In the absence of evidence to the contrary, we assume that mantle helium is transported into the crust in melts, from which it subsequently degasses, and that melting occurs principally in the hotter asthenospheric material which undergoes decompression beneath at least some sedimentary basins formed by thermal subsidence. Note, however, that non-magmatic fluids of mantle origin could satisfy the helium observations equally well.

In summary, it appears that the presence or absence of mantle-derived ^{3}He at shallow levels in Neogene sedimentary basins relates to their mechanism of formation. The presence of significant amounts of mantle helium in the Pannonian and Niigata basins and the Rhinegraben is related to the introduction and degassing of mantle-derived fluids produced as an integral part of the extensional process. Extension by dyke injection cannot be easily distinguished from extension by thinning, although some resolution may ultimately be possible because of predicted differences in the amounts of melt introduced. The Molasse basin, a classic loading basin, has developed without the introduction of identified mantle-derived melts or helium. The interpretation of the helium isotope composition in sedimentary basins whose development began in the Palaeozoic or Mesozoic, such as the Hugoton-Panhandle and North Sea basins, is less straightforward and requires some knowledge of the time required for the loss of helium in crustal regions undergoing basin development. Both of these basins have probably received late additions of mantle volatiles. Parts of this work were carried out under a research contract with the European Community, no. EN3G.004.UK(H). The ideas presented in this paper have been discussed with many colleagues, for whose comments we are extremely grateful.

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The c-*erb-A* protein is a high-affinity receptor for thyroid hormone

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Hormone binding and localization of the c-erb-A *protein suggest that it is a receptor for thyroid hormone, a nuclear protein that binds to DNA and activates transcription. In contrast, the product of the viral oncogene* v-erb-A *is defective in binding the hormone but is still located in the nucleus.*

THE avian v-*erb-A* oncogene does not by itself cause tumours in animals, but enhances the erythroblast-transforming potential of primary oncogenes, such as v-*erb-B* or other sarcoma-inducing oncogenes[1,2]. Two specific effects of v-*erb-A* have been characterized. First, erythroblasts transformed by the primary oncogene alone spontaneously differentiate into erythrocytes, and v-*erb-A* completely inhibits this maturation. Second, v-*erb-A* allows transformed erythroblasts to propagate in media with wide ranges in *p*H and salt concentration[1–3]. These observations suggest that v-*erb-A* in erythroid cells affects ion transport systems and regulatory factors of differentiation[3].

It was recently demonstrated that the v-*erb-A*-specific part of the p75$^{gag\text{-}v\text{-}erb\text{-}A}$ protein (encoded by the avian erythroblastosis virus, AEV-ES4) bears homology to receptors for steroid hormones[4–7], suggesting that the effects of v-*erb-A* could be due to direct transcriptional deregulation of genes responsible for the aforementioned effects[3]. To understand these processes better we have isolated and characterized complementary DNA clones of the cellular *erb-A* gene as well as their proteins, and demonstrate that the c-*erb-A* protein has biochemical properties very similar to those of the receptor for the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) (see ref. 8 for review).

Cloning and sequencing of cDNAs for c-*erb-A*

The avian c-*erb-A* gene is transcribed into two messenger RNAs, 4.5 and 3 kilobases (kb) long, that are present at low levels (5–10 copies per cell) in many adult and embryonic tissues (ref. 9 and B.V., unpublished data). We therefore screened with a v-*erb-A* probe a chicken embryonic cDNA library prepared in λ bacteriophage gt11 (refs 10, 11). Ten positive clones were isolated from the 10^6 plaques screened, and two (clones F1 and U1), that hybridized to probes representing 5′ and 3′ domains in v-*erb-A*, were subjected to nucleotide sequence analysis. Figure 1 shows the presence of a long open reading frame of 1,224 nucleotides homologous to v-*erb-A*, suggesting a relative

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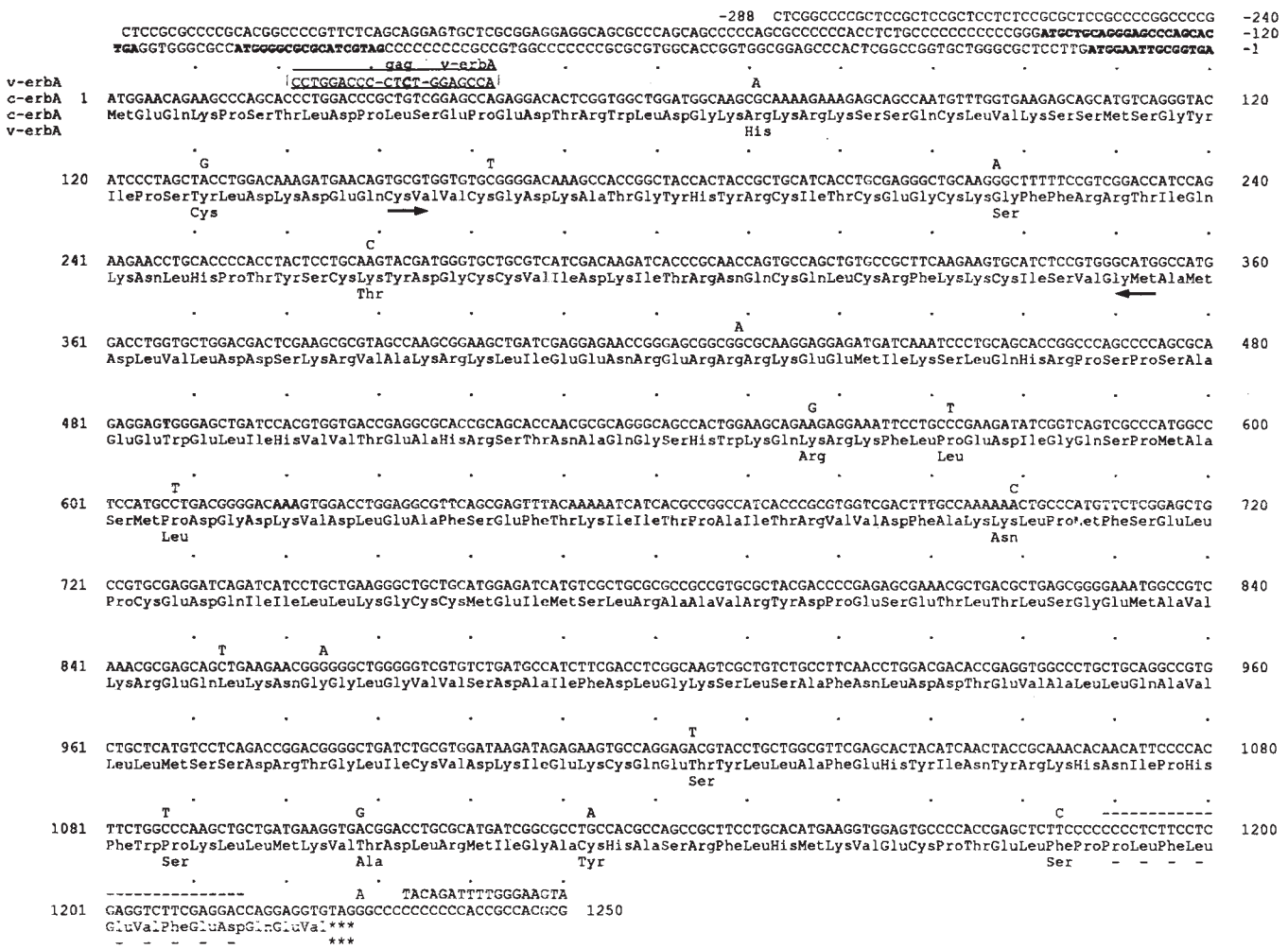

Fig. 1 Nucleotide and predicted amino-acid sequence of the c-*erb-A* cDNA clone F1, and comparison with v-*erb-A*. Only those nucleotides and amino acids in v-*erb-A* that differ from c-*erb-A* are shown, whereas deletions are indicated with bars. The three short open reading frames in the 5′ untranslated region are shown in bold. The *gag* sequence homologous to c-*erb-A* and which bridges the *gag*–v-*erb-A* junction is boxed; nucleotide differences in this sequence are shown in bold letters and deletions with bars. The region homologous to the DNA-binding domain of steroid receptors is indicated by arrows. The U1 cDNA clone starts at nucleotide −84.

Methods. Polyadenylated RNA was isolated from a pool of 10-day-old chicken embryos[9], and a cDNA library was constructed in λ gt11 essentially as described[10,11]. The library (~10^6 plaques) was screened with a nick-translated 2.5-kb *Pvu*II fragment encoding the viral *erb-A* and *erb-B* sequences from AEV. Ten c-*erb-A* positive clones were identified, which were further characterized by hybridization with probes specific for the 5′ and 3′ parts of v-*erb-A*. The inserts of two clones (F1 and U1) hybridizing to these probes were subsequently subcloned into the pTZ vectors[14] for nucleotide sequence analysis using the dideoxy method[50]. The F1 clone (1.55 kb) was sequenced in its entirety on both strands, whereas the U1 (2.1 kb) clone was subjected to partial analysis. No differences between the clones were detected in the coding or 5′ non-coding sequences.

molecular mass ~46,000 (M_r 46K) for the c-*erb-A* protein. The coding sequence is preceded by a 5′ untranslated region of at least 288 nucleotides, which contains three short open reading frames (shown in bold letters) 5′ to the long open reading frame. In addition, a fourth reading frame, lacking an AUG codon, is open for 413 nucleotides (position −287 to 126). Further analyses are required to determine if this reading frame is used for translation by any of the c-*erb-A* mRNAs. Interestingly, the 5′ untranslated sequence is very G+C rich, and a computer-assisted analysis suggested that it could form stable secondary structures (data not shown). Several of the cDNA clones isolated contained long (1–2 kb) sequences downstream of the open reading frame; their nucleotide sequences were not investigated in detail as a preliminary analysis revealed no long open reading frames, thus indicating that they represented 3′ untranslated sequences. These sequences were also heterogeneous in length, suggesting that most of the cDNA clones were generated by non-specific and internal priming of the first strand cDNA synthesis.

A comparison of the coding sequences of the cellular and viral[3,12] *erb-A* genes revealed that v-*erb-A* has undergone 17 point mutations, of which two led to amino-acid substitutions located in the putative DNA-binding domain and 11 in other regions (Fig. 1). Furthermore, the v-*erb-A* protein has lost nine amino acids (nucleotides 1,188–1,215) located three amino acids before the carboxy-terminus. Finally, a comparison between the c-*erb-A* coding sequence and the *gag* sequences of Rous sarcoma virus[13] or AEV (ref. 3) demonstrated a region with 18/22 and 19/22 nucleotide homology, respectively (boxed in Fig. 1), which bridges the junction between the *gag* and v-*erb-A* domains in $p75^{gag\text{-}v\text{-}erb\text{-}A}$. As a consequence, the amino-terminus of the c-*erb-A* polypeptide is 12 amino acids longer than the *erb-A*-specific part of $p75^{gag\text{-}v\text{-}erb\text{-}A}$, when taking into account the region homologous to *gag*. This also suggests that c-*erb-A* was fused to *gag* either by homologous recombination at the DNA level or during retrotranscription of c-*erb-A* mRNA packaged into virions.

Characterization of the c-*erb-A* protein

To determine whether the cDNA clones encode an authentic c-*erb-A* protein, one of them (clone F1) was cloned into the polylinker region of the pTZ19 plasmid vector[14] to allow *in vitro*

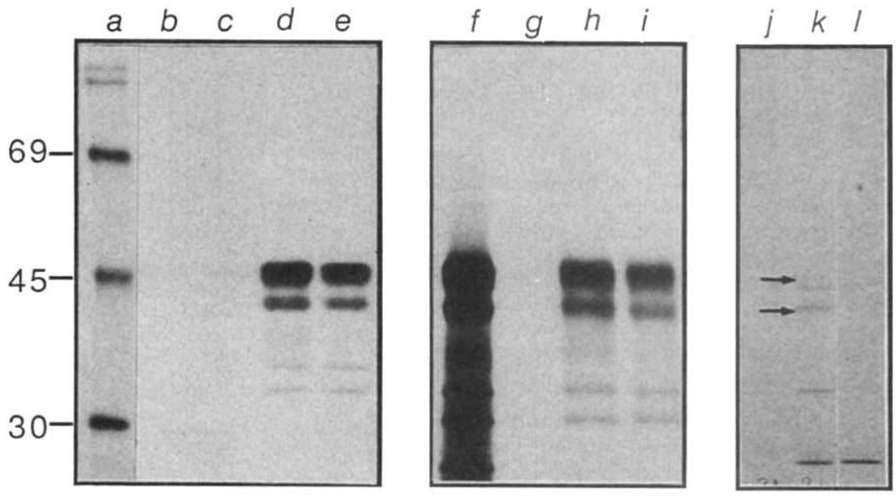

Fig. 2 Synthesis and immunoprecipitation of the c-*erb-A* protein. Capped mRNA, synthesized *in vitro* using T7 RNA polymerase, was translated in rabbit reticulocyte lysates. Lane *a*, marker proteins (sizes shown being relative molecular masses in thousands); lane *b*, unprogrammed lysate; lane *c*, translation with RNA from clone F1 containing the 288-nucleotides long 5′ untranslated sequence; lanes *d* and *e*, RNA from clones F1 and U1 with only 38 nucleotides of 5′ untranslated sequence remaining. Aliquots of the translation products were immunoprecipitated with two different anti-erb-A antisera. Total translation products with 5′-truncated RNA (lane *f*) were immunoprecipitated with preimmune serum (lane *g*) and with two different anti-erb-A antisera (lanes *h* and *i*). Chicken embryo fibroblasts, transiently transfected with expression plasmids, were labelled with [^{35}S]-methionine for 2 h. Immunoprecipitations using preimmune or anti-erb-A antiserum with lysates from cells receiving a c-*erb-A* expression plasmid are shown in lanes *i* and *k*, respectively. Immunoprecipitation with anti-erb-A antiserum of lysates from cells transfected with the plasmid lacking insert is shown in lane *l*.

Methods. To reduce the length of the untranslated leader sequence, plasmids containing the entire F1 and U1 cDNA clones in the appropriate orientation in the *Eco*RI site of pTZ 19 were cleaved with *Eag*I 38 nucleotides upstream of the translation initiation codon, and with *Sma*I in the polylinker region of the vector, treated with the Klenow fragment of DNA polymerase I in the presence of all four deoxynucleotide triphosphates, and religated at low DNA concentration. For *in vitro* transcription, plasmids were linearized with *Hin*dIII or *Eco*RI, treated with proteinase K, extracted with phenol-chloroform and precipitated. Transcription was done with T7 DNA polymerase (100 units ml^{-1}, Biolabs) at 37 °C in 40 mM Tris *p*H 7.9, 6 mM $MgCl_2$, 2 mM spermidine, 10 mM dithiothreitol (DTT), 0.5 mM of the four ribonucleotide triphosphates, 0.5 mM ^{7}mGpppG (Boehringer), 0.1 mg ml^{-1} bovine serum albumin (BSA), 1,000 U ml^{-1} Rnasin, at a DNA concentration of 0.1 mg ml^{-1}. Transcripts were translated in micrococcal-nuclease-treated rabbit reticulocyte lysates (NEN) in the presence of 3 mCi ml^{-1} [^{35}S]-methionine under conditions suggested by the manufacturer. Lysates were diluted 100-fold with lysis buffer before immunoprecipitation, kept on ice for 30 min and cleared by centrifugation at 15,000 r.p.m. for 30 min. Precipitations were essentially as described[3,51] using 5 μl of 1:10 diluted antiserum. Proteins were separated by SDS-polyacylamide gel electrophoresis on 10% gels, and the gels fluorographed and exposed at −70 °C. The anti-erb-A antisera were directed against the 5′ and 3′ domains of v-*erb-A* expressed in bacteria, and will be described in detail in a separate paper. To express c-*erb-A* *in vivo*, the 5′-truncated F1 clone was cloned into the expression plasmid pSV2 (ref. 15).

transcription with the T7 RNA polymerase and subsequent translation in reticulocyte lysates. In addition, to test if the 5′ untranslated sequence influenced the efficiency of translation, two constructs were made with cDNA from the clones F1 and U1, each lacking 250 nucleotides of this sequence. Figure 2 shows that RNA lacking the 5′ sequence (lanes *d* and *e*) directed the synthesis of a major 46K protein, whereas translation with RNA still containing this sequence gave only minor incorporation (lane *c*). Furthermore, the 46K protein as well as a minor 40K polypeptide were identified as c-*erb-A* proteins by immunoprecipitation with monospecific anti-v-erb-A antisera recognizing N- or C-terminal epitopes, respectively (Fig. 2, lanes *h* and *i*).

To demonstrate that the cDNAs directed the synthesis of c-*erb-A* protein also *in vivo*, chicken embryo fibroblasts were transiently transfected with a plasmid expressing c-*erb-A* from the simian virus 40 (SV40) promoter[15]. The cells were labelled after 48 h with [^{35}S]-methionine, lysed and subjected to immunoprecipitation with anti-erb-A antiserum. Figure 2 (lane *k*) shows that the c-*erb-A*-transfected cells contained 46 and 40K proteins, whereas no protein was specifically precipitated from cells transfected with the vector lacking an insert (lane *l*). The anti-erb-A antiserum also reacts with a 46K and a 40K protein expressed at very low levels in many types of avian cells (J.G., in preparation), suggesting that the polypeptides expressed from the cDNA represent authentic c-*erb-A* proteins.

The c-*erb-A* protein binds thyroid hormone

To identify a ligand for the c-*erb-A* protein we first searched the literature for a nuclear hormone receptor expressed in many types of cells that had M_r ~46K. The criteria were fulfilled by the nuclear receptor for the thyroid hormone triiodothyronine (T_3), as a 47K polypeptide that binds T_3 is expressed in many tissues[8,16,17]. Accordingly, c-*erb-A* protein produced by *in vitro* translation in reticulocyte lysates was incubated with [^{125}I]-labelled T_3 in the presence or absence of a 1,000-fold excess of unlabelled hormone, and tested for binding of hormone with a filter binding assay[18]. Figure 3*A* shows that lysates programmed with c-*erb-A* RNA containing the short 5′ untranslated sequence bound the radiolabelled hormone (column *a*, open), and that unlabelled T_3 (column *b*, open) or D-T_4, an analogue of T_3 which is biologically inactive but binds to the T_3 receptor[19], (column *c*, open) complete with most of this binding. In contrast, the binding exhibited by mock-programmed lysates in the presence or absence of competitor (stippled columns *a*, *b* and *c*) was similar to that determined for the c-*erb-A* lysate competed with unlabelled T_3, suggesting that the c-*erb-A* protein specifically binds T_3. Similar results (data not shown) were obtained when the binding was tested by chromatography on Sephadex G75 columns[20]. In addition, a 1,000-fold molar excess of unlabelled dexamethasone, oestradiol, progesterone, aldosterone, testosterone, vitamin D_3 (7-dehydrocholesterol), or tyrosine did not compete with radiolabelled T_3 for binding to c-*erb-A* containing lysates (open bars, Fig. 3*A*). Finally, an excess of these compounds did not compete with [^{125}I]-T_3 for nonspecific binding to mock-programmed lysates (compare the stippled columns *d*–*j* to *a*).

To demonstrate directly that the observed binding was due to c-*erb-A*, lysates containing *in vitro* translated c-*erb-A* protein were subjected to immunoadsorption with anti-erb-A or anti-erb-B antisera bound to protein A-Sepharose beads. The depleted samples were then assayed for binding of [^{125}I]-T_3 in the presence or absence of competitor. Column *m* in Fig. 3*A* shows that the adsorption with anti-erb-A antibodies removed about 70% of the specific binding activity, whereas extracts adsorbed with preimmune or anti-erb-B antisera exhibited no decreased

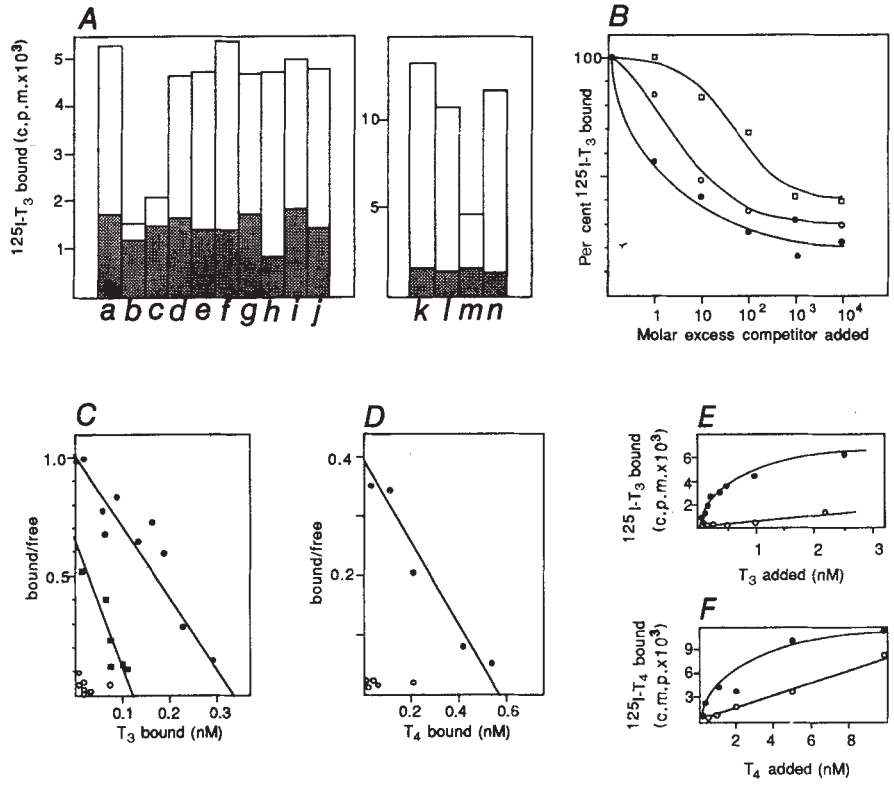

Fig. 3 Ligand-binding properties of the c-*erb-A* protein produced by *in vitro* translation. *A*, left, Reticulocyte lysates programmed with plus strand (open bars) or minus strand c-*erb-A* RNA (stippled bars) were incubated with 1 nM [^{125}I]-T_3 in the presence or absence of 1 μM of various unlabelled competitors: *a*, no competitor; *b*, T_3; *c*, D-T_4; *d*, dexamethasone; *e*, oestradiol; *f*, progesterone; *g*, aldosterone; *h*, testosterone; *i*, vitamin D_3; *j*, tyrosine. right, open bars show that the binding activity of c-*erb-A* protein for [^{125}I]-T_3 was reduced in lysates treated with anti-erb-A but not with anti-erb-B or preimmune sera. Antisera: *k*, no antibodies; *l*, pre-immune serum; *m*, anti-erb-A; *n*, anti-erb-B. Stippled bars show binding in the presence of a 1,000-fold excess of unlabelled T_3. *B*, Competition binding with analogues of T_3. Lysates containing c-*erb-A* were incubated with 1 nM [^{125}I]-T_3 in the presence of the indicated molar excesses of unlabelled competitor. Reverse T_3 (rT_3) is 3,3',5'-thyronine. ●, T_3; ○, T_4; □, rT_3. *C*, Scatchard analysis of T_3 binding. Lysate extracts were diluted (●) 7-fold (K_d = 0.33 mM) or (■) 20-fold (K_d = 0.21 nM); ○, extract of mock-programmed lysate. *D*, Scatchard analysis of T_4 binding. ●, 7-fold diluted extract (K_d = 1.4 nM); ○, extract of mock-programmed lysate. *e*, *f*, Saturation analysis of T_3 and T_4 binding. Binding measured in extracts of (●) c-*erb-A* protein-containing or (○) mock-programmed lysates. Nonspecific binding was determined by parallel incubations with 1 μM unlabelled T_3 or T_4.

Methods. All assays were done with protein synthesized *in vitro* essentially as described in Fig. 2, except that DNA was purified on small Sepharose CL6B columns before transcription, no label was included in the translation reactions, and translation lysates were obtained from Amersham. Aliquots of the lysate were incubated overnight at 0–4 °C with L-[3'-^{125}I]-triiodothyronine (3,000 mCi mg^{-1}) or L-[3',5'-^{125}I]-thyroxine (1,500 mCi mg^{-1}, both Amersham) at the indicated concentrations (generally 0.5–2.0 μl lysate in a total volume of 10 μl) in binding buffer (50 mM NaCl, 2 mM EDTA, 5 mM mercaptoethanol, 20 mM Tris *p*H 7.5, 10% glycerol). Radiolabelled hormone was dried under vacuum to remove ethanol when the final isotope concentration used would exceed 2 nM. Bound radiolabelled hormone was determined[18] by filtration at 4 °C using Millipore HAWP 02500 filters prewetted with 3.5 ml binding buffer. Filters were washed by suction with 5 ml binding buffer at 4 °C three times, and were counted in a gamma counter. Control lysates were programmed with RNA transcribed from a clone with the c-*erb-A* cDNA in the opposite orientation. For immunodepletion, 5 μl of programmed lysate was diluted fivefold with binding buffer, incubated with 2 μl undiluted antiserum for 4 h at 4 °C, and adsorbed for 30 min to protein-A-Sepharose (saturated with erythroblast extract and washed extensively with binding buffer).

binding (columns *l* and *n*). Finally, the proteins adsorbed to the anti-erb-A antibodies bound no T_3, presumably due to conformational changes or steric hindrance imposed by the antibodies (data not shown).

The receptors for thyroid hormone bind with lower affinity analogues[21,22] of T_3, such as thyroxine (T_4) and reverse T_3 (rT_3). We therefore tested if higher concentrations of these analogues would be required to compete with labelled T_3 for binding to c-*erb-A* protein. Figure 3*B* shows that T_4 was 5–10-fold and rT_3 was 50–100-fold less efficient than unlabelled T_3 in competing with [^{125}I]-T_3. These relative efficiencies are similar to those reported for competition of [^{125}I]-T_3 binding to the thyroid hormone receptors[21,22] and reflect the different biological activities of these products.

To determine accurately the affinity of c-*erb-A* protein for thyroid hormone, we performed Scatchard analyses[23] with labelled T_3 and T_4. Because these hormones have been reported to bind with low affinity to unspecific sites at high protein concentrations[24] which affect the dissociation constants measured, we determined the K_d of c-erb-A for T_3 using reticulocyte lysates diluted to different concentrations. Figure 3*C* (filled symbols) shows that the K_d for T_3 was between 0.21 and 0.33 nM with 20- and 7-fold diluted extracts, respectively, whereas the K_d for T_4, determined in a separate experiment with a 7-fold diluted extract, was 1.4 nM (Fig. 3*D*). In contrast, Scatchard analyses done with mock-programmed lysates only indicated the presence of low-affinity/high-capacity binding sites (open symbols). The affinities of the c-*erb-A* protein for T_3 and T_4 are similar to the published values obtained with the thyroid hormone receptor in whole cells and in crude nuclear extracts[22,25].

Finally, the results from the saturation binding experiments (Fig. 3*E* and *F*) showed that the high-affinity sites (closed symbols) were saturable with low concentrations of hormone, whereas the low-affinity sites present in the mock-programmed lysates were non-saturable (open symbols). By calculating the amount of [^{35}S]-methionine-labelled c-*erb-A* protein in the lysates and comparing to the amount of T_3 bound, we estimate that 5–10% of the total *in vitro* synthesized protein bound radiolabelled hormone, a result similar to those found for *in vitro* synthesized steroid receptors[26].

Cellular but not viral *erb-A* protein binds T_3

The T_3 receptor is nuclear regardless of whether or not it has bound hormone[8]. To verify that the c-*erb-A* protein binds T_3 also *in vivo*, and to test the hormone binding capacity of $p75^{gag\text{-}v\text{-}erb\text{-}A}$, chicken embryo fibroblasts containing c-*erb-A* or v-*erb-A* were tested for hormone binding. The c-*erb-A* gene was expressed from a replication-competent retrovirus vector[27], whereas a *wt* and an inactive v-*erb-A* gene (v-*erb-A2*$^-$, ref. 1) were expressed from retrovirus constructs also conferring resistance to the drug G418. To measure nuclear T_3 binding *in vivo*, transfected and fully infected cell cultures were incubated with [^{125}I]-T_3 for three hours, and the radioactivity in the isolated nuclei determined. Figure 4 (left) shows that the nuclei (open

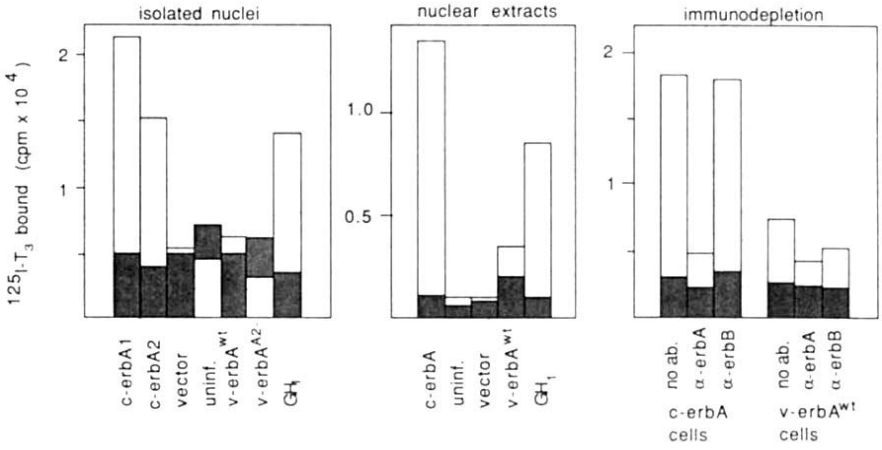

Fig. 4 Hormone-binding properties of c-*erb-A* and v-*erb-A* proteins produced *in vivo*. Left panel, Cells expressing c- or v-*erb-A* were incubated for 3 h with [^{125}I]-T$_3$, and the radioactivity in the nuclei determined. Middle, Hormone binding by nuclear extracts incubated with [^{125}I]-T$_3$ *in vitro*. Right panel, Reduction of *in vitro* binding activity in nuclear extracts by adsorption with anti-erb-A antisera. Open bars show binding of radiolabelled hormone in the absence of competitor, whereas stippled bars indicate binding of [^{125}I]-T$_3$ in the presence of a 1,000-fold excess of unlabelled hormone.

Methods. The F1 cDNA clone containing only the short 5′ leader sequence was cloned into a modification of the replication competent avian retrovirus vector 779/989, a derivative of a previously described vector[27]. Virus constructs expressing *wt* or mutant (v-*erb-A2*$^-$, ref. 1) v-*erb-A* genes were constructed by excising the v-*erb-B* gene from AEV with *Apa*I and *Eco*RI, followed by appropriate modification and insertion of the *Bgl*III–*Bam*HI fragment of the Tn*5 neo*^{R} element. The constructs were introduced into chicken embryo fibroblast cells by the $Ca_3(PO_4)_2$ precipitation technique[52,53]. In the case of the c-*erb-A* construct, virus infection was monitored by reverse transcriptase activity in the culture media, whereas cells containing the v-*erb-A-neo*^{R} viruses were superinfected with RAV1 helper virus and then isolated after selection for G418 resistance. Control experiments showed that these viruses expressed v-*erb-A* at levels similar to those found in AEV-transformed cells (not shown). Cells were grown in Eagle's medium plus 5% fetal calf serum and 2% heat-inactivated chicken serum. For analysis of nuclear T$_3$-receptor content, cells grown for 24–48 h in media containing T$_3$-depleted[54] serum were trypsinized, washed twice with Hank's and resuspended at $\sim 3 \times 10^6$ cells ml^{-1} in T$_3$-free medium supplemented with 0.15 nM [^{125}I]-T$_3$ for 3 h. Cells were subsequently washed with Hank's, and lysed in 1 ml 50 mM Tris *p*H 7.85, 1.1 mM $MgCl_2$, 0.5% Triton-X-100 at 0 °C. Nuclei were collected by centrifugation (1,500 r.p.m. for 5 min) and were washed once more in lysis buffer before counting. GH$_1$ cells were cultured in Eagle's medium containing 10% horse serum and 2.5% fetal calf serum. Isolation of nuclei and preparation of nuclear extracts were done employing techniques used for isolation of nuclear T$_3$ receptor[55], except that inhibitors of proteolysis were included in all steps of isolation. Experiments on [^{125}I]-T$_3$ binding with nuclear extracts and immunodepletion were done as described in Fig. 3.

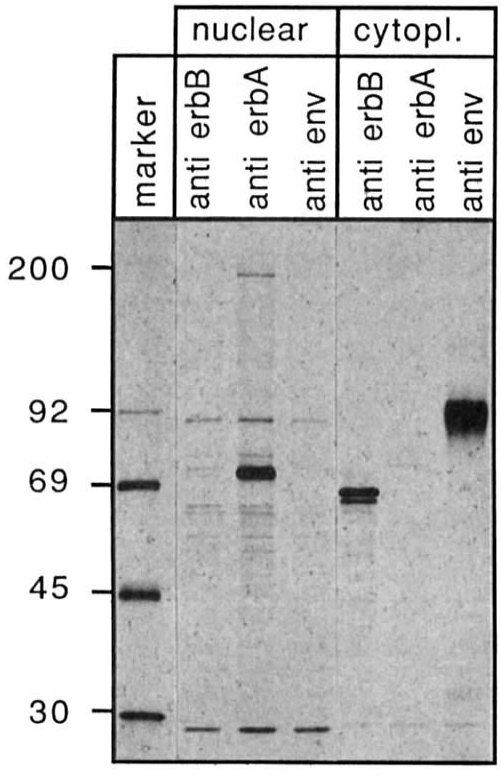

Fig. 5 Demonstration of a nuclear localization for the v-*erb-A* protein. Cell fractionation experiments with AEV-transformed erythroblasts were done as described in Fig. 4. Labelling of cells and immunoprecipitations were carried out as described in Fig. 2. Sizes for markers are relative molecular masses in thousands.

columns) from two different cultures of c-*erb-A* expressing cells (c-*erb-A*1 and 2) bound [^{125}I]-T$_3$ at levels similar to that found in the nuclei of GH$_1$ cells, a rat pituitary line expressing $1\text{–}2 \times 10^4$ T$_3$ receptors per cell[28,29]. In addition, a 1,000-fold excess of unlabelled T$_3$ competed with this binding (stippled columns). In contrast, only a low amount of specific T$_3$ binding was found in the nuclei from the *wt* and the mutant v-*erb-A* expressing cells, or the cells infected with the replication competent vector lacking c-*erb-A* insert. Similar results were obtained when nuclear extracts, prepared from unlabelled cells, were tested for binding of [^{125}I]-T$_3$ *in vitro* (middle panel). Again, receptors from the c-*erb-A* and the control GH$_1$ cells bound radioactivity at levels 8–12-fold higher (open bars) than that observed when binding was done in the presence of an excess of unlabelled T$_3$ (stippled bars). In contrast, the binding seen with extracts from uninfected cells or cells expressing v-*erb-A* was only 1.3–1.7 times background. Lastly, adsorption with anti-erb-A antisera of nuclear extracts from c-*erb-A* but not v-*erb-A* cells reduced the specific binding by 77% (Fig. 4, right panel), demonstrating that the high T$_3$ binding was dependent on c-*erb-A* protein.

To exclude the possibility that lack of nuclear hormone binding by v-*erb-A*-expressing cells was due to cytoplasmic localization of p75$^{gag\text{-}v\text{-}erb\text{-}A}$, a cell fractionation experiment was done. Erythroblasts transformed by AEV were labelled with [^{35}S]-methionine, and nuclear and cytoplasmic extracts, prepared using techniques described for the isolation of the T$_3$ receptor[17], were immunoprecipitated with antisera against v-erb-A, v-erb-B and the viral envelope glycoprotein. Figure 5 shows that the v-*erb-A* protein was present predominantly in the nuclear extract, whereas the v-*erb-B* and *env* proteins partitioned in the cytoplasmic fraction. Previous reports have suggested that v-*erb-A* is localized in the cytosol[30]; these cell fractionation experiments were however done under conditions that may not preserve the nuclear localization of at least the unoccupied oestradiol receptor[31,32].

Discussion

Several lines of evidence suggest that the avian c-*erb-A* gene codes for a thyroid hormone receptor: the molecular weight of the c-*erb-A* protein is the same as that of a previously characterized receptor; it binds T_3 and its analogues with affinities typical of the T_3 receptor; binding activity can be removed with anti-erb-A antibodies; and both the thyroid hormone receptor and c-erb-A are expressed at low levels in many tissues. Finally, Weinberger and collaborators have demonstrated that a human c-*erb-A* protein also binds thyroid hormone *in vitro*[33]. However, the evidence is so far based solely on hormone-binding experiments, and definite proof will require the demonstration that the c-*erb-A* protein exerts the same effects *in vivo* as the thyroid hormone receptor.

Thyroid hormone induces a wide variety of metabolic effects, for instance increased lipogenesis and ketogenesis. These effects have been studied in great detail in liver cells but have also been observed in many other types of tissues[8]. The hormone also plays key roles in differentiation, such as the rapid development of frogs from thyrectomized tadpoles, the induction of growth hormone production in the pituitary, and the development of the brain in neonatals by promoting dendrite formation and myelination[8]. Interestingly, T_3 does not induce the same physiological effects in the brain as in other tissues[34], suggesting that the brain receptor could be distinct from the receptor(s) found in other cells. In fact, several c-*erb-A* related genes appear to exist in the human genome. A c-*erb-A* gene highly homologous to the avian gene reported in this paper is located on human chromosome 17 (refs 35–38), whereas other distinct genes have been found on chromosome 3 (ref. 33).

The thyroid hormone receptor also has a nuclear localization in the unoccupied state as well, and is thought to act by binding to DNA (refs 39, 40) thus inducing or suppressing gene expression in a manner similar to that of steroid receptors[41]. The v-*erb-A* protein binds no hormone, yet it is nuclear, binds to DNA *in vitro* (H.B. and U. Gehring, unpublished data) and exerts specific physiological effects in erythroblasts[3]. It is therefore likely that $p75^{gag\text{-}v\text{-}erb\text{-}A}$ has lost its hormone-binding capacity as a result of one or several of the mutations it has accumulated. Moreover, it is unclear whether it affects the expression of the same genes and in the same manner as the normal receptor, as its presumed DNA-binding region has two amino-acid substitutions. It is possible that $p75^{gag\text{-}v\text{-}erb\text{-}A}$, although unable to bind ligand, acts in a similar fashion as the increased nuclear transfer (nt^i) mutants previously described for the glucocorticoid receptor[41].

The maturation of erythroid cells is accompanied by synthesis of several components important for proper red cell differentiation, such as band 3, the major anion transporter which also serves as an anchor for erythrocyte cytoskeletal proteins, and hemin, a possible regulatory factor of differentiation[42,43]. Interestingly, thyroid hormone regulates the induction of key liver enzymes involved in hemin synthesis[44], and induces increased levels of the Na^+/K^+ ATPase[45,46]. We are therefore currently investigating the possibility that v-erb-A exerts its effects in erythroblasts by suppressing or inducing the synthesis of ion transporters and hemin.

It has previously been demonstrated that thyroid status has a modulating effect on neoplasia. Administration of thyroid hormone to thyrectomized rodents is a prerequisite for the induction of hepatomas by certain chemicals, indicating a role in the initiating action of the carcinogen. In contrast, thyroid hormone appears to promote the growth and metastatic potential of other types of neoplasias, demonstrating an enhancement of tumour progression[47]. In addition, the transformation of cultured cells by radiation or adenovirus infection is facilitated by thyroid hormone[48]. The v-*erb-A* oncogene of AEV enhances the transforming activity of other oncogenes in erythroblasts but also in fibroblasts (refs 1–3, and M. Jansson and B.V., unpublished data). In addition, the integration of a hepatitis B viral genome close to a human *erb-A* related gene was recently reported[49]. The demonstration that the c-*erb-A* protein has properties similar to those of the T_3 receptor may thus represent the first identification of an oncogene that enhances oncogenesis without being able to induce detectable neoplastic effects on its own.

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