Calcium is a second messenger that controls a wide variety of cellular functions. Because of its multiple actions, there is a stringent requirement for calcium homestasis, and this is achieved in part by a system of transport and storage proteins such as calreticulin located in the endoplasmic reticulum. Calreticulin is also found in the nucleus, suggesting that it may have a role in transcriptional regulation. It has been reported that calreticulin can inhibit steroid-regulated gene transcription by preventing receptor binding to DNA. Here we report that overexpression of the calreticulin gene in B16 mouse melanoma cells resulted in a decrease in retinoic acid (RA)-stimulated reporter gene expression. Gel shift analysis showed that purified calreticulin inhibited the binding of endogenous RAR to a β-RAR response element oligonucleotide, only if added prior to the addition of the oligonucleotide. Co-immunoprecipitation studies suggest a physical interaction between RAR and calreticulin. Transfection of the calreticulin gene into B16 cells inhibited the RA induction of protein kinase Cα, a marker of RA-induced differentiation. We also found that cyclic AMP increased the expression of calreticulin. Cyclic AMP may act to antagonize RA action by both decreasing RA expression (Y. Xiao, T. Desai, T. Quick, and R. M. Niles, J. Cell Physiol., in press) and stimulating calreticulin levels.

Retinoic acid (RA), a metabolite of vitamin A, is essential for normal cell growth and differentiation (1, 2). RA is thought to achieve its biological action by altering the expression of genes through binding and activating nuclear receptors. These receptors (RARs) are similar to other steroid hormone receptors in that they have a ligand binding domain and a DNA binding domain (3, 4). Many genes are directly transcriptionally controlled by RA (5–8). Most of these genes share a common response element (RARE) in their 5′-flanking region consisting of a hexanucleotide direct repeat, usually separated by 5 base pairs. An additional class of retinoid receptors (RXRα, β, and γ), have been cloned (9). However, they are quite divergent from the RARs in the ligand binding domain and specifically bind 9-cis-RA (a naturally occurring metabolite of RA) but not all-trans-RA, whereas the RAR can specifically bind 9-cis-RA as well as all-trans-RA (9, 11). The RXRs form heterodimers with the RARs as well as several other receptors including thyroid hormone receptors and vitamin D3 receptors (12, 13). The heterodimer form appears to be more efficient for gene activation by RA (14). In addition to forming heterodimers, the RXR can under certain conditions form homodimers that bind to a hexanucleotide repeat separated by 1 or 2 base pairs (15). Also adding to the complexity of these retinoid receptors is the finding that different mRNAs can be produced due to alternate promoter usage and splicing (16).

Calcium is a second messenger that controls a wide variety of cellular functions. Its actions include the regulation of metabolic pathways, the synthesis and release of hormones, apoptosis, and mitosis (17). Because of its multiple actions there is a stringent requirement for the maintenance of cytosolic calcium by transport and storage proteins. Calreticulin fulfills some of these functions and is localized in the lumen of the endoplasmic reticulum in a wide variety of cells (18–21). By immunocytochemistry, calreticulin has also been localized to the nucleus (18), which has led to speculation that the protein may influence gene expression. This protein specifically binds to the synthetic peptide KLGFFKR (22), which is similar to the amino acid sequence in the DNA binding domain of the superfamily of steroid hormone receptors (23–25). Recently Burns et al. (26) and Dedhar (27) showed that calreticulin inhibited the action of glucocorticoid receptors and androgen receptors, respectively. The amino-terminal region of calreticulin was shown to interact with the DNA binding domain of the glucocorticoid receptor and prevent its binding to glucocorticoid response elements (26). Recombinant calreticulin also inhibited the binding of the androgen receptor to its hormone response element in a sequence specific manner and consequently inhibited ligand-stimulated transcription (27). In this report we demonstrate that calreticulin inhibits the transcriptional activity of endogenous RARs in B16 melanoma cells, co-immunoprecipitates with RAR, and inhibits RAR DNA binding. Interference with receptor transcriptional activity is accompanied by inhibition of an RA inducible gene. We also show that calreticulin expression is modulated by cyclic AMP.

**EXPERIMENTAL PROCEDURES**

Materials—Reagents were of the highest commercial grade available. PKCα monoclonal antibody was from Upstate Biotechnology, Inc. 

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**Inhibition of Retinoic Acid Receptor Function and Retinoic Acid-regulated Gene Expression in Mouse Melanoma Cells by Calreticulin**

A POTENTIAL PATHWAY FOR CYCLIC AMP REGULATION OF RETINOID ACTION*

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Calreticulin and Retinoid Action

Fig. 1. Transient transfection of the calreticulin gene inhibits RA-dependent reporter gene expression. B16 melanoma cells were transfected with a plasmid containing the luciferase gene with a thymidine kinase promoter and two copies of the RARE from the RARβ gene together with a Rous sarcoma virus-β-galactosidase plasmid to control for transfection efficiency. Each dish contained 1 μg of RARE-thymidine kinase-luciferase plasmid, 2 μg of Rous sarcoma virus-β-galactosidase plasmid, and 5 μg of pEUK-SV40 plasmid either with or without calreticulin cDNA. Plasmid vector (pEUK-SV40) alone does not have any effect on the luciferase activity. One day after the transfection, cells were split into dishes and treated with or without RA (10 μM) for 24 h. The cells were then harvested and assayed for luciferase and β-galactosidase activity. The data are presented as the means ± S.E. of triplicate dishes. The entire experiment was repeated three times with similar results.

Saranc Lake, NY; rabbit anti-human calreticulin polyclonal antibody was kindly provided by Dr. Sontheimer (Department of Dermatology, Southwestern Medical Center, Dallas, TX).

Cell Culture—B16-F1 melanoma cells were obtained from Dr. I. J. Fidler (M. D. Anderson Hospital and Tumor Clinic, Houston, TX). Cells under passage 25 were grown as a monolayer in 100-mm dishes in Dulbecco’s minimal essential medium with sodium pyruvate and 1000 mg/ml glucose (Irvine Scientific, CA) containing 10% bovine calf serum. Dulbecco’s minimal essential medium with sodium pyruvate and 1000 mg/ml streptomycin sulfate in a humidified atmosphere of 5% CO2/95% air. Routinely dishes were inoculated with 2 × 105 cells unless otherwise indicated. When the cells reached 60–70% confluence, they were split into 60-mm dishes 24 h after transfection and treated with RA for an additional 24 h before harvesting and determining luciferase activity via the commercial kit supplied by Promega.

Isolation of Nuclei and Preparation of Nuclear Extracts—B16 cells were washed with ice-cold phosphate-buffered saline, scraped into phosphate-buffered saline, and collected by centrifugation in a microfuge. Cells were disrupted in Nonidet P-40 lysis buffer (10 mM Tris-HCl, pH 7.4, 3 mM CaCl2, 2 mM MgCl2, and 1% Nonidet P-40). Nuclei were recovered by centrifugation (5 min at 500 × g) and washed twice with ice-cold Nonidet P-40 lysis buffer. The nuclear pellet was then resuspended in extraction buffer (0.05 M Tris-HCl, pH 7.4, 10% (v/v) glycerol, 0.01 M monothioglycerol, 0.001 M Na2 EDTA, 0.001 M phenylmethylsulfonyl fluoride, 0.6 mM KCI, and 2 μg/ml each of aprotinin, leupeptin, and pepstatin), sonicated 3 × 10 s, incubated for 2–3 h at 4°C, and centrifuged at 100,000 × g for 1 h to obtain a soluble nuclear extract.

Electrophoretic Mobility Shift Assay—Two 33-base DNA oligonucleotides were annealed to form a double-stranded oligonucleotide that contained a retinoic acid response element corresponding to that found within the human RARβ gene promoter (30). This β-RARE was labeled with [32P]dCTP (3000 Ci/mmol) by end-filling of the 5’ protruding termini with the Klenow fragment of DNA polymerase I. The labeled β-RARE was purified using a QIAGEN tip-20, precipitated with 10 mg of poly(dI-dC), and 2 μg/ml each of aprotinin, leupeptin, and pepstatin, sonicated 3 × 10 s, incubated for 2–3 h at 4°C, and centrifuged at 100,000 × g for 1 h to obtain a soluble nuclear extract.

Gel shift reactions typically contained B16 nuclear extract (25 μg) or S9 cell extracts from recombinant hRARβ baculovirus-infected cells (1 μg), 10% (v/v) glycerol, 10 × reaction buffer (0.2 mM HEPES, pH 7.9, 0.6 mM KCl, 0.01 mM Na2 EDTA, 0.1 mM monothioglycerol, and 0.01 mM phenylmethylsulfonyl fluoride), 25 μg poly(dI-dC), and 2 × 104 cpm of [32P]β-RARE in 20 μl of total volume. The reactions were incubated for...
20 min at 23–24 °C and immediately resolved at 20 mA at 23 °C in a 5% non-denaturing gel (60:1 acrylamide/bis) in 0.5 × TBE (0.045 M Tris borate, pH 8.0, 0.001 M Na2EDTA). Dried gels were exposed to film between intensifying screens at −80 °C.

Immunoblotting—B16 cells were washed twice with phosphate-buffered saline, scraped into phosphate-buffered saline, and transferred on ice to a 15-ml centrifuge tube. Cells were pelleted at 1000 × g for 5 min and resuspended in 200 μl of protein lysis buffer (20 mM Tris-HCl, pH 7.5, 2 mM EDTA, pH 8.2, 1% Triton X-100, 2 mM dithiothreitol, 50 μM NaF, 10 μM Na3PO4, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 50 μg/ml aprotonin, 87 μM phenylmethylsulfonyl fluoride). Cells were vortexed and sonicated at power setting 3.5 (Heat Systems Sonifier, NY) for 20 s. The cell lysate was transferred to 1.5-ml microfuge tubes and centrifuged for 5 min at 12,000 × g. Protein was determined by the BCA method (Pierce) according to the manufacturer's instructions. Equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis on 10% polyacrylamide gels and then electrophoretically transferred onto nylon membrane (Hybond C, Amersham Corp.) using a semi-dry blotting apparatus (Bio-Rad). The nylon membrane was incubated with 5% nonfat dry milk overnight at room temperature. The membrane was then washed three times with TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.5% Tween 20) for 5 min each at room temperature. The membrane was then incubated with the appropriate antibody for 1 h at room temperature. Subsequently, the membrane was washed as described above and further incubated with goat anti-mouse or goat anti-rabbit IgG labeled with horseradish peroxidase at room temperature for 1 h. The membrane was washed three times with TBST and developed using the ECL kit (Amersham Corp.). Molecular mass standards for SDS-polyacrylamide gel electrophoresis were phosphorylase b (135,000 kDa), bovine serum albumin (85,000 kDa), ovalbumin (50,000 kDa), carbonic anhydrase (39,000 kDa), soybean trypsin inhibitor (27,000 kDa), and lysozyme (17,000 kDa).

Co-immunoprecipitation—RARβ and calreticulin proteins were synthesized in vitro from full-length cDNAs cloned into a vector containing a T7 RNA polymerase promoter through the use of the transcription-and-translation-coupled reticulocyte lysate system (Promega). Co-immunoprecipitation was used to show physical interaction between RARβ and calreticulin. In vitro synthesized [35S]methionine labeled RARβ and calreticulin as well as unlabeled RARβ expressed in SF9 insect cells infected with recombinant baculovirus and purified calreticulin were used for these studies. 5 μl of [35S]methionine-labeled RARβ or calreticulin and 1 μl of purified unlabeled calreticulin or 2 μl of unlabeled SF-9 produced RARβ were mixed and incubated for 1 h at room temperature. At this time 500 μl of NET buffer (0.14 M NaCl, 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, pH 8.0, 0.05% Nonidet P-40) was added to the tubes. To this mixture was added 2 μl of calreticulin polyclonal antibody or 2 μl of RARα IIIB polyclonal antibody, and the tubes were incubated for 1 h at room temperature. At 50 μl of a 50% slurry of protein A-Sepharose (Sigma) was added to the antibody-antigen complex, and the mixture was incubated at room temperature for an additional 1 h. Protein A-Sepharose was collected by centrifugation in a microfuge, and the pellet was washed three times in NET buffer, 50 μl of SDS-polyacrylamide gel electrophoresis sample buffer was added to the pellet, the samples were treated at 100 °C for 5 min, and the resulting supernatant was separated on a SDS-polyacrylamide gel. Following electrophoresis, the gel was treated for fluorography and dried, and signals were visualized by autoradiography.

RESULTS

Transient Transfection of the Calreticulin Gene Inhibits RA-dependent Reporter Gene Expression—Because calreticulin has
been reported to inhibit the function of steroid receptors (26, 27), we determined whether overexpression of this gene in B16 melanoma cells would interfere with RA-dependent expression of a reporter gene containing the β-RARE. B16 cells were transfected with a thymidine kinase-luciferase plasmid containing a synthetic β-RARE showed strong stimulation of luciferase activity by RA (Fig. 1). Co-transfection of different amounts of a calreticulin expression plasmid and treated with 10 μM RA. Then labeled RA-12 were transfected with the calreticulin expression plasmid and treated with 10 μM RA, B, the autoradiogram was quantitated by laser scanning densitometry and the amount of PKCα in the different samples expressed in relative OD units. C, autoradiogram of PKCα in cells transfected with different amounts of calreticulin cDNA plasmid or RARβ12 cDNA plasmid. Control cells in this instance were transfected with 5 μg of pSG-5 plasmid and treated with 10 μM RA for 24 h to induce PKCα. D, autoradiogram in C was quantitated by laser densitometry, and the amount of PKCα protein was expressed in relative OD units.

**Fig. 5.** Transfection of the gene for calreticulin inhibits the RA induction of PKCα. Calreticulin and dominant-negative RARβ12 cDNAs were transfected into B16 cells by the lipofectamine method (Life Technologies, Inc.). The following day the cells were treated with RA (10 μM) for 24 h, and then the cells were harvested, and a total cell extract was prepared. 25 μg of protein from the extracts was analyzed for the amount of PKCα by Western blotting. A, autoradiogram of Western blot analysis of PKCα. The arrow indicates the 80,000 MW PKCα protein. Lanes labeled Ctrl were transfected with carrier plasmid not containing the calreticulin gene and were not treated with RA. Lanes labeled RA-12 represent two separate experiments in which cells were transfected with carrier plasmid not containing calreticulin and then treated with 10 μM RA. Lanes labeled Cal were cells transfected with the calreticulin expression plasmid and treated with 10 μM RA. B, the autoradiogram was quantitated by laser scanning densitometry and the amount of PKCα in the different samples expressed in relative OD units. C, autoradiogram of PKCα in cells transfected with different amounts of calreticulin cDNA plasmid or RARβ12 cDNA plasmid. Control cells in this instance were transfected with 5 μg of pSG-5 plasmid and treated with 10 μM RA for 24 h to induce PKCα. D, autoradiogram in C was quantitated by laser densitometry, and the amount of PKCα protein was expressed in relative OD units.

Purified Calreticulin Inhibits the Binding of RAR from B16 Cells to a β-RARE Oligonucleotide—Because it has been reported that calreticulin can inhibit steroid receptor activity by binding to a region within the DNA binding domain, we determined whether purified calreticulin would interfere with the ability of endogenous B16 RAR to bind to a β-RARE oligonucleotide (Fig. 2). Nuclear extracts from B16 cells contain a protein that specifically binds to the β-RARE oligonucleotide. This protein corresponds to RARs as judged by both co-migration with a band corresponding to authentic RARα from recombinant Baculovirus-infected Sf9 insect cells and by a supershift induced by incubation of the extracts with an polyclonal antibody that cross-reacts with all RAR subtypes (31). The addition of purified calreticulin at a concentration of 2.0 μg to nuclear extract from B16 cells decreased binding compared with control cells. Interestingly, the addition of an antibody to calreticulin reversed the inhibition of binding in the presence of 2 μg of purified calreticulin. The top band in this lane is due to non-specific binding of the calreticulin antibody.

**Fig. 6.** Transfection of the gene for calreticulin inhibits the RA induction of PKCα. Panel A shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA). Panel B shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA). Panel C shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA). Panel D shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA). Panel E shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA). Panel F shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA). Panel G shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA). Panel H shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA). Panel I shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA). Panel J shows a Western blot analysis of PKCα in cells transfected with CAL (control), RA (treated with RA), Cal (transfected with calreticulin), and RA (treated with RA).
radioactive protein.

Overexpression of the Calreticulin Gene Inhibits RA-induced PKCα Expression—Treatment of B16 melanoma cells with RA results in a time-dependent increase in PKCα (Fig. 4, A and B) protein. Because calreticulin decreased RA-dependent reporter gene expression, we tested its ability to inhibit the induction of this marker of RA-induced differentiation described above. Using the Lipofectin reagent we were able to routinely transfect up to 65% of the B16 cells (data not shown); therefore we used this procedure to transiently express the calreticulin gene. Fig. 5 shows that in cells transfected with the carrier plasmid, RA still induces a 6–9-fold increase in PKCα protein. However, transfection of B16 cells with the plasmid containing the calreticulin gene results in a greater than 70% decrease in the induction of PKCα. As a positive control we also transfected cells with RARβ12, a dominant/negative receptor that we have found to inhibit the function of RARα, β, and γ (data not shown). Expression of this dominant/negative receptor also inhibited the induction of PKCα by 70%. The decrease in RA induction of PKCα was proportional to the amount of transfected calreticulin cDNA (Fig. 5, C and D).

Calreticulin Levels Are Increased by Cyclic AMP—We have previously found that cyclic AMP can inhibit RA-dependent reporter gene expression.2 We investigated whether cyclic AMP could be achieving some of its effect by increasing calreticulin levels. B16 cells were treated for increasing times with RA, 8-bromo-cyclic AMP, or a combination of these compounds (Fig. 6). The amount of calreticulin, as determined by immunoblotting, in control cells increased at 48 h and then remained constant. RA induced a modest increase in calreticulin at 24 and 48 h but not at 72 h of treatment. 8-Bromo-cyclic AMP (0.5 mM), or both agents for the times indicated. Total cellular protein was harvested at various times and subjected to Western blot analysis using polyclonal antibodies against calreticulin (1:500 titer). A, autoradiogram of Western blot for calreticulin. The arrow indicates the immunoreactive calreticulin protein. B, the autoradiogram was subjected to laser densitometry, and the amount of calreticulin protein was expressed as relative OD units.

FIG. 6. Time-dependent effect of 8-bromo-cyclic AMP and RA on calreticulin protein levels in B16 melanoma cells. B16 melanoma cells were seeded and treated with RA (10 μM), 8-bromo-cyclic AMP (0.5 mM), or both agents for the times indicated. Total cellular protein was harvested at various times and subjected to Western blot analysis using polyclonal antibodies against calreticulin (1:500 titer). A, autoradiogram of Western blot for calreticulin. The arrow indicates the immunoreactive calreticulin protein. B, the autoradiogram was subjected to laser densitometry, and the amount of calreticulin protein was expressed as relative OD units.

FIG. 7. Concentration-dependent effect of 8-bromo-cyclic AMP on the expression of calreticulin protein in B16 melanoma cells. B16 cells were seeded and treated with the indicated concentrations of 8-bromo-cyclic AMP for 48 h. Cells were then harvested, and 50 μg of total cellular protein was analyzed for the amount of calreticulin protein by Western blotting. A, autoradiogram of Western blot. The arrow indicates the immunoreactive calreticulin protein. B, the autoradiogram was quantitated by laser densitometry, and the amount of calreticulin was expressed as relative OD units.

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AMP increased the amount of calreticulin at every time point examined. The greatest degree of stimulation was 9-fold at 24 h of treatment. There was no consistent pattern of change in calreticulin levels when both agents were incubated with the cells, although in all cases the level was higher than in control cells. The increase in calreticulin was dependent on the concentration of 8-bromo-cyclic AMP, with 0.5 mM inducing a 4.2-fold increase in the amount of this protein (Fig. 7).

DISCUSSION

These studies demonstrate that overexpression of calreticulin can inhibit RA stimulation of a luciferase reporter gene controlled by a consensus RARE (DR-5) in B16 mouse melanoma cells. These cells express RARα and γ constitutively, and RARβ expression is induced by RA. Therefore, it is likely that all of these endogenous receptors are affected by calreticulin. We did not definitively prove the mechanism of action of calreticulin in inhibiting RA-dependent reporter gene expression in B16 cells, but the gel shift and co-immunoprecipitation experiments suggest that inhibition of RAR binding to RARE is a likely explanation. This conclusion would agree with the proposed mechanism for calreticulin action on steroid hormone receptor, i.e., binding to a region in the zinc fingers and thus inhibiting receptor binding to the hormone response element (26).

We found that overexpression of calreticulin also decreased the induction of PKCa by RA. The endogenous RAR are involved in mediating the action of RA on the expression of these genes because a dominant-negative RAR (RARα2) also blocks the RA induction of these genes. Thus it is likely that calreticulin inhibits RA induction of PKCa expression by inhibiting RAR function. The only other report on calreticulin and RA-RAR action is that of Dedhar et al. (27), who established stable transfectants of teratocarcinoma cells expressing sense or antisense calreticulin cDNA. Analysis of these stable transfectants showed that expression of sense calreticulin cDNA inhibited RA-induced differentiation of these cells, whereas expression of antisense calreticulin cDNA enhanced RA-induced differentiation.

Previously we had observed that cyclic AMP antagonized RA function in B16 melanoma cells (32). In light of these findings, we tested the hypothesis that cyclic AMP might elicit some of its effect by increasing calreticulin expression. Our experiments showed that cyclic AMP increased the amount of calreticulin protein by 9-fold within 24 h of treatment. RA also induced a modest increase at 24 and 48 h of treatment. Calreticulin expression was previously studied in B16 cells where it was referred to as B50 (33). It was suggested that the expression of this protein could be associated with the proliferation of the melanoma cells. Because we have found that calreticulin can inhibit RA-RAR function and RA induces growth inhibition and differentiation in B16 melanoma, high levels of calreticulin should theoretically enhance growth. Expression of calreticulin in L6 myoblasts has been investigated by Opas et al. (18). They found that Ca2+ shock, inhibition of calmodulin, and activation of protein kinase C had no profound effect on calreticulin expression as measured by immunocytochemical techniques. Inhibition of protein kinase C with H-7 resulted in a small decrease in calreticulin, whereas differentiation of myoblasts to myotubes was accompanied by a large decrease in calreticulin staining in myotubes. Recent studies using a reporter gene under the control of calreticulin’s promoter indicate that expression of calreticulin is significantly affected by changes in intracellular Ca2+. In contrast, infection of mammalian cells with bovine papillomavirus type I results in a significant increase of calreticulin, with the 3’ end of the early region of the virus being responsible for this induction (34). The studies described in this report appear to be the first description of hormonal up-regulation of calreticulin expression.

We previously found that 8-bromo-cyclic AMP inhibited the RA induction of PKCa. Part of this decrease could be explained by the ability of cyclic AMP to inhibit the expression of the RARs. However, because cyclic AMP increases the amount of calreticulin, this pathway may also contribute to the inhibition of RA-regulated gene expression. Indeed we observed that although cyclic AMP decreased RAR mRNA expression, nuclear extracts from treated cells had an even greater decrease in protein binding to a consensus RARE in gel shift assays. This result might have been due to an increase in calreticulin, which bound to the endogenous RARs and prevented them from interacting with the DNA response element.

In summary we have found that calreticulin can negatively regulate RA function in B16 melanoma cells. We further showed that B16 cells express calreticulin and that its levels can be increased by cyclic AMP. This provides a means by which hormones working through the cyclic AMP signal transduction system can regulate a cell’s response to retinoids. Because retinoids play an important role in regulating growth and differentiation (10), inappropriate expression of the calreticulin gene either directly through mutation, translocation, etc., or indirectly through the cyclic AMP pathway could have deleterious consequences for the cell.

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Inhibition of Retinoic Acid Receptor Function and Retinoic Acid-regulated Gene Expression in Mouse Melanoma Cells by Calreticulin: A POTENTIAL PATHWAY FOR CYCLIC AMP REGULATION OF RETINOID ACTION
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