Identification and characterization of nuclear retinoic acid-binding activity in human myeloblastic leukemia HL-60 cells

(differentiation/gene expression/retinoic acid nuclear receptors)

CLARA NERVI*, JOSEPH F. GRIppo†, MICHAEL I. SHERMAN‡, MARGARET D. GEORGE*, AND ANTON M. JETTEN**

*Cell Biology Group, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709;
and †Department of Oncology and Virology, Roche Research Center, Nutley, NJ 07110

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ABSTRACT Specific [3H]retinoic acid (RA)-binding sites in nuclear and cytosolic extracts prepared from human myeloblastic leukemia HL-60 cells have been detected by sucrose density gradient sedimentation and size-exclusion high-performance liquid chromatography (HPLC) analyses. This RA-binding activity migrated as a single peak with an apparent molecular weight of 50,000 and >95% of the total binding activity was associated with the nuclear extract. Nuclear extracts prepared from COS-1 cells transfected with an expression vector for the nuclear RA receptors RARα or RARβ were enriched (20- to 100-fold) with a RA-binding activity that coeluted by size-exclusion HPLC with the putative RAR from HL-60 cells. The HL-60 nuclear receptor exhibited high-affinity binding of RA and its benzoic acid analogs Ch55, Ch30, Ro 13-7410, and SRI 6409-40 and low-affinity binding of retinol, Ro 8-8717, and SRI 5442-60, correlating well with the biological activity of these compounds in HL-60 cells. Saturation binding and Scatchard plot analyses of the binding of RA to the nuclear HL-60 receptor yielded an apparent dissociation constant of ~0.46 nM and 1400 ± 100 receptor sites per cell. Northern blot analyses of poly(A)* RNA with cDNA probes specific for RARα and RARβ indicated that HL-60 cells contain predominantly transcripts encoded by the RARα gene. Our results suggest that the observed nuclear RA-binding activity in HL-60 cells might mediate the action of RA in these cells.

Retinoids, analogs of vitamin A, are modulators of cellular proliferation and differentiation in many cell types in vitro as well as in vivo (1-3). In human myeloblastic leukemia HL-60 cells, precursor cells that are able to undergo either myeloid or monocytic differentiation (4-8), retinoids cause terminal differentiation into morphologically mature granulocytes (9-12). This induction of differentiation is accompanied by several molecular changes, including an increase in the expression of NAD glycohydrolase (13) and transglutaminase type II activity (14) and a reduction in the levels of the protooncogene c-myc (15, 16).

The mechanism by which retinoids induce differentiation in HL-60 cells remains to be established. Several studies have demonstrated that these cells do not contain detectable levels of either cellular retinol (CRBP)- or cellular retinoid acid (CRABP)-binding proteins (refs. 17, 18; A.M.J. and T. R. Breitman, unpublished observations). Additional studies with a specific set of benzoic acid analogs of RA, the Ch series, showed that these retinoids, which do not bind to CRABP or CRBP, are very effective inducers of differentiation in HL-60 cells (19-21). These observations indicate that CRBP and CRABP are not involved in the mechanism of action of retinoids in HL-60 cells. It was speculated that other, high affinity receptors are involved in this signal transduction of retinoids (21, 22). Recently, cDNAs of two nuclear RA receptors, RARα and RARβ, were cloned and sequenced (23-26). These RARs are part of a larger gene family of ligand-responsive transcriptional factors, including the steroid and thyroid hormone receptors (27).

In this paper, we demonstrate that nuclei isolated from HL-60 cells contain a receptor that exhibits high binding affinity for RA as well as for the retinoids of the Ch series, and we show that HL-60 cells contain predominantly transcripts encoded by the RARα gene. We hypothesize that this nuclear receptor activity is involved in mediating the action of retinoids in HL-60 cells.

EXPERIMENTAL PROCEDURES

Materials. All-trans-RA, RA analogs Ro 13-7410 and Ro 8-8717, and all-trans-retinol were synthesized by Hoffmann-La Roche. The analogs SRI 6409-40 and SRI 5442-60 were provided by M. I. Dawson (SRI International, Menlo Park, CA). The analogs Ch55 and Ch30 were a gift from K. Shudo (Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan). Medium, serum, and antibiotics were purchased from Gibco. All-trans-[3H]RA (50 Ci/mmol; 1 Ci = 37 GBq) was obtained from DuPont/NEN. The CRABP used in this study was prepared from PCC4.aza1R mouse embryonal carcinoma cells (28). The plasmids RARα0, RARβ0, and ER-RARα.CAS were provided by P. Chambon (Faculté de Médecine, Strasbourg, France).

Tissue Culture and Differentiation. Human myeloblastic leukemia HL-60 cells (passages 11-20) were obtained from T. R. Breitman (National Cancer Institute, Bethesda, MD) and grown as described (10). Granulocyte differentiation was quantitated by the nitroblue tetrazolium reduction assay or by counting the number of differentiated myeloid cells in Wright-Giemsa-stained cell preparations (10). Both methods gave comparable results.

Preparation of Nuclear and Cytosolic Extracts. The preparation of nuclear and cytosolic extracts was based on a procedure described by Eckert et al. (29, 30), with several modifications. Briefly, approximately 10⁶ HL-60 cells or 3-5 × 10⁷ COS-1 cells were collected by centrifugation at 1000 x g for 10 min and rinsed twice with phosphate-buffered isotonic salt solution containing 2 mM EDTA. The cells were then washed gently at 4°C in 10 ml of PTG buffer (5 mM sodium phosphate, pH 7.4/10 mM thioglycerol/10% glycerol) and the following protease inhibitors: phenylmethylsulfonyl fluoride (1 mM), aprotenin (10 units/ml), and leupeptin.

Abbreviations: RA, retinoic acid; RAR, nuclear RA receptor; CRBP, cellular RA-binding protein; CRABP, cellular retinol-binding protein.

†To whom reprint requests should be addressed.

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(10 units/ml). Cells were homogenized in 5 ml of PTG buffer with a Dounce homogenizer (pestle B, 60–80 strokes). The homogenate was centrifuged at 4°C for 15 min at 1000 × g. The pellet, containing the nuclei, was washed once with PTG buffer. The supernatant fractions were combined and centrifuged for 30 min at 130,000 × g at 4°C. The resulting supernate (cytosolic extract) was used in the RA-binding studies. The nuclear pellet was extracted in 3–6 ml of TTGG buffer (10 mM Tris-HCl, pH 8.5/1.5 mM EDTA/10 mM thioglycerol/10% glycerol/0.8 M KCl) and the same protease inhibitors as in the PTG buffer. The suspension was incubated for 1 h on ice with repeated resuspension every 10 min and then centrifuged at 130,000 × g for 30 min. The resulting supernate is referred to as the nuclear extract. Both extracts were used either immediately or after storage at −70°C.

**RA-Binding Assay.** In routine assays cytosolic or nuclear extracts (0.5 ml) were added to 1.5-ml Eppendorf tubes containing [3H]RA to give a final concentration of 3 nM. Nonspecific binding was measured in the presence of a 100- to 200-fold excess of unlabeled RA. After 3 h of incubation at 4°C, extracts were transferred to Eppendorf tubes containing dextran/charcoal pellets obtained after centrifugation of 50 µl of a charcoal/dextran suspension (3% acid-washed Norit A/0.3% dextran C in 10 mM Tris-HCl, pH 7.4/0.02% sodium citrate). After 10 min of incubation at 4°C, the suspension was centrifuged for 15 min at 15,000 × g and the supernate was analyzed for binding activity by sucrose gradient centrifugation or size-exclusion HPLC. Extracts were layered onto linear 5–20% sucrose gradients (11.5 ml) prepared in PTG buffer containing 0.4 M KCl. The gradients were centrifuged for 63 hr at 41,000 rpm (Beckman SW41 Ti rotor) at 4°C. Fractions (0.35 ml) were collected by using a Beckman fraction recovery system, and radioactivity was determined after the addition of Hydrofluor in a Beckman model LS 1800 liquid scintillation counter. Relative sedimentation was defined as the ratio of the fraction number at which protein sediments/total number of fractions. HPLC analysis was performed at 25°C with a Beckman model 3222MP programmable liquid chromatography system. For HPLC analysis, extracts were fractionated over a Superox 12 HR 10/30 size-exclusion column (Pharmacia) at a flow rate of 0.5 ml/min using PTG buffer containing 0.4 M KCl as eluent. Fractions of 0.5 ml were collected and radioactivity was measured. Binding data were calculated as the area under the peak using the trapezoidal rule. Relative elution was defined as the ratio Vv/Vo = volume at which protein is eluted/void volume. Linear least-square analysis of the Scatchard plot was performed with the aid of the computer program BDATAMEF (31, 32). In certain instances the RA binding was expressed per µg of DNA. DNA was determined by the fluorometric assay (33).

**Plasmid DNA.** RARα0 contains the full-length sequence [1770 base pairs (bp)] for the RARα protein cloned into the EcoRI site of the expression vector PSG5 (23, 25). RARβ contains the RARβ cDNA reading frame cloned into the EcoRI and BamHI sites of the expression vector PSG5 (25). ER- RARα.CAS is the inverse construct of the chimeric receptor RAR-ER.CAS described by Petkovich et al. (23)—i.e., the DNA-binding domain of the human estrogen receptor is replaced with the corresponding domain of the human RARα receptor. This construct is cloned into the EcoRI site of the expression vector PSG1. For Northern blot analysis the full-length RARα sequence and RARβ insert were used as probes.

**RNA Isolation and Northern Analysis.** Total RNA from 3–5 × 10⁸ HL-60 cells was isolated by the guanidine hydrochloride method of Cox (34) and Stroehman et al. (35). Poly(A)⁺ RNA was selected following two passages through an oligo(dT) cellulose (type 3, Collaborative Research) column, essentially as described by Jacobson (36). Fifteen micrograms of poly(A)⁺ RNA was fractionated by electrophoresis on a 1% agarose/2.2 M formaldehyde slab gel, stained with ethidium bromide, and transferred to GeneScreenPlus (DuPont) membranes as described by the manufacturer. The blots were baked at 80°C in vacuo for 2 hr and prehybridized at 42°C in 50% formamide/0.75 M NaCl/10% dextran sulfate. Hybridizations were carried out overnight at 42°C in the prehybridization mix described above, supplemented with RARα or RARβ probes (0.5 × 10⁶ cpm/ml), labeled by random hexamer priming (IBI, Prime Time kit) with [32P]-dCTP. Blots were washed twice in 2× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate) for 10 min at 25°C and once in 0.2× SSC/1% SDS for 20 min at 65°C. In the experiment described in the legend to Fig. 4, loading of equivalent RNA was confirmed by ethidium bromide staining and by cross-probing both blots with RARα or RARB.

**RESULTS**

**Analysis of Binding Activity by Density Gradient Sedimentation.** Fractionation of [3H]RA-labeled cytosolic extracts by sucrose density gradient sedimentation did not reveal any specific RA-binding activity (Fig. 1A). However, analyses of labeled nuclear extracts indicated the presence of specific RA-binding activity that sedimented as a single, symmetrical peak between fractions 15 and 22 (Fig. 1B). RA as well as the benzoic acid analog Ch55, which does not bind to CRABP (21), competed with [3H]RA for these binding sites (Fig. 1B). The nuclear extract could be stored for several weeks at −70°C without any loss of RA-binding activity. The omission of 10% glycerol and 10 mM thioglycerol in the PTG buffer used in nuclear extracts resulted in elimination of, or greatly

![Fig. 1. Analysis of [3H]RA binding in cytosolic and nuclear extracts prepared from HL-60 cells by sucrose gradient centrifugation. Cytosolic (A) and nuclear (B) extracts were incubated with 3 nM [3H]RA for 3 hr at 4°C in the absence (●) or presence of a 100-fold molar excess of unlabeled RA (○) or Ch55 (▲). The sedimentation positions of alcohol dehydrogenase (1; Mr 150,000), bovine serum albumin (2; Mr 66,000), ovalbumin (3; Mr 45,000), pepsin (4; Mr 35,000), mouse CRABP (5; Mr 16,000), and cytochrome c (6; Mr 12,400) are marked in A.](image-url)
reduced, RA-binding activity. Glycerol and thioglycerol may play a role in stabilizing the RA receptor.

**Size-Exclusion Chromatography.** [3H]RA-labeled cytosolic and nuclear extracts were also analyzed by HPLC on a Superose 12 size-exclusion column. The HPLC profile of the nuclear extract showed three peaks of radioactivity (Fig. 2B). The major, symmetrical peak eluted at the retention time (T_R) of 27 min. This peak was abolished when nuclear extracts were incubated in the presence of a 100-fold molar excess of either unlabeled RA or the analog Ch55, indicating that this binding of RA is specific. The two peaks eluting at 39 and 43 min consisted of nonspecific binding activity. The profile of the cytosolic extract showed several peaks of nonspecific binding and only a minor peak of specific binding activity that eluted at the same position as that in the nuclear extract (Fig. 2A). Analysis of the binding data obtained from several independent experiments indicated that 95% or more of the total specific [3H]RA-binding activity was associated with the nuclear extract. Maximal binding of [3H]RA to nuclear extracts was obtained after 18 hr of incubation at 4°C. Fractionation of nuclear extracts prepared from [3H]RA-labeled cells by size-exclusion HPLC gave similar profiles as shown in Fig. 2B (not shown).

**Molecular Weight Determination.** The approximate molecular weight of the nuclear receptor was determined by sucrose density sedimentation and HPLC analyses by comparing the relative sedimentation and elution time, respectively, of the nuclear receptor with that of several proteins of known molecular weight, including murine CRABP. From these values, an apparent Mr of 48,000–50,000 (Fig. 2) was calculated for this RA receptor. It should be noted that the molecular weight of the retinoid-binding activity is clearly distinct from that of CRABP.

**Saturation Binding and Scatchard Plot Analysis.** Fig. 3 shows the binding of [3H]RA to the nuclear extract as a function of the RA concentration. Optimal binding was observed at concentrations greater than 2 nM. Scatchard analyses (31, 32) of these data via the least-square method yielded a linear plot (Fig. 3 Inset) consistent with the presence of a single class of binding sites (r = -0.97). The apparent equilibrium dissociation constant (K_d) was calculated to be 0.46 ± 0.03 nM (mean ± SD, n = 4). HL-60 cells were estimated to contain 1400 ± 100 (mean ± SD, n = 4) RAR sites per cell.

**Binding Specificity.** In Table 1 the affinity of several retinoids for the HL-60 nuclear receptor is compared. Retinol, the phenyl analog of RA (Ro-8-8717), and the benzoic acid analog SRI 5442-60 did not compete effectively with [3H]RA for binding to the nuclear receptor, whereas the benzoic acid analogs Ro 13-7410, Ch55, Ch30, and SRI 6409-40 exhibited high affinity for the nuclear receptor. The efficacy with which these retinoids compete with [3H]RA for binding is listed in Table 1.

**Table 1.** Comparison of the ability of several retinoids to bind to the HL-60 RAR and to induce differentiation of HL-60 cells

<table>
<thead>
<tr>
<th>Analog</th>
<th>HL-60 RAR, IC_50</th>
<th>NBT-positive cells, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>3.0</td>
<td>70</td>
</tr>
<tr>
<td>Ro 13-7410</td>
<td>3.0</td>
<td>53</td>
</tr>
<tr>
<td>Retinol</td>
<td>&gt;3000</td>
<td>16</td>
</tr>
<tr>
<td>Ch55</td>
<td>6.9</td>
<td>68</td>
</tr>
<tr>
<td>Ch30</td>
<td>13.5</td>
<td>53</td>
</tr>
<tr>
<td>Ro 8-8717</td>
<td>&gt;3000</td>
<td>12</td>
</tr>
<tr>
<td>SRI 6409-40</td>
<td>15.9</td>
<td>57</td>
</tr>
<tr>
<td>SRI 5442-60</td>
<td>&gt;3000</td>
<td>19</td>
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</table>

Nuclear extracts prepared from HL-60 cells were incubated with 3 nM [3H]RA for 3 hr at 4°C in the presence of the indicated unlabeled analog at concentrations ranging from 0.3 nM to 3 μM. [3H]RA binding was analyzed by HPLC. The IC_50 value is the concentration at which the analog inhibits 50% of the total specific binding. The induction of differentiation by retinoids was measured as the percentage of the cells that stain with nitroblue tetrazolium (NBT) after 6 days of treatment with the indicated retinoid at 0.1 μM. Approximately 11% of the cells were NBT-positive in the absence of added retinoid.

![Fig. 2. HPLC analysis of [3H]RA binding in cytosolic and nuclear HL-60 extracts. Cytosolic (A) and nuclear (B) extracts were incubated as described in the legend to Fig. 1. The samples were fractionated over a Superose 12 HR 10/30 size-exclusion column and radioactivity in each fraction (0.5 ml) was determined. a, [3H]RA only; O, [3H]RA and 200-fold excess of unlabeled RA; a, [3H]RA and 200-fold excess of Ch55, TR, retention time. (Inset) The relative elution time (V_e/V_o) of the proteins was plotted as a function of M_r to estimate the molecular weight of the HL-60 receptor. 1, phosphorylase b (Mr, 97,000); 2, bovine serum albumin; 3, ovalbumin; 4, carbonic anhydrase (Mr, 29,000); 5, murine CRABP; 6, cytochrome c.]

![Fig. 3. Saturation binding and Scatchard plot analysis of the [3H]RA binding to HL-60 nuclear extracts. Nuclear extracts were incubated with the indicated concentration of [3H]RA (0.01-10 nM) in the presence or absence of a 200-fold excess of unlabeled RA. After 18 hr of incubation, [3H]RA binding was analyzed by size-exclusion HPLC. The calculated total ( ), specific ( ), and nonspecific ( ) binding was plotted against the RA concentration. (Inset) Scatchard plot analysis.]

The binding to this receptor was compared to the ability of these compounds to induce differentiation in HL-60 cells (Table 1). A strong qualitative correlation was found between the biological and binding activities of the retinoids tested.

**RARα and RARβ Expression.** To study the expression of the RARα and the RARβ genes in HL-60 cells, Northern blot analyses were performed with poly(A)^+ RNA isolated from HL-60 cells and 32P-labeled inserts of RARα0 and RARβ0 as probes (Fig. 4A and B). The RARα probe hybridized to two RNA species of approximately 3.5 and 2.6 kilobases (kb). Transcripts hybridizing to the RARβ probe were undetectable; however, the RARβ probe did hybridize strongly to RNA isolated from RARβ0-transfected COS-1 cells (not shown) and to RARβ DNA (Fig. 4D). Little cross-hybridization was observed when the RARα and -β probes were used in Northern analysis of RNA from RARα0- or RARβ0-transfected COS-1 cells (not shown) or in Southern analysis of RARα or RARβ inserts (Figs. 4 C and D).

**Transfection of RARα0 and RARβ0 into COS-1 Cells.** Following transfection of COS-1 cells with the expression vector RARα0, RARβ0, or ER-RARα.CAS, nuclear extracts were prepared and analyzed for specific [3H]RA-binding activity by HPLC on a Superose 12 column. The elution profile of extracts from mock-transfected cells (Fig. 5A) or cells transfected with ER-RARα.CAS, a chimeric receptor construct that lacks the RA-binding domain (Fig. 5B), showed very little specific [3H]RA binding. Only a minor peak eluting at 27 min was observed. The elution profile of nuclear extracts prepared from RARα0- and RARβ0-transfected COS-1 cells showed a major peak eluting at 27 min, representing the RARα and RARβ, respectively (Fig. 5 C and D), both exhibiting a Mₐ of 50,000. The expression of these receptors was 20- to 100-fold higher in RARα0- or RARβ0-transfected COS-1 cells than in mock- or ER-RARα.CAS-transfected cells. Both RA and Ch55 were able to compete effectively for the binding of [3H]RA to RARα and RARβ (Fig. 5), whereas retinol, Ro 8-8717, and SRI 5442-60 did not compete (not shown). Particularly in the HPLC profile of nuclear extracts from RARβ0-transfected COS-1 cells, we observed consistently two minor peaks containing specific RA-binding activity, which eluted at 23.5 min (approximate Mₐ 110,000) and near the void volume. The RA-binding activity of the Mₐ 110,000 peak comprised 14 ± 3% of the total RA-binding activity and may constitute a dimeric form of the RARβ, as has been demonstrated for the estrogen receptors (38, 39).

**DISCUSSION**

In the present study, the specific binding of RA to cytosolic and nuclear extracts isolated from human promyeloblastic leukemia HL-60 cells was examined. A single class of specific receptor sites with high affinity for RA was identified in nuclear and cytosolic extracts. More than 95% of total binding activity was associated with the nuclear extract. The minor activity in the cytosol may be due to lysis of nuclei during the isolation procedure or may reflect newly synthesized receptors. In agreement with previous findings (refs. 17, 18; A.M.J. and T. R. Breitman, unpublished observations), AD₃ and detectable levels of the CRABP were found in HL-60 cells. Scatchard plot analysis indicated a dissociation constant of 0.46 nM for the RA receptor complex. The RA receptor possessed an apparent Mₐ of ≈50,000. These characteristics distinguish this RAR from CRABP, which has a Mₐ of 16,000, exhibits >50-fold lower affinity for RA (Kₐ of ≈20–30 nM), and is localized primarily in the cytosolic fraction. Studies on the structure of several benzoid acid analogs of RA (Ch series) demonstrated that these analogs do not bind to CRABP (21). These analogs, however, compete effectively with RA for the binding to the HL-60 nuclear receptor, indicating the existence of different binding specificities of CRABP and the nuclear receptor for retinoids.

Recently, cDNAs encoding two proteins, RARα and RARβ, that bind RA with high affinity have been cloned and sequenced (23–26). Certain domains of these proteins show close homology to the DNA-binding domains of the receptors for thyroid and steroid hormones and vitamin D₃. It appears that the encoded proteins are RA-dependent transcriptional factors. Northern blot analyses of poly(A)^+ RNA isolated from HL-60 cells indicated the presence of predominantly RARα transcripts. Our results differ from those by de Thé et al. (40), which showed the presence of hap (RARβ) gene transcripts in HL-60 cells. RARα and RARβ are homologous (25, 40) and one might find cross-hybridization if the stringency is inadequate. In our study (Fig. 4), the hybridization conditions were such that the RARα and RARβ probes hybridized specifically to the RARα and RARβ DNA, respectively. Similar results were obtained with RARα and

![Fig. 4](image-url) Northern blot analysis of the expression of the genes for RARs in HL-60 cells. Poly(A)^+ RNA (15 μg per lane), isolated from HL-60 cells, was fractionated, blotted to GeneScreen, and hybridized with [32P]RARα (A) and [32P]RARβ (B). The specificity of the RAR probes was verified by Southern analysis of 0.5 μg of full-length RARα (lanes 1) or RARβ (lanes 2) DNA inserts. (C) Hybridization with [32P]RARα. (D) Hybridization with [32P]RARβ.

![Fig. 5](image-url) Analysis of [3H]RA binding to nuclear extracts prepared from COS-1 cells transfected with expression vector RARα0, RARβ0, or ER-RARα.CAS. Extracts were prepared and analyzed as described in the text and the legend to Fig. 2. (A) Extract from mock-transfected COS-1 cells. (B) Extract from ER-RARα.CAS-transfected cells. (C) Extract from COS-1 cells transfected with RARα0. (D) Extract from COS-1 cells transfected with RARβ0. * [3H]RA alone; o, [3H]RA plus 200-fold molar excess of unlabeled RA; △, [3H]RA plus 200-fold molar excess of Ch55. Tₐ, retention time.

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RARβ transcripts in RNA from COS-1 cells. No cross-hybridization was detected. These observations suggest that HL-60 cells contain predominantly transcripts of the RARα gene.

Analysis of nuclear extracts prepared from COS-1 cells, transfected with the expression vector RARα0 or RARβ0, showed that the RARα and RARβ receptors elute at the same position as the HL-60 receptor, exhibiting a Mr, 48,000–50,000, in agreement with the size predicted by the RARα and RARβ cDNA sequence (23). Both RARs, like the HL-60 receptor, bind RA and Ch55, but not retinol, with high affinity. These observations, together with the finding that HL-60 cells contain predominantly RARα transcripts, strongly support the view that the binding activity in HL-60 cells represents the product of the RARα gene. Of course, since both RARα and RARβ proteins copurify under our conditions (Fig. 5) we cannot eliminate the possibility that HL-60 cells also possess some of the latter protein.

During the course of this study, Hashimoto et al. (41) identified a specific receptor activity with Mr, 95,000 in the cytosolic fraction of HL-60 cells. Although it was claimed that this receptor activity was largely nuclear, RA-binding activity in the nuclear fraction was not clearly demonstrated, and the relationship between this protein and the RAR proteins is not clear. Steroid hormone receptors have been shown to form dimers under certain conditions (38, 39) and to bind as a dimer to their responsive elements (42, 43). Since the RARs belong to the same family of ligand-dependent transcriptional factors as the steroid hormone receptors, it is likely that RARs also form dimers that bind tightly to their responsive elements. It is therefore conceivable that the receptor identified by Hashimoto et al. (41) represents a dimeric form of the RAR. The RA-binding activity found in nuclear extracts from RARβ0-transfected COS-1 cells that elutes with Mr, 110,000 may also represent a dimeric form of RARβ.

Studies on the activity of several RA analogs of the Ch series, which exhibited high biological activity in several cell systems, including HL-60 cells (19–21), but which did not bind to CRABP (21), have led to the conclusion that CRABP is not essential in the induction of differentiation by retinoids. The absence of CRABP in HL-60 cells supported this hypothesis (17). It had been predicted that the relevant receptors would be able to bind the retinoids of the Ch series and would have a higher binding affinity for RA (21, 22). These conditions have been met, as demonstrated here, by the RARs. Our studies, therefore, provide support for the hypothesis that the nuclear receptor mediates the action of retinoids in HL-60 cells.

We thank Dr. P. Champon for providing the RARα0 and RARβ0 plasmids and Dr. K. Korach for his advice during this study.