46 are large fusion proteins, we exclude the possibility that the cytoplasmic localization is a result of diffusion caused by a smaller size. Recently, Dobbelstein et al. demonstrated that two adenovirus proteins modify nuclear export and import [36]. Intrinsic signals that direct a protein into the cytoplasm [17] have not been found in the TR molecule; moreover, our experiments suggest that the energy dependent nuclear shuttling mechanism described for the progesterone receptor (PR) [37,38] is not recruited by cTRα-p46 (data not shown).

The biological significance of expressing the two N-terminal variants cTRα-p46 and cTRα-p40 in a cell is unclear. In the PR, the N-terminal region is important for tissue target gene specificity [39]. A short N-terminal isoform of PR, expressed in most cells, activates transcription of target genes in some cell types but differs from the full length PR in its function as a repressor in other tissue types [40]. The TR p40 isoform is abundantly expressed in chicken embryonic erythroid cells [21], and it functions as a T_3 dependent transcription factor via thyroid hormone receptor response elements [22,41]. Hadzik and collaborators have shown that cTRα-p46 in HeLa cells enhances transcription more efficiently than cTRα-p40 due to its N-terminal AF-1 domain [18]. It is thus likely that the transactivation domain unique to cTRα-p46 strongly activates or represses certain target genes, perhaps in a tissue specific manner. The short isoforms of either TRα or TRβ would accordingly have a lower transactivation potential and perhaps fewer gene specific activities. Another alternative is provided by reports that suggest a cytoplasmic or mitochondrial function for thyroid hormones [42,43].

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References

[1] Evans, R.M. (1988) Science 240, 889–895.
[2] Carson-Jurica, M.A., Schrader, W.T. and O'Malley, B.W. (1990) Endocr. Rev. 11, 201–220.
[3] Laudet, V., Hänni, C., Coll, J., Catzeflis, F. and Stéhelin, D. (1992) EMBO J. 11, 1003–1013.
[4] Tjian, R. and Maniatis, T. (1994) Cell 77, 5–8.
[5] Mangelsdorf, D.J. et al. (1995) Cell 83, 835–839.
[6] Picard, D., Salser, S.J. and Yamamoto, K.R. (1988) Cell 54, 1073–1080.
[7] Dalman, F.C., Koening, R.J., Perdew, G.H., Massa, E. and Pratt, W.B. (1990) J. Biol. Chem. 265, 3615–3618.
[8] Wrenn, C.K. and Katzenellenbogen, B.S. (1990) Mol. Endocrinol. 4, 1647–1654.
[9] Picard, D.R., Kumar, V., Chambon, P. and Yamamoto, K. (1990) Cell. Regul. 1, 291–299.
[10] Spindler, B.J., MacLeod, K.M., Ring, J. and Baxter, J.D. (1975) J. Biol. Chem. 250, 4113–4119.
[11] Newmeyer, D. (1993) Curr. Opin. Cell Biol. 5, 395–407.
[12] Rexach, M. and Blobel, G. (1995) Cell 83, 683–692.
[13] Boulikas, T. (1993) Crit. Rev. Eukaryot. Gene Express. 3, 193–227.
[14] Kalderon, D., Roberts, B.L., Richardson, W.D. and Smith, A.E. (1984) Cell 39, 499–509.
[15] Picard, D.R. and Yamamoto, K.E. (1987) EMBO J. 6, 3333–3340.
[16] Rihs, H.P., Jans, D.A., Fan, H. and Peters, R. (1991) EMBO J. 10, 633–639.
[17] Gerace, L. (1995) Cell 82, 341–344.
[18] Hadzic, E. et al. (1995) Mol. Cell. Biol. 15, 4507–4517.
[19] Glineur, C., Bailly, M. and Ghysdael, J. (1989) Oncogene 4, 1247–1254.
[20] Goldberg, Y., Glineur, C., Gesquière, J.-C., Ricouart, A., Sap, J., Vennström, B. and Ghysdael, J. (1988) EMBO J. 7, 2425–2433.

[21] Bigler, J. and Eisenman, R.N. (1988) Mol. Cell. Biol. 8, 4155–4161.
[22] Wahlström, G.M., Sjöberg, M., Andersson, M.L., Nordström, K. and Vennström, B. (1992) Mol. Endocrinol. 6, 1013–1022.
[23] Sap, J., Munoz, A., Damm, K., Goldberg, Y., Ghysdael, J., Leutz, A., Beug, H. and Vennström, B. (1986) Nature 324, 635–640.
[24] Barettino, D., Vivanco Ruiz, M.M. and Stunnenberg, H.G. (1994) EMBO J. 13, 3039–3049.
[25] Sjöberg, M. and Vennström, B. (1995) Mol. Cell. Biol. 15, 4718–4726.
[26] Damm, K., Thompson, C.C. and Evans, R.M. (1989) Nature 339, 593–597.
[27] Munoz, A., Zenke, M., Gehring, U., Sap, J., Beug, H. and Vennström, B. (1988) EMBO J. 7, 155–159.
[28] Fuerstenberg, S. et al. (1990) J. Virol. 64, 5891–5902.
[29] Beug, H., Mullner, E.W. and Hayman, M.J. (1994) Curr. Opin. Cell Biol. 6, 816–824.
[30] Sap, J., Munoz, A., Schmitt, J., Stunnenberg, H. and Vennström, B. (1989) Nature 340, 242–244.
[31] Lerner, E.A., Lerner, M.R., Janeway, C.A. and Steitz, J.A. (1981) Proc. Natl. Acad. Sci. USA 78, 2737–2741.
[32] Forrest, D., Sjöberg, M. and Vennström, B. (1990) EMBO J. 9, 1519–1528.
[33] Boucher, P., Koning, A. and Privalsky, M.L. (1988) J. Virol. 62, 534–544.
[34] Dang, C.V. and Lee, W.M. (1989) J. Biol. Chem. 264, 18019–18023.
[35] Hamy, F., Helbecque, N. and Henichart, J.P. (1992) Biochem. Biophys. Res. Commun. 182, 289–293.
[36] Dobbelstein, M., Roth, J., Kimberly, W.T., Levine, A.J. and Shenk, T. (1997) EMBO J. 16, 4276–4284.
[37] Guiochon-Mantel, A., Lescop, P., Christin-Maitre, S., Loosfelt, H., Perrot-Applanat, M. and Milgrom, E. (1991) EMBO J. 10, 3851–3859.
[38] Vancurova, I., Jochova-Rupes, J., Lou, W. and Paine, P.L. (1995) Biochem. Biophys. Res. Commun. 217, 419–427.
[39] Tora, L., Gronemeyer, H., Turkotte, B., Gaub, M.P. and Chambon, P. (1988) Nature 333, 185–188.
[40] Zhang, Y., Beck, C.A., Poletti, A., Edwards, D.P. and Weigel, N.L. (1994) J. Biol. Chem. 269, 31034–31040.
[41] Andersson, M.L., Nordström, K., Demczuk, S., Harbers, M. and Vennström, B. (1992) Nucleic Acids Res. 20, 4803–4810.
[42] Sterling, K., Brenner, M.A. and Sakurada, T. (1980) Science 210, 340–342.
[43] Wrutniak, C. et al. (1995) J. Biol. Chem. 270, 16347–16354.