MOLCEL 02538

At the Cutting Edge

Protein–protein interactions involving erbA superfamily receptors: through the TRAPdoor

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(Accepted 23 March 1991)

Introduction

Modulation of transcription in response to hormonal signals is mediated in part by a large family of structurally related receptor proteins, the erbA superfamily. This superfamily includes the receptors for steroid and thyroid hormones, vitamin D and retinoic acid, as well as a rapidly enlarging array of so-called 'orphan' receptors that lack identifiable ligands (for reviews, see Evans, 1988; Beato, 1989). The homology amongst erbA superfamily members is particularly evident upon examining the modular structure of these proteins (Fig. 1). Briefly, they consist of an amino terminal segment of variable length and sequence, a more or less centrally located DNA binding domain featuring two zinc finger motifs, and a carboxy-terminal hormone binding domain. Other functions such as transcriptional activation and nuclear localization have been delimited to various subdomains in different receptors (Giguere et al., 1986; Fawell et al., 1990). The cis-acting response elements to which erbA proteins bind typically contain two inverted or directly repeated 'half sites' (Brent et al., 1989; Nordeen et al., 1990). Current evidence suggests these receptors generally bind to response elements as dimers. However, there is an emerging awareness of the existence of interactions between erbA superfamily members and relatively poorly characterized nuclear proteins. These interactions, which are the topic of this review, appear to play a role in receptor-DNA binding, and perhaps in transcriptional activation. Due to space limitations, this review will not address interactions of erbA superfamily proteins with other known erbA superfamily members (Glass et al., 1989), with other well-characterized transcription factors such as fos and jun (Jonat et al., 1990; Lucibello et al., 1990; Yang-Yen et al., 1990), or with heat shock proteins (Pratt, 1990).
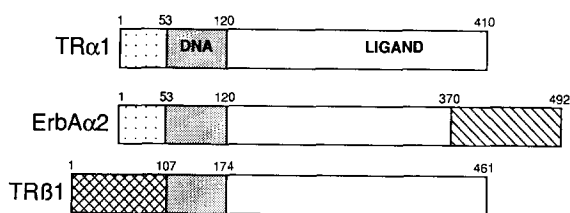

Fig. 1. Domain structure of erbA superfamily proteins. Thyroid hormone receptor α1 is shown on top, with the central 66 amino acid DNA binding domain and the C-terminal ligand binding domain noted. The non-hormone binding splice variant erbAα2 is identical to TRα1 through the first 370 amino acids, but then the proteins diverge completely. The β1 thyroid hormone receptor is 85–90% identical to TRα1 in DNA and ligand binding domains, but unrelated in amino terminal sequence. In general, other members of the erbA superfamily share similarities in their DNA binding domains (45–90% identity), but there is little conservation amongst ligand binding domains and no conservation amongst amino terminal regions.

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Nuclear proteins enhance binding of erbA superfamily members to DNA

Nuclear extracts have been shown to enhance or stabilize DNA binding by several erbA superfamily members, including the receptors for glucocorticoids (Cavanaugh and Simons, 1990), progesterone (Edwards et al., 1989), estrogens (Feavers et al., 1987), vitamin D (Liao et al., 1990), retinoic acid (Glass et al., 1990), and thyroid hormone (Murray and Towle, 1989), as well as the orphan receptor COUP-TF (Tsai et al., 1987). The most recent information comes from experiments involving thyroid hormone, retinoic acid and vitamin D receptors, which are believed to comprise a subfamily based primarily upon sequence homology within their DNA binding domains.

Murray and Towle (1989) found, using an electrophoretic mobility shift assay (EMSA), that in vitro translated thyroid hormone receptors (TRs) were unable to bind to a T3 response element (TRE). However, cellular extracts containing TRs did bind to this TRE. An extract from rat liver nuclei was able to restore the ability of the in vitro translated receptor to bind the TRE, leading to the formation of two gel-shifted complexes containing TR, non-TR nuclear protein, and DNA. It appeared that at least two non-TR proteins were responsible for this activity, one of which was detected in all tissues tested, while the other was detected primarily in liver. Thyroid hormone was found not to influence the formation of these complexes. For all parameters mentioned, the $\alpha1$ form of the TR behaved similarly to the $\beta1$ form.

These findings were confirmed and extended by other groups. By the use of EMSA and the more quantitative ABCD assay, nuclear protein(s) with the ability to enhance the binding of TRs to TREs have been discovered in many tissues and cell lines (Burnside et al., 1990; Lazar and Berrodin, 1990; O'Donnell et al., 1991). A variety of TRE sequences have been tested, and all support this activity to some degree, although some are more potent than others. Irrelevant DNA sequences do not support either basal TR binding or nuclear protein-induced enhancement of DNA binding. The factor(s) necessary for the enhancement of TR-DNA binding has been designated T3 Receptor Auxiliary Protein, or TRAP (Darling et al.,

1991; O'Donnell et al., 1991). By gel filtration, TRAP activity in GH3 cells was associated with a broad molecular weight range centered at ~65 kDa (Burnside et al., 1990). In accord with this, a protein of approximately 63 kDa from JEG3 cells has been crosslinked to the TRs (O'Donnell et al., 1991).

To date, TRAP activity has not been associated with any known proteins. TRβ itself, for example, does not replace TRAP activity when added exogenously to the ABCD assay in place of nuclear extract (O'Donnell et al., 1991). Similarly, RARβ, which is known to heterodimerize with TR (Glass et al., 1989), does not replace TRAP activity. Other proteins which have proven not to have TRAP activity are BSA, ovalbumin, histones, and high mobility group protein-1. The last is a known enhancer of DNA binding of other transcription factors (Watt and Molloy, 1988). Heat shock of cultured cells does not increase the amount of TRAP activity within those cells (E.D.R. and R.J.K., unpublished), arguing against a role for heat shock proteins in TRAP activity.

The vitamin D receptor also requires nuclear extract from any of several cell types to allow binding of in vitro translated receptor to a vitamin D response element (Liao et al., 1990). Yeast extracts cannot mimic the activity of these mammalian cell nuclear extracts (Sone et al., 1990).

In addition, retinoic acid receptors (RARs) respond to TRAP or a TRAP-like activity. RARs have been shown to exhibit dependence on nuclear extracts for binding to TRE/RARE sequences as well as RARE sequences from the RARβ gene (Glass et al., 1990). Although all vertebrate cells tested were positive for this activity, different sources of extract led to a shift in binding preference of RAR for various response elements. Thus, in the presence of extract from HL60 cells, RAR bound tighter to a palindromic TRE/RARE than to the RARβ gene RARE. This preference was reversed in the presence of HeLa extracts. Crosslinking studies have revealed that RARα was physically associated with proteins which varied in size and number depending upon their source. For example, HeLa extracts yielded crosslinked products of 55 and 65 kDa (after subtraction of the mass of the RAR), while HL60 cells exhibited a single major product of ~45 kDa. This suggests

that a family of TRAP-like proteins exists. Cell specific expression of these TRAP-like proteins might determine which genes are actually RAR responsive in those cells.

What parts of the receptor molecule are important for TRAP interaction?

The only receptors for which published data exist to help answer this question are the TRs and RARs. Sequences at the N-terminus appear not to be important for mediating the effect of TRAP on TR (Hudson et al., 1990; Darling et al., 1991). Similarly, Glass et al. (1990) noted that a truncated RARα that had lost its 187 amino terminal residues, including the entire DNA binding domain, was still able to be crosslinked to the 55 and 65 kDa proteins from HeLa cell extracts.

Sequences at the carboxy terminus of the molecule, on the other hand, do appear to be important for TRAP action to occur. Truncations of TRβ at amino acid 230 (E.D.R. and R.J.K., unpublished) and TRα at amino acid 210 (Lazar and Berrodin, 1990), which remove the majority of the ligand binding domains while leaving the DNA binding domains intact, ablate the ability of TRAP to complex with TR and DNA as assessed by EMSA. In contrast with these results, Darling et al. (1991) have shown using the ABCD assay that similar mutations reduce basal binding of receptor to DNA, but have little effect on the response to TRAP. They have localized a domain immediately adjacent to the second zinc finger in TRα (aa 122–149) which is critical for the enhancement of DNA binding due to TRAP. The reason for the discrepancy is not known, but may relate to the increased sensitivity of the ABCD assay or differing sources of nuclear extracts. From a variety of deletion mutants, the region of interaction of RARα was delimited to a relatively broad area encompassing amino acids 187–404 (Glass et al., 1990).

O'Donnell and Koenig (1990) investigated the function of a 20 amino acid segment within the ligand binding domain of TRβ (amino acids 286–305) that is well conserved among all members of the receptor superfamily (Fig. 2). Point mutations of this segment were found to impair the ability of TRβ to transactivate in transfected JEG3 cells.

Protein	Amino Acid Sequence	
rT3R-β1	FAKKLPMFCELPCEDQIILL	(286-305)
hVDR	FAKMIPGFRDLTSEDQIVLL	(244-263)
hRAR	FAKRLPGFTGLTIADQITLL	(235-254)
hER	WAKRVPGFVDLTLHDQVHLL	(360-379)
hGR	WAKAIPGFRNLHLDDQMTLL	(577-596)
rRev-ErbA	FAKQIPGFRDLSQHDQVTLL	(347-366)
mPPAR	FAKAIPGFANLDINDQVTLL	(290-309)
hCOUP-TF	WARNIPFFPDLQITDQVSLL	(228-247)
mH-2RIIBP	WAKRIPHFSSLPLDDQVILL	(230-249)

Fig. 2. Conservation of a 20 amino acid sequence in the ligand binding domains of erbA superfamily members. The sequence of rat TRβ1 aa 286–305 is shown, along with homologous regions of the VDR, RARβ, ER, GR, reverse erbA, peroxisome proliferator activated receptor, COUP-TF, and H-2RIIBP. Residues that are identical to TRβ1 are in bold; residues that are identical or conserved are within the shaded area.

Further studies revealed no defects in ligand binding, nuclear translocation, or basal DNA binding to account for the inability to transactivate. Subsequently, these 20 amino acids were found to be essential for TRAP interaction to occur (Darling et al., 1991; O'Donnell et al., 1991; E.D.R. and R.J.K., unpublished). In both EMSA and ABCD assays, deletion of the entire 20 amino acid stretch (Δ20) was found to abolish enhancement of DNA binding when cell extract was added, and point mutations dramatically reduced the ability of TRAP to enhance DNA binding. Wild-type TR could be crosslinked to a 63 kDa protein which presumably is TRAP. This crosslinking was not observed with the Δ20 mutant. Thus, the following three attributes of TRs appear to correlate with each other: the ability to transactivate target genes, the ability to physically interact with TRAP, and the ability to respond to this TRAP interaction with increased binding to TREs. The broad region necessary for RARα to interact with TRAP-like proteins includes this 20 amino acid domain.

Interpretation of these results, however, is complicated by other data. The c-erbA-α gene encodes at least one other product besides TRα1, an alternatively spliced form called erbAα2. ErbAα2 diverges from TRα1 in its C-terminal domain (Fig. 1) and thus does not bind T3 (Lazar et al., 1988). Although TRα1 and erbAα2 are identical in all domains that have been shown to interact with TRAP, TRAP does not enhance erbAα2-TRE binding (Darling et al., 1991; O'Donnell et al.,

1991). Darling et al. (1991) truncated TRα at amino acid 378, providing a close approximation of an α-generic protein. This construct was fully able to interact with TRAP, indicating that the erbAα2 specific domain is likely to disrupt this interaction. The viral oncogene product v-erbA also is unable to respond to TRAP (O'Donnell et al., 1991), despite the fact that it is highly homologous to TRα1 and does not contain a long α2-like C-terminal extension. It is interesting to note that v-erbA closely resembles erbAα2 in function; neither protein binds T3, and both proteins are constitutive repressors of T3 responsive genes (Koenig et al., 1989; Sap et al., 1989). It is tempting to speculate that the failure to interact with TRAP may play a role in the inability of these proteins to transactivate target genes (see below).

How might TRAP increase the affinity of nuclear hormone receptors for their cognate response elements?

(1) A catalytic mechanism. Although nuclear proteins such as TRAP may act enzymatically on TR (e.g. to enhance phosphorylation) and thus to enhance binding to the TRE, several lines of evidence argue against such a role. Burnside et al. (1990) found no change in the electrophoretic mobility of TR after the addition of extract. These authors also subjected their nuclear extracts to dialysis prior to use in DNA binding assays; this would presumably remove ATP, thus making phosphorylation a less likely candidate. We have found that performing binding studies at 4°C does not alter the ability of TRAP to enhance TR-TRE binding, further arguing against an enzymatic process. Finally, the crosslinking studies with TR and RAR argue strongly for a stoichiometric association between TRAP-like proteins and the receptors they act upon.

(2) A direct DNA response element binding mechanism. TRAP and TRAP-like proteins may stabilize the DNA binding of receptors by directly binding response element sequences themselves, thus forming receptor-TRAP heterodimers. Hudson et al. (1990) demonstrated that a HeLa cell extract enhanced the binding of TR and RAR to the EGF receptor promoter. This same DNA fragment, however, was unable to bind the nuclear

factor in the absence of added receptor. Some of these same authors were later able, however, to modify this experiment with very different results (Glass et al., 1990). In this case, the RAR DNA binding enhancement activity was purified ~ 40-fold after high salt elution from a column made by coupling a concatamerized TRE to Sepharose. Using a similar protocol with a different TRE (the TSHα promoter TRE), Darling et al. (1991) were able to demonstrate binding of TRAP to DNA.

This same group was able to delineate a sequence motif within the TRE-containing oligonucleotide that was necessary for TRAP action to occur (Beebe et al., 1991). The authors found that the sequence (T/A)GGGA was the critical cis-acting determinant of TRAP activity. This sequence represents one of the half-sites from the rate growth hormone promoter TRE. The authors point out that previous footprinting studies of TR binding to this TRE (Glass et al., 1987; Koenig et al., 1987) were performed in the presence of contaminating nuclear proteins that might have included TRAP, raising the possibility that some components of these footprints are actually due to TRAP binding rather than TR. These authors also found that the (T/A)GGGA motif was not sufficient to allow TRAP binding; an additional half-site for TR binding was required. These data support the notion of TR-TRAP heterodimerization on the response element.

(3) A physical association of proteins with receptor dimers. In this model, a receptor dimer would sit on the response element and interact with one or more TRAP molecules; TRAP itself would not bind the response element. While no data exist to support such a model for TRAP, this type of interaction appears to occur between COUP-TF and the nuclear protein S300-II (Tsai et al., 1987).

What role does the TRAP–receptor interaction play in receptor physiology?

Ultimately, this is the most important question one can pose regarding the interactions described above. The TRAP–receptor interaction may simply stabilize receptor binding to low affinity response elements in vivo. This could occur by altering receptor conformation and thereby directly enhancing its affinity for DNA, or by TRAP itself

binding to DNA with very high affinity and then dimerizing with the receptor.

A more ambitious view is that TRAP-like proteins may be important for the ability of the receptor to interact with the transcription apparatus. It is commonly held that receptors influence transcription by interacting directly or indirectly with components of the basal transcription machinery. These interactions appear to stabilize the preinitiation complex (Klein-Hitpass et al., 1990). To accomplish this, a protein(s) is likely to form a bridge between the receptor and the transcription apparatus; parsimony would argue TRAP may play such a role, because otherwise yet another protein would have to interact with the receptor. One issue relevant to this is that TRAP–receptor interactions are not influenced by the presence of ligand (Darling et al., 1991; O'Donnell et al., 1991). How then could TRAP transmit a ligand dependent signal from the receptor to the transcription machinery? Here we might take advantage of the fact that the TRAP binding site of the TR includes part of the ligand binding domain. Ligand binding would then induce a conformational change in this region of the receptor, which would in turn cause a conformational change in TRAP. This ligand-dependent conformational change in TRAP may then be required for optimal interaction with the transcription machinery (Fig. 3).

It is interesting that TRAP does not enhance binding of TRs to every DNA sequence capable of basal TR binding. Thus, while TR exhibits basal binding to the vitellogenin estrogen response element (ERE), TRAP is unable to enhance that binding (Darling et al., 1991). TR has previously been shown to be transcriptionally inactive when cotransfected with a reporter gene driven by this ERE (Glass et al., 1988). Thus, TRAP appears to enhance binding of TR to functionally active TREs, but not to other DNA sequences that bind TR in vitro. Perhaps when the TR binds to ERE it does not achieve a conformation favorable for interaction with TRAP. If TRAP helps form the bridge between the TR and the transcription machinery, this would explain why this ERE is not a functional TRE. Finally, it is also possible that TRAP plays a role not only in hormone dependent transcriptional activation, but in hormone dependent repression as well, since TRAP also enhances TR binding to negative acting TREs in vitro (Burnside et al., 1990).

This review has emphasized the roles played by TRAP and TRAP-like proteins in receptor-DNA binding and transcriptional activation. Regulated expression of these auxiliary proteins can be expected to add flexibility to the transcriptional response. Further studies in this area should help unravel the complex mechanisms whereby hormones regulate specific genes, and may lead to new therapeutic modalities for diverse conditions ranging from hormone resistance to hormone responsive malignancies.
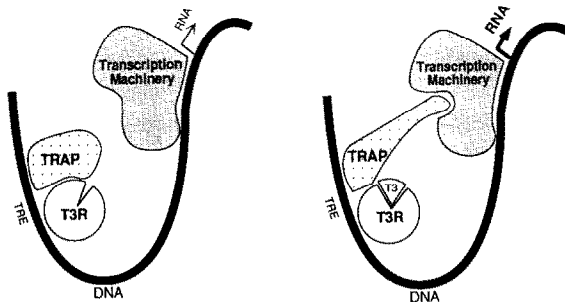

Fig. 3. A model for TRAP activation of transcription. In the absence of hormone (left) TRAP and TR form a heterodimer on the TRE, but do not interact with the transcription machinery, and the transcription rate is low. In the presence of ligand (right), a conformational change in TR induces a change in TRAP, allowing the latter to interact with the transcription machinery and stimulate RNA synthesis. This is but one of numerous potential models for TRAP–receptor interactions.

References

Beato, M. (1989) Cell 56, 335–344.

Beebe, J.S., Darling, D.S. and Chin, W.W (1991) Mol. Endocrinol. 5, 85–93.

Brent, G.A., Harney, J.W., Chen, Y., Warne, R.L., Moore, D.D. and Larsen, P.R. (1989) Mol. Endocrinol. 3, 1996–2004.

Burnside, J., Darling, D.S. and Chin, W.W. (1990) J. Biol. Chem. 265, 2500–2504.

Cavanaugh, A.H. and Simons, S.S. (1990) Biochemistry 29, 989–996.

Darling, D.S., Beebe, J.S., Burnside, J., Winslow, E.R. and Chin, W.W. (1991) Mol. Endocrinol. 5, 73–84.

Edwards, D.P., Kuhnel, B., Estes, P.A. and Nordeen, S.K. (1989) Mol. Endocrinol. 3, 381–391.

Evans, R.M. (1988) Science 240, 889–895.

Fawell, S.E., Lees, J.A., White, R. and Parker, M.G. (1990) Cell 60, 953–962.

Feavers, I.M., Jiricny, J., Moncharmont, B., Saluz, H.P. and Jost, J.P. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 7453–7457.

Giguere, V., Hollenberg, S.M., Rosenfeld, M.G. and Evans, R.M. (1986) Cell 46, 645–652.

Glass, C.K., Franco, R., Weinberger, C., Albert, V.R., Evans, R.M. and Rosenfeld, M.G. (1987) Nature 329, 738–741.

Glass, C.K., Holloway, J.M., Devary, O.V. and Rosenfeld, M.G. (1988) Cell 54, 313–323.

Glass, C.K., Lipkin, S.M., Devary, O.V. and Rosenfeld, M.G. (1989) Cell 59, 697–708.

Glass, C.K., Devary, O.V. and Rosenfeld, M.G. (1990) Cell 63, 729–738.

Hudson, L.G., Glass, C.K. and Gill, G.N. (1990) Cell 62, 1165–1175.

Jonat, C., Rahmsdorf, H.J., Park, K., Cato, A.C.B., Gebel, S., Ponta, H. and Herrlich, P. (1990) Cell 62, 1189–1204.

Klein-Hitpass, L., Tsai, S.Y., Weigel, N.L., Allan, G.F., Riley, D., Rodriguez, R. Schrader, W.T., Tsai, M. and O'Malley, B.W. (1990) Cell 60, 247–257.

Koenig, R.J., Brent, G.A., Warne, R.L., Larsen, P.R. and Moore, D.D. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 5670–5674.

Koenig, R.J., Lazar, M.A., Hodin, R.A., Brent, G.A., Larsen, P.R., Chin, W.W. and Moore, D.D. (1989) Nature 337, 659–661.

Lazar, M.A. and Berrodin, T.J. (1990) Mol. Endocrinol. 4, 1627–1635.

Lazar, M.A., Hodin, R.A., Darling, D.S. and Chin, W.W. (1988) Mol. Endocrinol. 2, 893–901.

Liao, J., Ozono, K., Sone, T. and Pike, J.W. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 9751–9755.

Lucibello, F.C., Slater, E.P., Jooss, K.U., Beato, M. and Muller, R. (1990) EMBO J. 9, 2827–2834.

Murray, M.B. and Towle, H.C. (1989) Mol. Endocrinol. 3, 1434–1442.

Nordeen, S.K., Suh, B.J., Kuhnel, B. and Hutchison, C.A.I. (1990) Mol. Endocrinol. 4, 1866–1873.

O'Donnell, A.L. and Koenig, R.J. (1990) Mol. Endocrinol. 4, 715–720.

O'Donnell, A.L., Rosen, E.D., Darling, D.S. and Koenig, R.J. (1991) Mol. Endocrinol. 5, 94–99.

Pratt, W.B. (1990) Mol. Cell. Endocrinol. 74, C69–C76.

Sap, J., Munoz, A., Schmitt, J., Stunnenberg, H. and Vennstrom, B. (1989) Nature 340, 242–244.

Sone, T., McDonald, D.P., O'Malley, B.W. and Pike, J.W. (1990) J. Biol. Chem. 265, 21997–22003.

Tsai, S.Y., Sagami, I., Wang, H., Tsai, M.O. and Malley, B.W. (1987) Cell 50, 701–709.

Watt, F. and Molloy, P.L. (1988) Nucleic Acids Res. 16, 1471–1486.

Yang-Yen, H., Chambard, J., Sun, Y., Smeal, T., Schmidt, T.J., Drouin, J. and Karin, M. (1990) Cell 62, 1205–1215.