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# IDENTIFICATION AND QUANTITATION OF INTRACELLULAR RETINOL AND RETINOIC ACID BINDING PROTEINS IN CULTURED CELLS

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**Although the mechanism whereby vitamin A mediates normal cell differentiation and inhibits tumor cell proliferation is unknown, intracellular receptor-like proteins for retinol and retinoic acid have been implicated in the molecular action of vitamin A. We have assayed these two binding proteins, cellular retinol binding protein (protein R) and cellular retinoic acid binding protein (protein RA), in the cytosolic fraction of various normal and tumor cells via sucrose density gradient centrifugation and saturation analysis. Employing charcoal separation of bound and free tritiated retinoid, the saturation analysis yields an approximate $K_d$ for ligand binding and an estimate of the number of protein R and protein RA molecules per cell. Unique protein R and protein RA macromolecules sedimenting at 2 S with $K_d$ values of 7–42 nM are detected in murine cells (1° epidermal, 3T6 fibroblasts and melanoma) and human neuroblastoma cells. Concentrations of the intracellular binding proteins range from 55000 to 3000000 copies per cell. When one cell line (C-127 mouse mammary) is transformed by bovine papilloma virus, protein RA levels increase from undetectable to 193000 copies per cell. Assessment of growth inhibition by $10^{-6}$ M retinol or retinoic acid in the culture medium reveals that there exists a partial, but not absolute, correlation between the presence of protein R or protein RA and the antiproliferative effect of the particular retinoid in the tested cell lines. We conclude that the 2 S intracellular binding proteins for the retinoids are present in most vitamin A responsive cells, but may not be essential for biologic actions of the vitamin such as growth inhibition in monolayer culture.**

## Introduction

The two specific intracellular binding proteins for retinol and retinoic acid, denoted protein R and protein RA, respectively, are cytosolic macromolecules of molecular weight approx. 15000 and have N-terminal sequence homology with each other as well as with myelin protein P-2 [1]. The most compelling evidence that protein R and protein RA participate in the biochemical action of retinoids is the strong relationship between the binding affinities of vitamin A congeners for the proteins and the retinoids' respective in vivo and in vitro bioactivities [2,3]. Protein R and protein RA selectively bind their endogenous ligands with high affinity: purified protein R has a $K_d$ of 16 nM for retinol binding [4] and pure protein RA binds retinoic acid with a $K_d$ of 4 nM [5]. The binding proteins have been detected in a wide variety of normal and tumor cells [6], but few

Abbreviations: Protein R, cellular retinol binding protein; protein RA, cellular retinoic acid binding protein.

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quantitative data are available. The proteins are usually identified by sucrose density gradient centrifugation after labeling with the appropriate [$^3$H]retinoid.

Efforts to determine if protein R and protein RA represent true receptors, analogous to the receptors for steroid hormones [7] and vitamin D [8], have met with conflicting results. Using cultured cells as model systems and attempting to correlate the presence of protein R or protein RA with vitamin A responsiveness has led to a positive relationship in the cases of 3T3 and 3T6 fibroblasts [9], MCF-7 and other human breast cancer cells [10], and embryonal carcinoma cell lines [11]. Conversely, human leukemic (HL-60) cells possess undetectable levels of protein RA but are growth inhibited [12] and induced to differentiate into granulocytes by retinoic acid [13]. One group [14] has studied a number of cell lines and found no strict correlation between retinoid mediated growth inhibition and the presence or concentrations of protein R/protein RA.

Subcellular distribution analyses of protein R and protein RA reveal fundamental differences between the retinoid binding proteins and steroid and vitamin D receptors, the latter of which exist as cytoplasmic molecules when unoccupied and translocate to the nuclear chromatin when occupied with hormonal ligand. Radioimmunoassay data [15] indicate that the majority of liver and testis protein R exists in the cytoplasm — whether occupied or unoccupied. Cellular uptake data using [$^3$H]retinoic acid show that only 3–8% of the ligand is localized in target cell nuclei. Moreover, unlike occupied steroid and vitamin D receptors [16], protein RA is not a DNA binding protein (Chandler, J.S., Mangelsdorf, D.J. and Haussler, M.R., unpublished data). Recent work has clarified the possible role of protein R, by demonstrating that the protein delivers retinol to a second binding site on target cell nuclear chromatin [17]. Thus protein R, and possibly protein RA, may constitute delivery proteins instead of primary receptors that influence the biochemical machinery of target cells.

Regardless of the exact molecular role of protein R and protein RA, the presence of these molecules in human tumors [18,19], coupled with the promise displayed by vitamin A as a natural antineoplastic agent in epithelial cells [20,21], makes it of interest to elucidate their biologic function. In the present communication, we present new results on the occurrence, quantitation and correlation of protein R/protein RA and the antiproliferative effects of retinoids in a number of cultured cell lines.

## Material and Methods

*Materials.* All-*trans*-retinol, all-*trans*-retinoic acid and activated charcoal were purchased from the Sigma Chemical Co. (St. Louis, MO). Dextran T-70 was obtained from Pharmacia (Piscataway, NJ). 13-*cis*-retinoic acid and [$^3$H]retinoic acid (28.7 Ci/mmol) were gifts from Dr. A. Liebman, Hoffmann-LaRoche Inc. (Nutley, NJ). [$^3$H]Retinol (4.5 Ci/mmol) was supplied by Dr. Carl Smith of the Division of Cancer Cause and Prevention, National Cancer Institute. [$^3$H]Retinoid purity was routinely assessed by reverse phase high performance liquid chromatography as described elsewhere [22]. [$^3$H]Retinoic acid (28.7 Ci/mmol) was found to be 94% radiochemically pure and consisted of an equal mixture of *cis*- and *trans*-isomers. [$^3$H]Retinol (4.5 Ci/mmol) was determined to be 93% radiochemically pure. ACS aqueous counting solution was purchased from Amersham International (Arlington Heights, IL). Media and sera for cell culture were purchased from Grand Island Biological (Santa Clara, CA). Tissue culture plasticware was obtained from Costar (Cambridge, MA) or Falcon (Oxnard, CA). Antibiotics were purchased from Eli Lilly and Co. (Indianapolis, IN).

*Cells and culture methods.* Primary mouse epidermal cultures were isolated and cultivated according to reported methods [23,24]. Cells were grown in Eagle's minimum essential medium with reduced calcium (0.02–0.07 mM) supplemented with 10% chelexed fetal bovine serum. 3T6 (mouse embryo fibroblast) cells were obtained from the American Type Culture Collection (ATCC), (Rockville, MD). Cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum. CHO (Chinese hamster ovary) cells were a gift of Dr. John Duffy, University of Arizona, College of Medicine, Department of Pharmacology. Cells were main-

tained in minimum essential medium alpha supplemented with 5% fetal bovine serum. LA-N-1 (human neuroblastoma) cells were a generous gift of Dr. Robert Seeger, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA. Cells were maintained in Waymouth's MB752/1 medium supplemented with 10% fetal bovine serum. IMR-32 (human neuroblastoma) cells were obtained from the ATCC (Rockville, MD). Cells were maintained in Eagle's minimum essential medium supplemented with 10% fetal bovine serum. ROS 17/2.8 cells (a subclone of the osteoblastic rat osteosarcoma described elsewhere [25,26]) were a generous gift of Dr. Gideon Rodan, Department of Oral Biology, University of Connecticut, School of Dental medicine, Farmington, CT. Cells were maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum. C127 (mouse mammary) cells were kindly provided by Dr. C. Heilman, NIH. Cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum. C127 B200 (bovine papilloma virus type 1 (BPV-1) transformed mouse mammary cells) were maintained as were the parent line (C127). Cloudman S-91 Clone M-3 (mouse melanoma) cells were obtained from the ATCC (Rockville, MD). Cells were maintained in Ham's F-10 medium supplemented with 10% horse serum and 2% fetal bovine serum. MEL-11A (S91 melanotic subclone) was a generous gift of Dr. Bryan Fuller, Department of Biology, Texas Tech University, Lubbock, TX. Cells were maintained as were the parent line (Cloudman S91-Clone M-3). AM-7 (S91 amelanotic subclone), also provided by Dr. Bryan Fuller, was maintained as was the parent line. All cell culture media were additionally supplemented with 100 units/ml penicillin and 100 $\mu$g/ml streptomycin. All cells were grown in monolayer cultures in tissue culture plasticware. Cells were routinely subcultured and media changed as necessary. Cultures were maintained at 37°C in a humidified 5% $CO_2$/95% air atmosphere.

*Assay for cell growth.* To examine the effects of retinoic acid or retinol on cell growth, cells ($10^5$) were plated in 25 $cm^2$ flasks with 5 ml of culture medium. One day after plating, the culture medium was replaced with medium containing retinoic acid, retinol, or the ethanol vehicle. The culture medium was replaced every fourth day with medium containing either retinoid or vehicle throughout the course of the experiment. Cells were detached with trypsin-EDTA in Dulbecco's phosphate-buffered saline (see below) and counted with a hemacytometer. Cell viability was determined via trypan blue exclusion.

*Assay for protein R/protein RA.* At least $100 \cdot 10^6$ cells from confluent cultures were released with Dulbecco's phosphate-buffered saline ($Ca^{2+}$ and $Mg^{2+}$ free 5 mM sodium phosphate, pH 7.4) containing 0.25% trypsin and 0.02% EDTA. Cells were washed free of serum with Dulbecco's phosphate-buffered saline before assays were performed. The cells were then sonicated in 4 ml of buffer 1 (1 mM EDTA, 10 mM Tris-HCl, pH 7.4) and centrifuged at $159\,000 \times g$ for 45 min to obtain a supernatant (cytosol) fraction. For qualitative identification of protein R or protein RA, 0.4 ml of cytosol was incubated 4–6 h at 4°C with 50 nM of the appropriate [$^3$H]retinoid alone, or in combination with a 100-fold excess of radioinert retinol or retinoic acid. The incubate was then analyzed by centrifugation on 5–20% sucrose gradients at $234\,000 \times g$ for 22 h. Five drop fractions taken from the top of the gradient were counted in 5 ml of ACS scintillation cocktail (26% efficiency). Sedimentation markers used were lactoglobulin (2.0 S), ovalbumin (3.7 S) and bovine serum albumin (4.4 S).

Quantitative assessment of protein R and protein RA in cytosol was accomplished by labeling 100 $\mu$l aliquots with 0–100 nM [$^3$H]retinoid for 24 h at 4°C to determine total binding. Nonspecific binding at each concentration of [$^3$H]retinoid was assessed via parallel incubations in the presence of a 100-fold excess of unlabeled retinoid. Specific binding was assumed to be the difference between the total and nonspecific values. All points were run in duplicate and bound and free retinoid were separated by adding 1 ml of 2.5% dextran T-70-coated charcoal [27] in gelatin-phosphate buffer (0.15 M NaCl, 0.015 M $NaN_3$, 0.1 M $Na_2HPO_4$, 0.039 M $NaH_2PO_4$, pH 7.03 and 0.1% gelatin). After 15 min, the charcoal suspension was centrifuged at $4000 \times g$ for 15 min and the supernatant fluid counted in 10 ml of ACS (26% efficiency).

## Results

Initially, we examined primary cultured cells from a classic vitamin A target tissue-skin [28]. The results of sucrose gradient analysis of protein RA/protein R in cultured epidermal cell cytosol are depicted in Fig. 1. Distinct 2 S peaks of protein RA (Fig. 1, left) and protein R (Fig. 1, right) were present in the cytosol. They displayed saturability, because they were abolished by inclusion of a 100-fold excess of radioinert ligand in the incubation, and specificity because they were unaffected by the inclusion of a 100-fold excess of the alternate nonradioactive retinoid. Quantitation of protein RA and protein R via saturation analysis employing the technique described in Methods resulted in the values of 320 000 molecules of protein RA and 160 000 molecules of protein R per epidermal cell (Table I). Protein RA and protein R displayed dissociation constants of 30 nM and 25 nM for their respective ligands (Table I). The detection of protein RA and protein R in

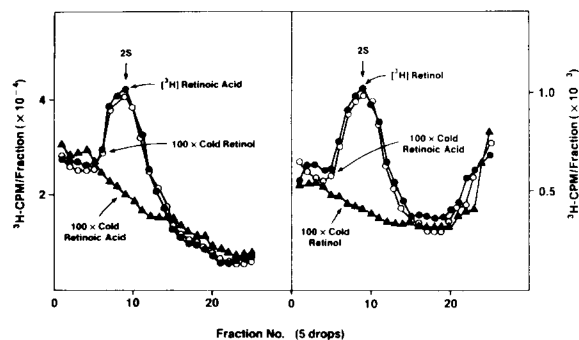


Fig. 1. Sucrose gradient detection of protein RA (left) and protein R (right) in newborn mouse primary epidermal cells. Culture of epidermal cells and sucrose gradient analysis was carried out as described except that cells were Dounce homogenized instead of sonicated. The cytosol contained the equivalent of $30 \cdot 10^6$ cells/ml. Left: cytosol was incubated with 50 nM [$^3$H]retinoic acid alone (●) or in the presence of 5 $\mu$M radioinert retinoic acid (▲) or 5 $\mu$M radioinert retinol (○). Right: Cytosol was incubated with 50 nM [$^3$H]retinol alone (●), or in the presence of 5 $\mu$M nonradioactive retinol (▲) or 5 $\mu$M nonradioactive retinoic acid (○).

TABLE I

CONCENTRATION OF PROTEIN RA AND PROTEIN R IN CULTURED CELLS VERSUS GROWTH INHIBITION BY THE RESPECTIVE RETINOID

B.D. below detectable level by both sucrose gradient and saturation analysis; N.D. = not determined; N.A. = not applicable; + = growth inhibition; − = no growth inhibition.

| Cell line | Retinoic acid | | | Retinol | | |
|---|---|---|---|---|---|---|
| | Protein RA (molecule/cell) | $K_d$ (nM) | Growth inhibition | Protein R (molecule/cell) | $K_d$ (nM) | Growth inhibition |
| 1° Epidermal (mouse skin | 320 000 | 30 | N.D. | 160 000 | 25 | N.D. |
| 3T6 Fibroblast (mouse embryo) | 3 000 000 | 7 | + | 1 300 000 | 12 | + |
| LA-N-1 (human neuroblastoma) | 150 000 | 42 | + | 140 000 | 10 | N.D. |
| IMR-32 (human neuroblastoma) | 211 000 | 21 | + | B.D. | N.A. | − |
| CHO (Chinese Hamster Ovary) | B.D. | N.A. | − | B.D. | N.A. | − |
| ROS 17/2.8 (rat osteosarcoma) | B.D. | N.A. | N.D. | B.D. | N.A. | N.D. |
| Clone M3 Melanoma (mouse) | B.D. | N.A. | + | 450 000 | 38 | − |
| MEL 11-A (mouse melanoma) | 136 000 | N.D. | + | 520 000 | 17 | − |
| AM-7 (mouse melanoma | 55 000 | N.D. | N.D. | 350 000 | 38 | N.D. |

these epidermal cells validates our assay procedures and also correlates with the known ability of retinoids to cause the differentiation of epidermal cells into mucus secreting cells [29].

We next investigated mouse mammary (C-127) cells, a line which does not grow in soft agar, for the presence of protein RA via sucrose gradient analysis. The parent line (C-127) contains only a trace of protein RA-like material (Fig. 2, left panel). However, the level of protein RA is dramatically enhanced after transformation [30] by bovine papilloma virus (Fig. 2, right panel). We estimate that transformed C-127 cells, which are growth inhibited in monolayer by treatment with $10^{-6}$ M retinoic acid (data not shown), contain 193000 copies of protein RA per cell, as opposed to the less than 5000 copies present in the parent line, the growth of which is not regulated by retinoic acid. These results are in contrast to data on BALB/c 3T3 fibroblasts, where protein RA disappears after SV-40 virus-induced transformation [9], but like the study in SV-40 transformed cells [9], they suggest a correlation between the presence of protein RA and the action of retinoic acid to suppress the anchorage-dependent growth of cells.

In order to further evaluate the levels of protein RA and protein R in cultured cells and their possible association with the action of retinoids to regulate growth in monolayer, we have probed a number of established lines. These include the 3T6 embryonic mouse fibroblast, several melanoma and neuroblastoma lines, CHO cells and a rat osteogenic sarcoma line. Fig. 3 illustrates typical examples of three point binding curves that were generated for the quantitation of protein R/protein RA by saturation analysis in all cell lines studied. In mouse melanoma AM-7 cell cytosol (Fig. 3A and B), saturable specific binding of retinol is observed which displays an apparent $K_d$ of 38 nM and corresponds to 350000 protein R molecules per cell. A representative protein RA containing line, LA-N-1 neuroblastoma (Fig. 3C and D) exhibits saturable specific binding of retinoic acid with an apparent $K_d$ of 42 nM and 150000 copies of protein RA per cell. Table I summarizes data on the apparent $K_d$ values and number of molecules per cell for all the tested cell types. $K_d$ values range from 7 to 42 nM and the numbers of in-

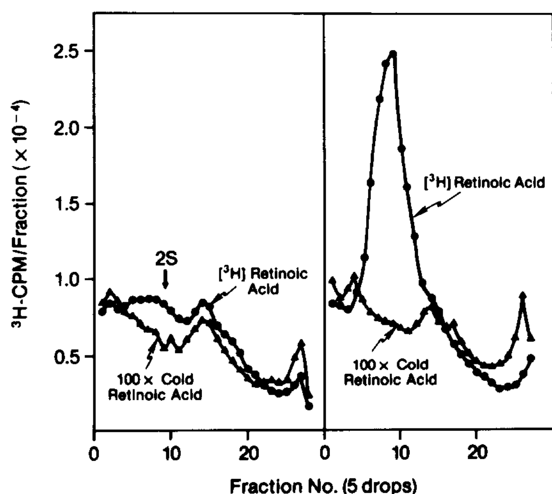


Fig. 2. Apparent induction of protein RA in C-127 cells by viral transformation. Sucrose gradient analysis of protein RA was performed on cytosol from both lines ($23.5 \cdot 10^6$ cells/ml). Left: control C-127 parent line. Right: bovine papilloma virus transformed C-127.

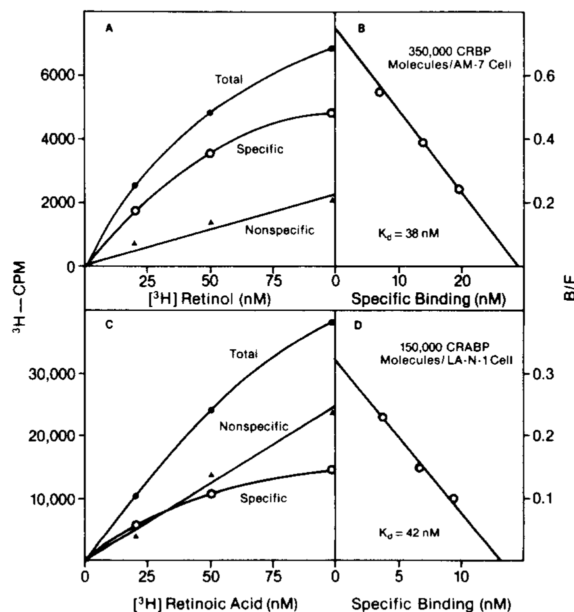


Fig. 3. Saturation-quantitation of protein R in mouse melanoma AM-7 cells (panels A and B, CRBP) and protein RA in human neuroblastoma LA-N-1 cells (panels C and D, CRABP). Cytosols containing $50 \cdot 10^6$ cells/ml were analyzed for specific binding of [$^3$H]retinol or [$^3$H]retinoic acid. Panels B and D represent Scatchard analysis of specific binding from A and C, respectively.

tracellular binding proteins per cell lie between 55 000 and 3 000 000. In all cases where protein R or protein RA were below detectable levels, negative results were obtained by both saturation and sucrose gradient analysis.

Consistent with previous reports [6,9], we observe impressive levels of both protein RA and protein R in embryonic mouse 3T6 cell cytosol (Table I). Also, as depicted in Fig. 4, 3T6 cell proliferation is strongly inhibited by the addition of $10^{-6}$ M retinoic acid or retinol to the culture medium. Thus there is a correspondence between the existence of the intracellular retinoid binding proteins and growth inhibition by retinoids in 3T6 cells. A similar correspondence appears to exist in human neuroblastoma cells, which have recently been shown to respond to retinoic acid by differentiation into mature neuronal cells [31]. Table I indicates that both LA-N-1 [32] and IMR-32 [33] neuroblastoma cells contain appreciable levels of protein RA, but only LA-N-1 possesses detectable protein R. IMR-32 cells, which selectively contain protein RA, are uniquely growth inhibited by retinoic acid in culture (Fig. 4). In two other established lines, CHO and ROS 17/2.8 (osteosarcoma), retinoid binding proteins were undetectable and, at least in the case of CHO cells, this apparent absence of protein RA/protein R agrees with the lack of retinoid effect on cell proliferation (Table I).

Although the above results suggest a positive correlation between the occurrence of the intracellular retinoid binding proteins and the antiproliferative effects of the appropriate retinoid, this association breaks down in the case of melanoma cells. Fig. 5 pictures sucrose gradient analysis of protein RA and protein R in three mouse melanoma cell lines. All three possess significant concentrations of protein R (Fig. 5, lower panels), as evidenced by distinct 2 S peaks of [$^3$H]retinol

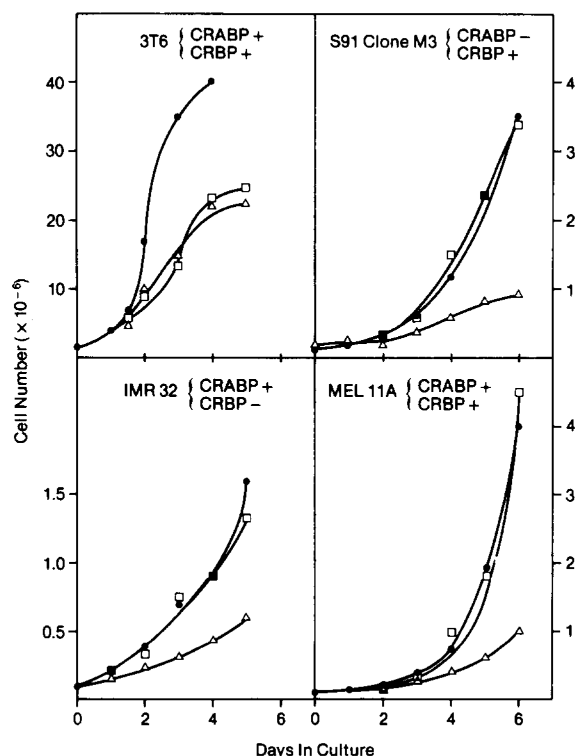


Fig. 4. Growth inhibition by retinoic acid and retinol in cell lines grown in monolayer culture. Growth studies were carried out with $10^{-6}$ M retinoic acid (△) or retinol (□) being introduced at day 1 and day 5 (where appropriate). Control cells received ethanol vehicle (●). In the case of 3T6 (upper left panel), the cells were placed in 55 $cm^2$ plates at a starting density of $1.5 \cdot 10^6$ cells; all other cells were grown as detailed in Methods.

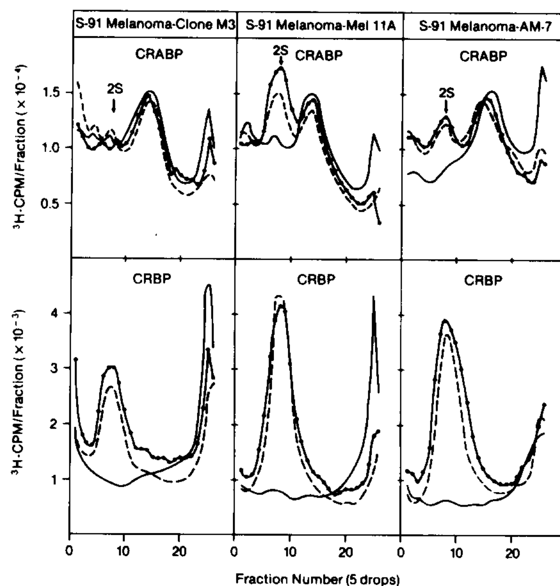


Fig. 5. Sucrose gradient analysis of protein RA (CRABP) and protein R (CRBP) in cloned mouse melanoma cell lines. Cytosols contained the following equivalent numbers of cells per ml: Clone M3 = $32 \cdot 10^6$, MEL-11A = $31 \cdot 10^6$ and AM-7 = $52 \cdot 10^6$. In all panels, the symbols are as follows: (●) 50 nM radioactive ligand, (———) 100-fold excess of same ligand, and (— — —) 100-fold excess of opposite ligand.

binding which are obliterated by excess unlabeled retinol but relatively unaffected by excess radioinert retinoic acid. Protein RA is below detectable levels in the clone M3 melanoma cells and present in small amounts in the melanin-laden (MEL 11-A) and amelanotic (AM-7) lines (Fig. 5, upper panels). The approximate number of protein RA and protein R molecules per cell determined by saturation analysis is summarized in Table I. Various melanoma cell lines are known to contain cellular retinoid binding proteins [6], and therefore the apparent absence of protein RA in clone M3 is somewhat puzzling. The influence of retinoids on the proliferation of two of the melanoma lines in question is illustrated in Fig. 4. S-91 melanoma clone M3 (which appears to lack protein RA) is strongly growth inhibited by retinoic acid, but it is unaffected by retinol, in spite of the fact that it contains high concentrations of protein R. Similarly, MEL 11-A cells, which contain both protein RA and protein R, are only growth inhibited by retinoic acid. Table I contains a compilation of all growth inhibition data we have obtained. We conclude that there is a partial, but not absolute, correlation between the presence of protein RA and protein R and the effects of retinoic acid and retinol, respectively, on the rate of cell growth.

**Discussion**

The present study reports the detection and quantitation of protein R and protein RA in a variety of murine and human cultured cell lines, many of which exhibit a biological response(s) to retinoids. Sucrose gradient analysis (Figs. 1, 2 and 5) demonstrates the presence of 2 S cytoplasmic retinoid binding moieties, which display the specificities of ligand binding typical of protein R and protein RA. Further analysis using saturation binding techniques reveals that between 55000 to 3000000 copies of protein RA and protein R, which bound their ligands with high affinity ($K_d$ = 7–42 nM), were present in each of the positive cells tested. The $K_d$ values reported here range from 2 to 10-fold higher than those determined for pure rat testis protein RA and protein R via fluorimetric titration [4,5]. The larger $K_d$ values reported here can probably be attributed to either the crudeness of the cytosolic fractions employed for these studies, species variation, or (in the case of protein RA) the fact that the [$^3$H]retinoic acid used in this study was approximately an equal mixture of *cis*- and *trans*-isomers. Independent competition and saturation analyses reveal that 13-*cis*-retinoic acid binds protein RA with approximately one-fifth the affinity of all-*trans*-retinoic acid (data not shown). The ability of *cis*-retinoic acid to compete for binding with all-*trans*-[$^3$H]retinoic acid suggests, but does not prove that these ligands bind to the same site on protein RA. Moreover, quantitation of protein RA via Scatchard analysis in 3T6 cell cytosols employing either pure 13-*cis*- or pure all-*trans*-[$^3$H]retinoic acid yield identical numbers of binding sites in either case (data not shown). These data further indicate that both 13-*cis*- and all-*trans*-isomers of retinoic acid bind to the same site on protein RA although with different affinities. Therefore, the concentrations of both protein R and protein RA reported here for the cell lines examined are valid despite the fact that the [$^3$H]retinoic acid used was a mixture of isomers. The number of copies of protein RA/protein R per cell as determined in this study does not allow us to make any conclusive statements regarding their functional role in these cell types. Our finding (Table I) that the presence or levels of protein RA/protein R is not sufficient to predict the susceptibility of cultured cells to growth inhibition by retinoids is consistent with the results of Lotan et al. [14]. It is also in concert with our recent independent report that in a series of human neuroblastoma cells, protein RA correlates with retinoic acid-induced differentiation to the more normal phenotype, but not with growth inhibition in monolayer culture [34].

That protein RA/protein R appears not to be essential for the antiproliferative effects of retinoids can be explained in several ways. It is possible that the lower limits of detection of protein RA and protein R, which are respectively 5000 and 25000 copies per cell at the specific activities of [$^3$H]retinoids employed, preclude our detecting a small, but functionally significant population of protein RA/protein R in apparently 'negative' cell lines. Future experiments with very high specific activity [$^3$H]retinoids should answer this question. A second possibility is that proteases or other enzymes inactivate protein RA/protein R

upon release during sonication of the cells. This hypothesis has been rendered unlikely, at least in the case of HL-60 leukemic cells which lack protein RA but respond to retinoic acid, by an experiment demonstrating that mixing of 3T6 cell cytosol with HL-60 cell cytosol did not destroy 3T6 cell protein RA [12]. In the case of the growth inhibition data (Table I), apparent discrepancies between retinoid bioresponsiveness and the presence/absence of the binding protein could result from cellular metabolism of retinoids. For instance, retinol might be converted to retinoic acid by a specific cell type which appears to respond to both forms, but is actually growth-inhibited only by retinoic acid. A second possibility is that certain cells may rapidly inactivate a particular retinoid in culture, causing negative results in terms of growth regulation. To investigate this, extensive studies of vitamin A metabolism in each of the cell lines in question will be required. In contrast to the above explanations, another possibility is that retinoids function to control cell growth directly at cellular membranes (independently of protein RA/protein R), either via a cofactor-like action in mediating glycosylation of cell surface proteins [35], or by influencing the transmembrane transfer of growth signals or mediators [36]. If this is true, then protein R and protein RA are left to some function other than control of cell growth in the biologic action of retinoids. This could consist of a carrier role, with protein RA/protein R serving to deliver the hydrophobic ligands to metabolizing enzymes on intracellular membranes. Alternatively, protein R and protein RA might serve to transfer retinoids to intracellular sites such as nuclear chromatin, where they could in turn influence replication and transcription to ultimately affect the program for cell differentiation.

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