tation, and perhaps some evergreen conifers could have provided a source of food during the winter. Many of the palynomorph taxa represent plants of unknown affinity that may also have provided a year-round source of food for the hadrosaurids. Hadrosaurs had well-developed dentitions capable of coping with diverse feeding habits to accommodate available forage (*20*). Their wide muzzles with upper and low horny rhamphothecae (pads) might have been useful in stripping off leaves from branches or shrubs or in rooting up and pulling off pieces of rhizome mats. The massive dental battery could accommodate large amounts of tooth wear, a consequence of eating subaquatic rhizome mats full of silt, mud, and abrasive, silica-rich *Equisetites* vegetation.

Current hypotheses suggesting an extraterrestrial cause of the extinction of dinosaurs, such as an asteroid's impact (*21*), invoke lethal effects that include a period of darkness lasting a few weeks or months and a great decrease in ambient temperature. If the North Slope dinosaurs were not migratory, their occurrence at high northern, Late Cretaceous latitudes provides direct evidence of the ability of some species to tolerate up to several months of darkness and to cope with cold air temperatures. Thus, some of the proposed effects of impacts of an asteroid or comets, increased volcanism, or related hypotheses may not have been the direct cause of the demise of the dinosaurs.

REFERENCES AND NOTES

1. D. B. Weishampel, in preparation; A. R. Milner and D. B. Norman, in *Third Symposium on Mesozoic Terrestrial Ecosystems, Short Papers*, W.-E. Reif and F. Westphal, Eds. (ATTEMPTO Verlag, Tubingen, 1984), pp. 145–150.
2. H. Roehler and G. Stricker, *J. Alaska Geol. Soc.* **4**, 35 (1984).
3. K. Davies, *J. Paleontol.* **61**, 198 (1987).
4. Liscomb's site was relocated in 1984 and 1986 by E.M.B., L.D.C., and T.A.A., who collected additional vertebrate fossils and sediments that have yielded microfossil assemblages. In 1985, a field party under W.A.C. made extensive collections of vertebrate fossils. Locality data are on file at the U.S. Geological Survey (Reston, VA; Denver, CO; and Menlo Park, CA); the Museum of Paleontology, University of California, Berkeley; and the Museum, University of Alaska, Fairbanks. The National Museum of Natural History, Washington, DC, will be the final repository of the collections. Some of the 1985 collections are curated in the Museum, University of Alaska, Fairbanks.
5. W. A. Clemens and C. W. Allison, *Abstr. Programs Geol. Soc. Am.* **17**, 548 (1985).
6. W. P. Brosge and C. L. Whittington, *U.S. Geol. Surv. Prof. Pap. 303-H* (1966), p. 501.
7. Several tephra layers below the stratigraphically lowest dinosaur occurrence are being analyzed by means of K-Ar, ^{39}Ar-^{40}Ar, fission-track, and chemical characterization. One of these tephras has yielded a fission-track age of 50.9 ± 7.7 million years, now considered a minimum age [L. D. Carter *et al., U.S. Geol. Surv. Circ. 751-B* (1971), p. B12].
8. Based on 1986 field studies by E.M.B., L.D.C., R. L. Phillips, and L. Marincovich.
9. Total organic carbon (TOC) analyses of sediments associated with the Liscomb bone bed indicate an average of 6.85% TOC for the bone bed and concretions, 1.05% TOC for the siltstone immediately above the bone bed, and 0.58% TOC for the siltstone immediately below the bone bed (analyses provided by J. T. Parrish).
10. M. Feist and N. Grambast-Fessard, *Paleobiol. Continent.* **13**, 1 (1982).
11. F. P. C. M. van Morkhoven, *Post-Palaeozoic Ostracoda* (Elsevier, Amsterdam, 1963).
12. T. D. Fouch *et al.*, in *1979 Basin and Range Symposium*, G. W. Newman and H. D. Goode, Eds. (Rocky Mountain Association of Geologists, Denver, CO), pp. 305–312.
13. H. Tappan, *U.S. Geol. Surv. Prof. Pap. 236-C* (1962).
14. W. G. E. Caldwell *et al.*, *Geol. Assoc. Canada Spec. Pap. 18* (1978), p. 495; D. H. McNeil and W. G. E. Caldwell, *Geol. Assoc. Canada Spec. Pap. 21* (1981).
15. N. O. Frederiksen, in *Am. Assoc. Stratigr. Palynol. Program Abstr. 19th Annu. Meeting* (1986), p. 11; V. D. Wiggins, *Geosci. Man.* **15**, 51 (1976); B. D. Tschudy, *U.S. Geol. Surv. Prof. Pap. 643-A* (1969).
16. J. A. Wolfe and G. R. Upchurch, Jr., *Palaeogeogr. Palaeoclim. Palaeoecol.*, in press; R. A. Spicer and J. T. Parrish, *Geology* **14**, 703 (1986).
17. J. T. Parrish *et al.*, *Abstr. Programs Geol. Soc. Am.* **19**, 326 (1987).
18. W. K. Witte *et al.*, in *North Slope Geology*, I. L. Tailleur and P. Weimer, Eds. (Society of Economic Paleontologists and Mineralogists, Tulsa, OK, in press); L. D. Carter and J. W. Hillhouse, unpublished paleomagnetic data.
19. D. I. Axelrod, *Palaeogeogr. Palaeoclim. Palaeoecol.* **45**, 105 (1984); R. T. Bakker, *Sci. Am.* **232**, 58 (April 1975); N. Hotton, in *A Cold Look at Warm-Blooded Dinosaurs*, R. D. K. Thomas and E. C. Olson, Eds. (American Association for the Advancement of Science, Washington, DC, 1980), pp. 311–350.
20. D. B. Weishampel, *Adv. Anat. Embryol. Cell Biol.* **87**, 1 (1984); R. Krausel, *Palaeont. Z.* **4**, 80 (1922).
21. L. W. Alvarez, W. Alvarez, F. Asaro, H. V. Michel, *Science* **208**, 1095 (1980); W. Alvarez, *Eos* **67**, 649 (1986).

22. We thank R. Emmons (Shell Oil Company) for his assistance in relocating the late R. L. Liscomb's field notes and collections. W. Langston, Jr., and K. Davies (University of Texas) made available their analyses of Liscomb's collection and a preprint of Davies' paper. The assistance of P. Currie (Tyrrell Museum, Drumheller, Alberta) and J. Horner (Museum of the Rockies, Bozeman, MT) with identifications and discussions of ontogenetic change in dinosaurs is gratefully acknowledged. C. A. Repenning (U.S. Geological Survey, Denver, CO) and D. Weishampel (Johns Hopkins University) provided insights on hadrosaur jaw and tooth structure and possible seasonal food sources. Fieldwork for W.A.C. during 1985 was supported by USGS grant 14-08-0001-G-1122. Funding for 1986 fieldwork for R.A.S. was provided by British Petroleum.

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Identification of a Novel Thyroid Hormone Receptor Expressed in the Mammalian Central Nervous System

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A complementary DNA clone derived from rat brain messenger RNA has been isolated on the basis of homology to the human thyroid hormone receptor gene. Expression of this complementary DNA produces a high-affinity binding protein for thyroid hormones. Sequence analysis and the mapping of this gene to a distinct human genetic locus indicate the existence of multiple human thyroid hormone receptors. Messenger RNA from this gene is expressed in a tissue-specific fashion with highest levels in the central nervous system.

THYROID HORMONES ARE INVOLVED in a complex array of developmental and physiological responses in many tissues of higher vertebrates (*1*). Their numerous and diverse effects include the regulation of important metabolic enzymes, hormones, and receptors (*2*). The actions of thyroid hormones are mediated through a nuclear receptor, which modulates the expression of specific genes in target cells (*3–5*). These properties are similar to the interactions of steroid hormones with their receptors and are consistent with the recent observation of structural relatedness between steroid and thyroid hormone receptors (*6*).

Despite the diversity of thyroid hormone action, it is generally accepted that thyroid hormone function occurs through a single high-affinity nuclear receptor. However, the recent characterization of the thyroid hormone receptor as the cellular homolog of the v-*erb*A oncogene product (*6, 7*), along with the previous identification of multiple c-*erb*A genes on human chromosomes 3 and 17 (*6, 8*), predicts the existence of multiple thyroid hormone receptors. To examine the possibility that the mechanisms underlying the multiple thyroid hormone responses

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may be derived from the expression of structurally distinct thyroid hormone receptors, we have isolated a complementary DNA (cDNA) clone that encodes the product of one of these related loci.

A putative neuronal form of the thyroid hormone receptor was isolated by screening a cDNA library prepared from rat brain messenger RNA (mRNA) with a 1500-bp fragment of the human thyroid hormone receptor cDNA (*6*). From ~10^6 phage, three positive clones were isolated, and the complete nucleotide sequence of the largest of these, rbeA12, was determined (Fig. 1). The sequence is 2079 bp long and contains a long open reading frame of 1230 bp with a potential initiator methionine at nucleotide position 325 and a terminator codon at position 1554. This open reading frame is preceded by a 5′ untranslated region of at least 320 bp that contains three short open reading frames upstream of the putative initiator methionine and encodes a protein of 410 amino acid residues, with a calculated molecular mass of 45 kD.

Comparison of the deduced amino acid sequence from rbeA12 with that of the human thyroid hormone receptor (*6*) reveals that the two proteins have distinct amino termini (Fig. 2). The first 41 amino acids of the neuronal protein and the first 90 amino acids of the human thyroid hormone receptor show no significant homology, whereas the carboxyl terminal 367 amino acids share 75% nucleotide and 82% amino acid identities. The rat protein is more related to the chicken thyroid hormone receptor (*7*) both in predicted size and homology, and shares 82% nucleotide and 89% amino acid identity. For reference, the chicken thyroid hormone receptor is designated alpha (cTRα) because of its homology to previously isolated *erb*A genes (*8*), and the human thyroid hormone receptor is designated beta (hTRβ). Because the rat neuronal form is more related to the chicken receptor, it has been designated alpha (rTRα).

By analogy to the steroid hormone receptors, a cysteine-rich region in the thyroid hormone receptor is predicted to be the DNA-binding domain (*6, 9, 10*). In this region, the rTRα protein has 97% amino acid identity with the cTRα protein and 90% amino acid identity with the hTRβ protein. The proteins are also well conserved in the carboxyl terminal portion that is presumed to be the hormone-binding domain, again by analogy with the steroid receptors (*9, 11*). This region of rTRα shows 94% amino acid identity with cTRα and 85% amino acid identity with hTRβ.

On the basis of the sequence data, it appears that the cDNA we have isolated encodes a protein different from the previously characterized human thyroid hormone receptor (*6*). To demonstrate that the neuronal clone is a distinct gene product, rbeA12 was used to identify human homologs by Southern blot and chromosome analyses. Human placenta DNA digested with various restriction enzymes was separated on an agarose gel, transferred to nitrocellulose, and hybridized with either rat or human TR-specific probes derived from overlapping regions of their respective genes (Fig. 3). Different hybridization patterns were revealed for all of the restriction enzymes tested, which indicates that the two cDNAs represent distinct genes. The same probe from rbeA12 was hybridized to laser-sorted chromosomes prepared from human lymphoid cells (Fig. 3C). Hybridization was observed only to chromosome 17, consistent with previous mapping studies that localized c-*erb*A genes to human chromosome 17 (*8*). This distinguishes rTRα from hTRβ, which is found on human chromosome 3 (*6*).

Fig. 1. Restriction map and nucleotide and predicted amino acid sequence of thyroid hormone receptor cDNA from rat brain. (**A**) Schematic representation of thyroid hormone receptor cDNA from rat brain; some common restriction endonuclease cleavage sites are indicated. The hatched box indicates the predicted coding region. The 500-bp Pvu II fragment (corresponding to nucleotide positions 607 to 1113) used for the hybridization studies is represented by the solid bar below the restriction map. (**B**) The complete nucleotide sequence of rbeA12 is shown with the predicted amino acid sequence given above the long open reading frame. The three short open reading frames in the 5′ untranslated region are shown in bold type with termination codons underlined. RbeA12 was isolated by using the entire Eco RI insert of pheA4 (*6*) as a nick-translated probe to screen ~10^6 phage from a rat brain cDNA library obtained from J. Arriza (*19*). Three positive clones were isolated, and the complete nucleotide sequence of the largest of these, rbeA12, was determined on both strands by the chemical cleavage method of Maxam and Gilbert (*20*).

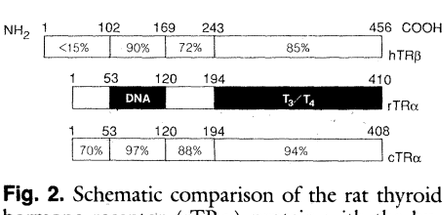

Fig. 2. Schematic comparison of the rat thyroid hormone receptor (rTRα) protein with the human thyroid hormone receptor (hTRβ) and chicken thyroid hormone receptor (cTRα) proteins. Numbers above the boxes indicate amino acid residues; numbers inside the boxes indicate the percent amino acid identity within the enclosed region with the rTRα protein. DNA designates the putative DNA-binding domain predicted by analogy with the human glucocorticoid receptor (amino acids 421 to 486 of the human glucocorticoid receptor), while T_3/T_4 designates the putative hormone-binding domain.

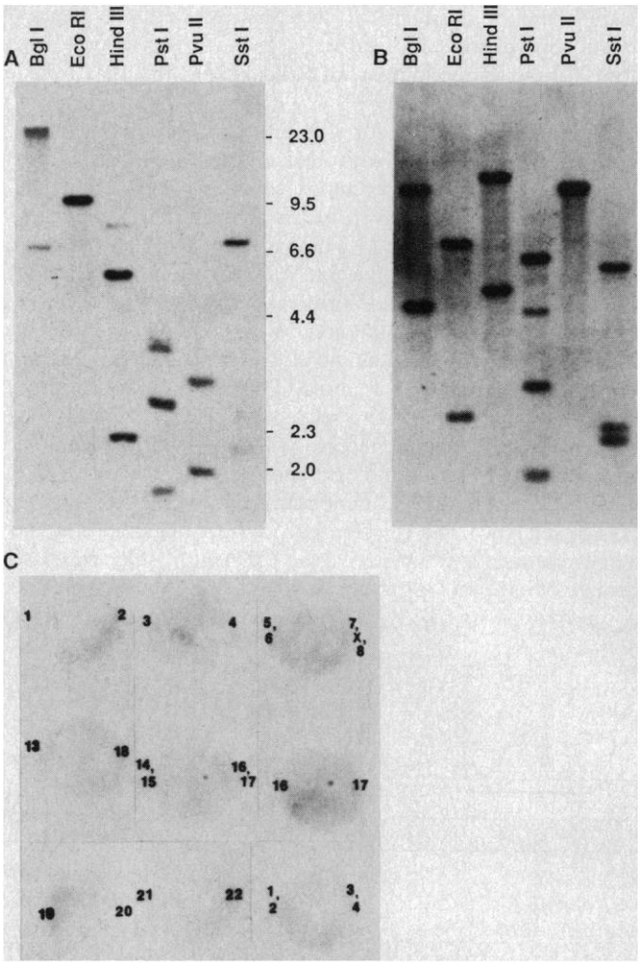

Fig. 3. Southern blot analysis and human chromosomal localization of the rTRα gene. Human placenta DNA was digested with various restriction enzymes, separated on a 0.8% agarose gel, transferred to nitrocellulose, and hybridized to either a 500-bp Pvu II fragment from rbeA12 (**A**) or a 450-bp Sst I fragment from hTRβ (*6*) that encompasses the DNA-binding region (**B**). Both blots were hybridized in 50% formamide, 5× SSPE (0.15*M* NaCl, 0.01*M* NaH_2PO_4, 0.001*M* EDTA), 1× Denhardt's solution, 0.1% SDS, and salmon sperm DNA (100 μg/ml) at 42°C, and washed in 2× SSC (standard saline citrate) and 0.1% SDS at 68°C. Sizes of λ Hind III markers in kilobase pairs are indicated. (**C**) Chromosome mapping of the rTRα gene. Human lymphocyte chromosomes were separated by laser cytofluorometry (*21*) and hybridized under the same conditions as above with the 500-bp Pvu II fragment of rbeA12.

Expression studies were performed to determine whether the rTRα cDNA encodes a functional receptor protein. The product of the rTRα gene was first characterized by in vitro transcription followed by in vitro translation. For in vitro transcription, the Eco RI insert of rbeA12 was linked to the bacteriophage SP6 promoter by subcloning into the expression vector pGEM1. A second construction, rbeA12B, was created in an attempt to increase the efficiency of translation. The 5′ untranslated region up to nucleotide position 97 was deleted, which removed two of the three short open reading frames in this region. Transcripts synthesized with SP6 polymerase were translated in vitro with rabbit reticulocyte lysates, and the [^{35}S]methionine-labeled products were analyzed on an SDS-polyacrylamide gel (Fig. 4A). Four proteins of approximately 52, 48, 35, and 33 kD were observed only when the sense strand was translated. The same four bands were observed for rbeA12 and rbeA12B. These translation products were then used to test thyroid hormone binding.

Thyroid hormone binding was measured with [^{125}I]3,5,3′-triiodo-L-thyronine (^{125}I-T_3). Only samples that contained the rTRα specific proteins exhibited T_3 binding. Hormone affinity was determined by Scatchard analysis, which gave a dissociation constant (K_d) of $2.9 \times 10^{-11}M$ (Fig. 4B), similar to the K_d observed for the hTRβ protein ($5 \times 10^{-11}M$) (*4, 5*) and an order of magnitude lower than that determined for the cTRα protein (2.1×10^{-10} to $3.3 \times 10^{-10}M$) (*6*). The different K_d values obtained may be due to differences in the assay systems used. In competition experiments, the rTRα proteins translated in vitro showed the same characteristic affinities for L-T_3 and L-thyroxine (L-T_4) as the hTRβ protein but revealed a different pattern for 3,5′,3′-triiodothyroacetic acid (TRIAC) (Fig. 4C). TRIAC competed better for T_3 binding with the hTRβ protein, whereas it competed about as well as T_3 for binding to the rTRα protein. As with the hTRβ and cTRα proteins, there was no competition for T_3 binding to the rTRα protein by excess aldosterone, estrogen, progesterone, testosterone, or vitamin D_3. Thus, it appears that we have isolated a thyroid hormone receptor with binding properties similar to but not identical to those of the thyroid hormone receptors previously described (*6, 7*).

The tissue specificity of metabolic responses to thyroid hormone led us to consider that this thyroid hormone receptor might be expressed in a restricted set of tissues. Therefore, the pattern of expression of the rTRα gene was determined by Northern blot analysis (Fig. 5). Total RNA isolated from various rat tissues was separated on a formaldehyde-agarose gel, transferred to nitrocellulose, and hybridized to the same fragment of rbeA12 used for the Southern blot analysis and chromosome mapping. A 2.6-kb RNA was observed in all tissues tested except liver. This message is also present in pituitary and muscle and is expressed in GC, rat-1, and PC12 cell lines. Densitometric scanning indicated that the level of expression of rTRα was 10- to 20-fold as high in brain as in any other tissue tested. Two additional RNAs of approximately 5.0 and 6.0 kb are present in about equal amounts in all tissues, although they are much less abundant than the 2.6-kb message. These bands may represent precursors of the 2.6-kb message or may be products of a related gene.

The isolation of a second mammalian thyroid hormone receptor is surprising because previous biochemical studies have not predicted the existence of more than a single receptor for thyroid hormones. In retrospect, much of the clinical and physiological studies can be interpreted as indicating the existence of multiple receptors. A form of functional heterogeneity has been suggested by the identification of patients with familial thyroid hormone resistance in which peripheral response to thyroid hormones is lost or diminished, while neuronal functions are maintained (*12, 13*). Furthermore, severe developmental effects associated with low circulating thyroid hormone levels (cretinism) have been classified into types severely affecting the nervous system and those more dramatically affecting peripheral functions (*13, 14*).

In addition to demonstrating the existence of structurally distinct forms of the thyroid hormone receptor, the form that we have characterized is expressed at high levels in the rat central nervous system. Preliminary studies utilizing in situ hybridization have revealed high levels of expression in the hippocampus, hypothalamus, cortex, and amygdala. RNA hybridization studies indicate exceptionally high levels in the cerebellum as well. Although it is known that thyroid hormones play a critical role in early brain development (*14*), this high level of expression is unexpected because biochemical studies have shown that brain has fewer thyroid hormone receptors than many other tissues (*5, 16*), and the adult brain is not responsive to thyroid hormone by traditional

criteria (oxygen consumption, or α-glycerophosphate dehydrogenase activity) (*17*).

The second interesting result from the expression studies is that this transcript is not present in liver, which is the tissue from which thyroid hormone receptors usually have been isolated. This absence suggests the existence of yet another form of the thyroid hormone receptor. This proposal would be consistent with the data of Underwood *et al.* (*18*), which indicates the existence of pharmacologically distinguishable thyroid hormone responses between liver and heart. Furthermore, data from DNA hybridization studies indicate the existence of multiple genetic loci that hybridize with the cDNA clones for the mammalian thyroid hormone receptor and suggest that there may be as many as five different related loci (*6, 8*). It seems likely that some of these loci will encode additional functional molecules, which leads us to propose the existence of a family of thyroid hormone receptors that coordinately regulate overlapping networks of genes to control developmental and homeostatic function.

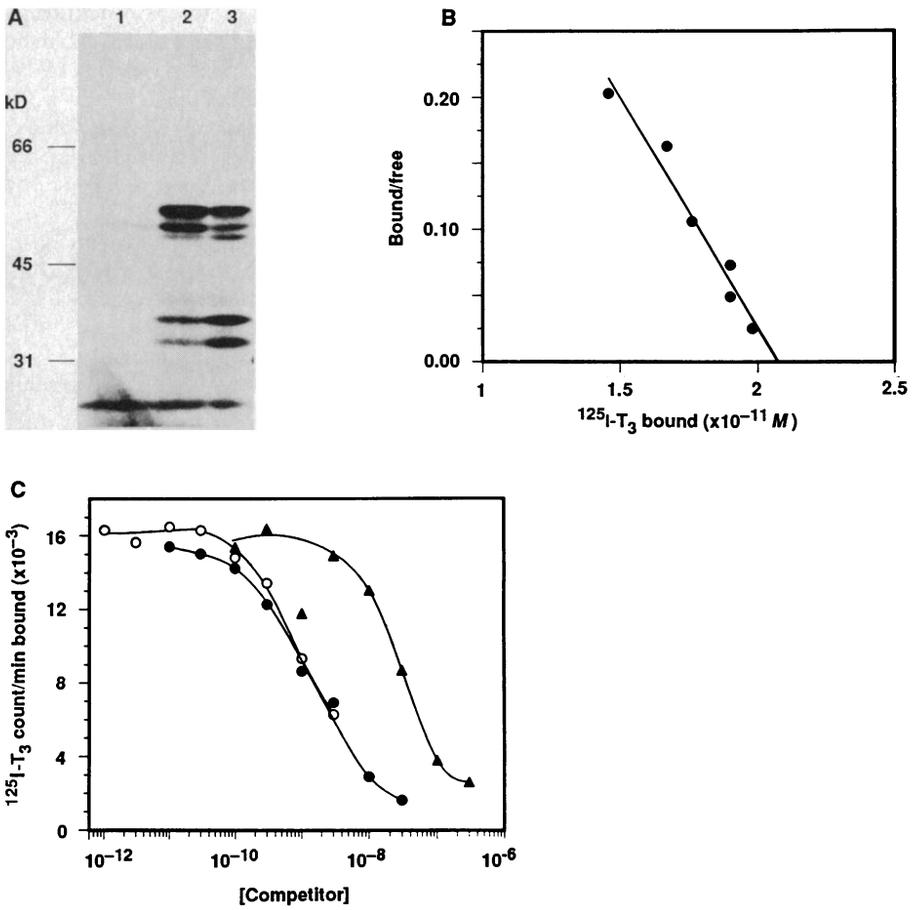

Fig. 4. In vitro translation and thyroid hormone binding of rTRα. (**A**) rTRα was transcribed in vitro and translated in a rabbit reticulocyte lysate. The [^{35}S]methionine–labeled products were separated on a 7.5% SDS-polyacrylamide gel and visualized by fluorography. Lane 1, no added RNA; lane 2, rbeA12, which contains the entire 5′ untranslated region; lane 3, rbeA12B, which contains only 97 bp of 5′ untranslated sequence. Sizes of protein markers: bovine serum albumin, 66.2 kD; ovalbumin, 45 kD; carbonic anhydrase, 31 kD. (**B**) Scatchard analysis of ^{125}I-T_3 binding to in vitro translated rTRα. Lysates containing in vitro translated rbeA12B transcripts were assayed for specific thyroid hormone-binding activity by measuring the amount of hormone bound at different concentrations of ^{125}I-T_3. $K_d = 2.9 \times 10^{-11} M$. (**C**) Competition of thyroid hormone analogs for ^{125}I-T_3 binding to in vitro translated rTRα. Samples from rbeA12B programmed lysates were mixed with increasing concentrations of unlabeled thyroid hormone or analogs to compete with labeled hormone. Specifically bound ^{125}I-T_3 is plotted versus concentration of competitor compound. The same competition pattern was observed in four separate experiments. In vitro transcription and translation and hormone binding were performed as described (*22, 23*). ○, TRIAC; ●, L-T_3; ▲, L-T_4.

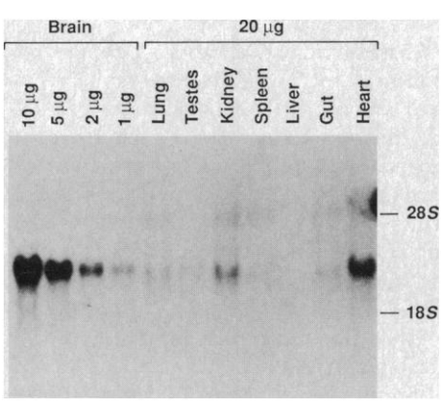

Fig. 5. Tissue distribution of rTRα mRNA. Total RNA was isolated from various rat tissues with guanidine thiocyanate (*24*), separated on a 1% agarose-formaldehyde gel, transferred to nitrocellulose, and hybridized with a nick-translated 500-bp Pvu II fragment from rbeA12. The tissue type and the amount of total RNA loaded are indicated above each lane. A cDNA of CHO-B, a Chinese hamster ovary cell mRNA expressed at equivalent levels in all tissues examined (*25*), was used as an internal standard. Positions of 28*S* and 18*S* ribosomal RNA are indicated.

REFERENCES AND NOTES

1. E. C. Wolff and J. Wolff, in *The Thyroid Gland*, R. V. Pitt-Rivers and W. R. Trotter, Eds. (Butterworths, London, 1964), vol. 1, pp. 237–282; H. L Schwartz, in *Molecular Basis of Thyroid Hormone Action*, J. H. Oppenheimer and H. H. Samuels, Eds. (Academic Press, New York, 1983), pp. 413–444.
2. N. L. Eberhardt, J. W. Apriletti, J. B. Baxter, in *Biochemical Actions of Hormones*, G. Litwack, Ed. (Academic Press, New York, 1980), vol. 7, pp. 311–394.
3. J. H. Oppenheimer, D. Koerner, H. L. Schwartz, M. I. J. Surks, *J. Clin. Endocrinol. Metab.* **35**, 330 (1972); H. H. Samuels and J. S. Tsai, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3488 (1973).
4. J. R. Tata and C. C. Widnell, *Biochem. J.* **98**, 604 (1966); J. A. Martial, J. D. Baxter, H. M. Goodman, P. H. Seeburg, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1816 (1977); R. M. Evans, N. C. Birnberg, M. G. Rosenfeld, *ibid.* **79**, 7659 (1982).
5. H. L. Schwartz and J. H. Oppenheimer, *Endocrinology* **103**, 267 (1978).
6. C. Weinberger *et al.*, *Nature (London)* **324**, 641 (1986).
7. J. Sap *et al.*, *ibid.*, p. 635.
8. N. K. Spurr *et al.*, *EMBO J.* **3**, 159 (1984); A. I. Dayton *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4495 (1984); S. C. Jhanwar, R. S. K. Chaganti, C. M. Croce, *Somatic Cell Mol. Genet.* **11**, 99 (1985).
9. V. Giguere, S. M. Hollenberg, M. G. Rosenfeld, R. M. Evans, *Cell* **46**, 645 (1986).
10. S. M. Hollenberg, V. Giguere, P. Segui, R. M. Evans, *ibid.* **49**, 39 (1987).
11. V. Kumar, S. Green, A. Staub, P. Chambon, *EMBO J.* **5**, 2231 (1986).
12. M. M. Menezes-Ferreira, C. Eil, J. Wortsman, B. D. Weintraub, *J. Clin. Endocrinol. Metab.* **59**, 1081 (1984).
13. G. N. Burrow, in *Endocrinology and Metabolism*, P. Felig, J. D. Baxter, A. E. Broadhus, L. A. Frohman, Eds. (McGraw-Hill, New York, 1981), pp. 351–385.
14. L. J. DeGroot and J. B. Stanbury, *The Thyroid and Its Diseases* (Wiley, New York, ed. 4, 1975).
15. J. T. Eayrs, *Br. J. Anim. Behav.* **1**, 144 (1953); D. H. Ford and E. B. Cramer, in *Thyroid Hormones and Brain Development*, G. Grave, Ed. (Raven, New York, 1977), pp. 1–17.
16. J. H. Oppenheimer, H. L. Schwartz, M. I. Surks, *Endocrinology* **95**, 897 (1974).
17. S. P. Barker and H. M. Klitgaard, *Am. J. Physiol.* **170**, 81 (1952); Y. P. Lee and H. A. Lardy, *J. Biol. Chem.* **240**, 1427 (1965).
18. A. H. Underwood *et al.*, *Nature (London)* **324**, 425 (1986).
19. The rat brain cDNA library was constructed by means of oligo(dT)-selected RNA from whole rat brain. Moloney murine leukemia virus (M-MuLV)

reverse transcriptase (RT) was used for first strand synthesis; second strand synthesis was with the Klenow fragment of DNA polymerase I followed by M-MuLV RT. cDNAs were treated with S1 nuclease, methylated, size-fractionated on Sepharose 4B, ligated to λgt11 arms, and packaged. The library consists of 25×10^6 independent recombinants with inserts >500 bp.
20. A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
21. R. V. Lebo *et al.*, *Science* **225**, 57 (1984).
22. P. A. Krieg and D. A. Melton, *Nucleic Acids Res.* **12**, 7057 (1984); S. M. Hollenberg *et al.*, *Nature (London)* **318**, 635 (1985).
23. For in vitro transcription, the entire Eco RI insert of rbeA12 was cloned into pGEM1 (Promega Biotec). A seond construction deleting 227 bp of 5′ untranslated sequence, rbeA12B, was made by inserting the T_4 DNA polymerase–filled Bgl I–Sma I fragment of rbeA12 (nucleotide position 227 to the polylinker) into the Sma I site of pGEM3. For thyroid hormone binding, transcriptions were performed with SP6 polymerase and 5 to 10 μg of rbeA12B linearized with Sst I. Transcripts were purified by P60 chromatography and translated in 150 to 200 μl of rabbit reticulocyte lysate (Promega Biotec) in conditions suggested by the manufacturer. Thyroid hormone binding for both the Scatchard and competition analyses were determined in the same manner, except that unlabeled protein was used for the Scatchard analysis. [^{125}I]3,3′,5-Triiodothyronine (New England Nuclear, 2200 Ci/mmol, 0.3 n*M* final concentration) was mixed with rTRα polypeptides synthesized in vitro (5 to 8 μl of the 200 μl of lysate per binding reaction) in T_3-binding buffer at 0°C for 2 hours in a final volume of 250 μl. Specific hormone binding was determined by adding a 1000-fold excess of unlabeled hormone and assayed by counting radioactivity eluting in the excluded volume from a Sephadex G-25 fine (Pharmacia) 0.9- by 4.0-cm column.
24. J. M. Chirgwin, A. F. Przybyla, R. J. MacDonald, W. F. Rutter, *Biochemistry* **18**, 5294 (1979).
25. M. M. Harpold, R. M. Evans, M. Salditt-Georgieff, J. E. Darnell, *Cell* **17**, 1025 (1979).
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Evidence for Dispensable Sequences Inserted into a Nucleotide Fold

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Previous experimental results along with the structural modeling presented indicate that a nucleotide fold starts in the amino-terminal part of *Escherichia coli* isoleucyl–transfer RNA synthetase, a single chain polypeptide of 939 amino acids. Internal deletions were created in the region of the nucleotide fold. A set of deletions that collectively span 145 contiguous amino acids yielded active enzymes. Further extensions of the deletions yielded inactive or unstable proteins. The three-dimensional structure of an evidently homologous protein suggests that the active deletions lack portions of a segment that connects two parts of the nucleotide fold. Therefore, the results imply that removal of major sections of the polypeptide that connects these two parts of the fold does not result in major perturbation of the nucleotide binding site.

IT IS POSSIBLE TO DISSECT PROTEINS into pieces and to investigate activities and structures of fragments (*1–3*). The delineation of substructures within a structure provides a starting point for making chimeric enzymes, in which components of two or more enzymes are recombined to give a new species. There is evidence that such recombination occurs in nature (*4, 5*).

Aminoacyl–transfer RNA synthetases are a class of proteins that catalyze aminoacylation of specific transfer RNAs in a two-step reaction: condensation of adenosine triphosphate (ATP) with amino acid to give an enzyme-bound aminoacyl adenylate, and reaction of the bound adenylate with transfer RNA (tRNA) to give aminoacyl-tRNA (*6*). Although these enzymes catalyze the same reaction for each amino acid, there is considerable variability in the gross structural features of the proteins (for example, different quaternary structures or subunit sizes). Structural analysis has shown that a domain resembling a nucleotide binding fold (Rossmann fold) (*7*) occurs in the NH_2-terminal part of the *Escherichia coli* methionine (*8–10*) and *Bacillus stearothermophilus* tyrosyl-tRNA synthetases (*11, 12*). There is further evidence that a similar fold occurs in the NH_2-terminal parts of several other aminoacyl-tRNA synthetases (*6*).

Apart from the delineation of dispensable sequences in the COOH-terminal halves of two aminoacyl-tRNA synthetases (*1, 13, 14*), there is no evidence for insertions of expendable sequences that interrupt a structural or functional domain within these enzymes. We now present evidence that a large, dispensable sequence is inserted into the nucleotide fold of a large aminoacyl-tRNA synthetase.

The α-β structure that forms the nucleotide binding fold of *E. coli* methionyl-tRNA synthetase (*8–10*) (Fig. 1) is made up of approximately the first 360 residues of the 677 amino acid subunit (*10, 15, 16*). We adopted the nomenclature for lactate dehydrogenase and assigned letter designations to the individual elements of β structure and α helix (*7*). In this prototypical representation of the Rossmann nucleotide fold, there are six strands of parallel sheet with two α helices on either side of the β structure (*7*). The secondary structural element boundaries indicated in Fig. 1 are in accordance with the most recent structural analyses by Brunie and co-workers (*10*).

The nucleotide fold is divided more or less into two halves, each of which comprises three β strands and two α helices. The first half is made up of approximately the NH_2-terminal 97 amino acids and the second half starts around amino acid residue 226 and continues to about amino acid residue 361. The two halves are separated by a segment of roughly 127 amino acids which starts at the COOH-terminal end of β_C. We designate this segment connective polypeptide one (CP1). While this segment contains elements of defined secondary structure, which includes a helix that lies parallel to the β sheet, it is not part of the prototypical nucleotide fold of Fig. 1. The second half of the methionine synthetase nucleotide fold is further interrupted by a segment (designated CP2) of about 70 amino acids, which starts at the COOH-terminal end of β_D.

In contrast to the dimeric methionyl-tRNA synthetase (*6*), isoleucyl-tRNA synthetase is a monomer of 939 amino acids (*6, 17*). While there is no three-dimensional structural information on this enzyme, amino acids 58 to 68 have ten identities and one conservative substitution when aligned with amino acids 14 to 24 of the methionine enzyme (*17*) (Fig. 1). The match includes the connecting segment between the COOH-terminal end of β_A and the NH_2-terminal end of α_B and extends somewhat into the latter structure.

This element is a signature sequence for a subclass of aminoacyl-tRNA synthetases and is likely to correspond to the same three-dimensional structural component in these enzymes (*17, 18*). In the case of the *B. stearothermophilus* tyrosine enzyme, close structural homology with the *E. coli* methionyl-tRNA synthetase in this general region has been demonstrated. This homology of structure extends over β_A, α_B, and β_B, even though the two enzymes have no significant sequence match beyond that of the signature

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