Retinoic Acid Down-regulation of Fibronectin and Retinoic Acid Receptor \( \alpha \) Proteins in NIH-3T3 Cells

BLOCK OF THIS RESPONSE BY ras TRANSFORMATION* (Received for publication, August 30, 1995, and in revised form, November 14, 1995)

Giorgio Scita, Nadine Darwiche, Eileen Greenwald, Miriam Rosenberg, Katerina Politi, and Luigi M. De Luca‡

From the Laboratory of Cellular Carcinogenesis and Tumor Promotion, NCI, National Institutes of Health, Bethesda, Maryland 20892

All-trans-retinoic acid (RA) markedly reduced the level of intracellular fibronectin (FN) in a time- and concentration-dependent fashion in NIH-3T3 cells, but not in NIH-3T3 cells transformed by an activated Ha-ras oncogene. Pulse/chase experiments indicated that RA affects FN biosynthesis rather than its turnover rate. Steady state levels of FN transcripts did not change after treatment of the cells with RA for various times or concentrations, suggesting that RA acts at the translational level. Similar effects were observed in other fibroblasts.

In NIH-3T3 cells, RA had distinct effects on different receptors; it down-regulated retinoic acid receptor (RAR) \( \alpha \) protein and transcript levels, it up-regulated RAR\( \beta \) transcripts, and it had no effect on RAR\( \gamma \). Transformation of NIH-3T3 cells with an activated Ha-ras oncogene down-modulated RAR expression and abolished responsiveness to RA. We identified the retinoid signal transduction pathways responsible for the effects of RA on FN and RAR\( \alpha \) proteins by the use of the retinoid X receptor-selective compound, SR11237, by stable over-expression of a truncated form of the RAR\( \alpha \) gene, RAR\( \alpha \)403, with strong RAR dominant negative activity, and by overexpression of RAR\( \beta \). We conclude that: 1) RA-dependent FN down-modulation is mediated by RARs, 2) retinoid X receptors mediate the observed reduction of RAR\( \alpha \) by RA, and 3) the block of RA responsiveness in Ha-ras cells cannot be overcome by overexpression of RAR\( \alpha \).

These studies have defined fibronectin and RAR\( \alpha \) as targets of RA in fibroblast cells and have shown that oncogenic transformation renders the cells resistant to RA action.

All-trans- and 9-cis-retinoic acid are the mediators of vitamin A action on growth and differentiation of normal, premalignant, and malignant cells (1, 2). Their effects are mediated by two classes of nuclear receptors, the RARs\(^1\) and RXRs, for which they function as respective ligands. Distinct RAR- and RXR-dependent gene pathways exist, and individual receptor subtypes may control distinct gene expression patterns by interacting with RAREs, or RXREs, in the promoter region of different responsive genes (3, 4). RA has proven effective in transformation therapy of acute promyelocytic leukemia, a disease characterized by a t(15;17) translocation with breakpoint in the RAR\( \alpha \) gene (5). In vitro, overexpression of RAR\( \alpha \) has been shown to suppress transformation by v-myb in monoblasts (6) and by polyomavirus in rat fibroblasts (7, 8). In addition RA treatment of NIH-3T3 cells transformed by the introduction of an activated Ha-ras oncogene inhibited focus formation (9). Various reports have shown opposing effects of RA and ras on the regulation of the expression of different genes (10–13), suggesting that an interaction between the signal transduction pathways mediated by RA and ras may take place. Therefore, studies of this interaction may provide insight into the mechanism whereby RA inhibits transformations.

FN is a large transformation-sensitive glycoprotein composed of two non identical subunits of 220 kDa. It exists in the extracellular matrix and in soluble form in the plasma. Cellular FN is produced in large amounts by fibroblasts and is implicated in a wide range of cellular processes including cell adhesion, migration, morphology, differentiation, and transformation (14, 15). It is modulated by a variety of effectors including cytokines like transforming growth factor \( \beta \) and hormones like glucocorticoids and RA. Loss of cell surface FN is a hallmark of transformation, and it has been correlated with acquisition of tumorigenic and metastatic potential. This effect has been observed with many oncogenic stimuli, among which are the ras oncogenes (14, 16, 17).

The ras genes encode a 21-kDa plasma membrane protein that binds guanine nucleotides and is involved in signal transduction, cell growth, and differentiation (18). Many types of tumors express mutated forms of the ras protein, resulting in constitutive activation of this protein and altered gene expression (18).

In this study, we identified fibronectin as target of RA action in NIH-3T3 cells but not in ras-transformed fibroblasts. We also identified retinoid receptors involved in this process.

EXPERIMENTAL PROCEDURES

Cell Culture—NIH-3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum, 400 units/ml penicillin, 100 \( \mu \)g/ml streptomycin, and 2 \( \mu \)M glutamine. Cells were grown to subconfluence and treated with retinoids. Activated Ha-ras-transfected cells were generated and cultured as described (10). All comparative studies were done with matched pair dones, i.e. all the cells were always transfected with vector plus/minus the activated ras construct. Furthermore identical results were obtained in four NIH-3T3 clones compared to their ras-transformed counterparts. C3H10T1/2 cells were obtained from ATCC (Rockville, MD) and cultured as described (19). Primary mouse skin fibroblasts were isolated and cultured as described (20).
Retinoids—RA was obtained from Sigma. The RXR-selective compound, SR11237 (21), was kindly provided by Dr. A. Levin (Hoffman La Roche). RA and SR11237 were dissolved in Me2SO at 10 mM and in ethanol at 1 mM, respectively. The maximum concentration of the solvent used was 0.03% for Me2SO and 0.1% for ethanol. Subconfluent fibroblasts were incubated in the presence of the media containing the respective solvents and treated as described. G418-resistant colonies were selected in medium containing 1 mg/ml G418, and positive clones (one expressing RARα protein) were isolated and expanded.

Northern Blot Analysis—Isolation of total RNA was performed by using a Total RNA isolation kit (Tet-Test "b", Inc, Friendswood, TX). The full-length fragments of the mouse RARs were excised from the expression plasmid pSG5/RAR (29, 30). The 1.4-kilobase human RARα fragment was obtained from Life Technologies, Inc. The probes were labeled with [32P]dCTP using random primer labeling methods. Total RNA (40 μg) was fractionated on a 1% agarose gel and blotted overnight onto Schleicher & Schuell nitrocellulose. The membranes were prehybridized for 2 h at 65°C in a buffer of 5× SSC, pH 7.0, 5× Denhardt's, 0.05% sodium phosphate, pH 6.8, 0.1% SDS, 5× EDTA, 0.1% sodium deoxycholate, 0.5% sodium deoxycholate, 1.0% Nonidet P-40, 0.5% phenylmethylsulfonyl fluoride, and 10 μM leupeptin. Cell lysates were centrifuged at 15,000 × g for 5 min at 4°C, and the insubile pellets were discarded. Volumes containing equal amounts of radioactivity (precipitation with 10% trichloroacetic acid) were subjected to immunoprecipitation. Polyclonal rabbit mouse FN antibodies (5 μl) were added. After 2 h of incubation at room temperature, 25 μl of prewashed Pansorbin (Calbiochem, La Jolla, CA) was added and the samples were incubated overnight at 4°C on a rotary shaker. The immunoprecipitates were washed three times with TTBS washing buffer (20 mM Tris-HCl, pH 8.5, 150 mM NaCl, 0.5% sodium deoxycholate), solubilized in SD sample loading buffer, and loaded onto 4–15% polyacrylamide gels (22).

Pulse/Chase—The reduced level of newly synthesized FN in RA-treated cells may be due to either a reduced rate of biosynthesis or an increased turnover rate. To explore these possibilities we performed pulselabel experiments. Fibroblasts were pulsed for 20 min with [35S]methionine and [35S]cysteine and chased with unlabeled amino acids for 60 min and centrifuged to get rid of insoluble cell debris. Protein concentration was determined by the bicinchoninic acid method (23) (Pierce). Reaction products were denatured in sample buffer containing 3× SDS, 2 mM phenylmethylsulfonyl fluoride, and 10 μg/ml trypsin for 5 min to remove extracellular cell surface FN. The reaction was stopped by adding 2 μg/ml trypsin to the samples. The samples were washed three times with TTBS washing buffer (20 mM Tris-HCl, pH 8.5, 150 mM NaCl, 0.5% sodium deoxycholate), solubilized in SD sample loading buffer, and loaded onto 4–15% polyacrylamide gels (22).

Immunoblot Analysis—To determine intracellular FN levels, retinoic- and Me2SO-treated cells were washed and trypsinized as described in the pulse/chase section. Cells were lysed in Laemmli buffer without reducing agent and bromphenol blue. Lysates were boiled and run immediately on a 7.5% polyacrylamide gel. The proteins were transferred to nitrocellulose (Schleicher & Schuell) on a Bio-Rad electroblot apparatus. The membranes were stained with Ponceau S (Sigma) to ensure the integrity of the membrane preparations. The membranes were prehybridized for 2 h with buffer B (20 mM Tris-HCl, pH 8.5, 150 mM NaCl, 0.5% sodium deoxycholate), solubilized in SD sample loading buffer, and loaded onto 4–15% polyacrylamide gels (22).

RESULTS

Inhibition of FN Biosynthesis by RA in NIH-3T3 but Not in Ha-ras NIH-3T3 Cells—FN levels were markedly reduced by RA in pSVneo, but not in Ha-ras-transfected cells (Fig. 1A). The effect was specific for FN, since neither collagen type IV nor laminin B1 and B2 chains (the only ones to be detected in NIH-3T3 cells) were affected. RA inhibition of FN was time-dependent (Fig. 1B) and concentration-dependent (Fig. 1C). A 3-fold reduction of intracellular FN was already observed after 12 h of treatment (Fig. 1B). RA caused a 90% down-regulation at 2 × 10^-6 M.

Mechanism of RA Inhibition of Newly Synthesized FN—RA-treated cells were metabolically labeled with a mixture of [35S]methionine/cysteine for 20 min, lysed, and immunoprecipitated with specific anti-FN antibodies. The choice of the labeling time was crucial because FN takes about 30 min to reach the cell surface from the cytosolic site of synthesis (data not shown and Ref. 35). RA inhibited the newly synthesized FN in a time-dependent fashion. An 80% reduction was observed.
after 4 h of RA treatment (Fig. 2A).

In pulse/chase experiments, the rate of disappearance of labeled FN was not altered by RA treatment of the cells (Fig. 2B). The time required to reduce the amount of $^{35}$S-FN by 50% was 28 ± 2 and 31 ± 3 min in RA- and Me$_2$SO-treated cells, respectively. Therefore, a reduction of FN biosynthetic rate likely accounts for the effects of RA.

Northern blots of total cellular RNA from RA-treated and control cells were hybridized to FN and GAPDH probes. The FN mRNA band intensities, which represent relative steady-state levels, were normalized to GAPDH mRNA band intensities. The results (Fig. 3) reveal that the accumulated levels of FN mRNA were not altered either by different RA concentration or by times of RA treatment. RA failed to alter FN mRNA in Ha-ras NIH-3T3 cells.

**RAR modulation of RARs in pSVneo NIH-3T3 but Not in ras fibroblasts**

RA Modulation of RARs in pSVneo NIH-3T3 but Not in Ha-ras NIH-3T3 Cells—In pSVneo NIH-3T3 cells, RARα transcripts were constitutively expressed and slightly (30%) down-regulated by RA (data not shown). RARγ mRNAs were expressed to a lower extent than RARα mRNAs and were not substantially altered by RA. RARβ transcripts were induced in pSVneo cells to a similar extent (data not shown) as in primary mouse skin fibroblasts (36). In Ha-ras NIH-3T3 cells, the levels of RARs mRNA were generally lower than in control cells and were not responsive to RA treatment.

RARα protein levels were strongly down-regulated by RA in NIH-3T3 cells, but not in Ha-ras cells (Fig. 4A). RARγ proteins were not altered by RA in either type of cells and could be detected only after overexposing the blots (data not shown). We were unable to detect RARβ proteins. The effect of RA on the levels of RARα protein was time- and dose-dependent (Fig. 4B).
A concentration of 0.1 nM RA was sufficient to bring about an 80% inhibition of RARα protein, and a 40% inhibition could be already observed after 2 h of RA treatment.

RA Inhibition of FN and RARα Proteins in Fibroblast Cells—We investigated the effects of RA on FN and RARα proteins in C3H10T1/2 mouse embryo fibroblasts and in primary mouse skin fibroblasts. RA markedly reduced the level of FN and RARα in these two cells (Fig. 5).

Role of RAR Signal Transduction Pathways on RA Inhibition of FN and RARα Proteins—SR11237, an RXR-selective compound, specifically activates reporter genes fused to the RXR-responsive element of the CRBPII promoter, to which only RXR-RXR homodimers bind, and is unable to induce genes driven by an RAR-responsive element, like the RARE of RARβ and CRBPI (21). The RAR-mediated signaling pathways can be blocked by overexpressing a mutated form of the RARα gene, RARα403, which shows strong dominant negative activity (31, 32). The truncated form of the RARα gene, RARα403, inserted into the retroviral vector LXRARα403SN and the corresponding control retrovirus LXSN were used. We infected NIH-3T3 cells with these amphotropic retroviruses containing the neomycin resistance gene. Neomycin-resistant cells were isolated and expanded. Northern blot analysis was performed to detect the expression of the typical 4.7-kilobase retroviral transcripts containing the RARα403 mRNA (Fig. 6). The dominant negative activity of such mutated receptor was confirmed in RARα403 NIH-3T3 cells by transient transfection with a vector carrying the luciferase reporter gene fused to two copies of the RARE of RARβ2 gene (33). RA-dependent transactivation of luciferase was then evaluated. Cells overexpressing RARα403 transcripts showed an almost negligible 1.2-fold induction of luciferase activity in comparison to a 5-fold induction in control LXSN cells (Fig. 6B). Similar data were obtained by others in tk− NIH-3T3 cells infected with the very same retroviral vectors (31).
RA failed to down-regulate the intracellular FN levels in the LXRα403SN cells (Fig. 7B, left panel). Furthermore, the RXR-selective compound, SR1237, also failed to reduce FN protein (Fig. 7B, right panel). These results indicate that an RAR-mediated signaling pathway likely accounts for the RA-dependent FN down-regulation.

Similar experiments were performed, looking at the RA modulation of RARα (Fig. 7A). In LXRαSN NIH-3T3, treatment with RA caused a marked reduction of the levels of RARα, ruling out the involvement of RAR signal transduction pathways. This finding was strengthened by the inhibition of RARα in cells treated with SR1237 to a similar extent as that achieved by RA treatment (Fig. 7A, right panel). This suggests the involvement of RAR in this effect.

Effect of RA in NIH-3T3 Cells Overexpressing RARα—To further investigate the role of RARs, we generated cell lines overexpressing the RARα gene. Cotransfections of NIH-3T3 cells with the plasmid pSVneo, harboring the neomycin resistance gene, and the pSG5RARαf vector, containing the full-length RARα gene, were performed. G418-resistant clones were isolated, expanded, and tested for RARα protein overexpression. Two representative clones are shown in Fig. 8. Both clones expressed very high levels of RARα protein, however, the levels of RARα were down-modulated by RA only in clone 3A. Along the same trend, when FN sensitivity to RA was determined, RA failed to reduce the level of FN in the resistant clone.

Effect of RA on Ha-ras NIH-3T3 Cells Overexpressing RARα and RARα403—A similar series of experiments was performed in Ha-ras NIH-3T3 cells. Since the levels of RARs were generally lower than in control cells, we generated Ha-ras NIH-3T3 cell lines, which overexpressed RARα. The RA effects on FN biosynthesis were evaluated. Overexpression of RARα protein was not sufficient to overcome the block on RA responsiveness (Fig. 9A). Similarly, disruption of the RAR signaling pathways by the introduction of the dominant negative RAR construct, RARα403, into Ha-ras NIH-3T3 cells, was ineffective in altering the responsiveness of FN protein to RA.

The involvement of RXR in this effect.

Effect of RA on FN synthesis in NIH-3T3 cells overexpressing RARα. Cells were co-transfected with pSG5RARαf and p5Vneo plasmids. Neomycin-resistant clones, 13A and 3A, overexpressing RARα proteins were treated with 3 μM RA for 48 h. RARα protein levels and intracellular FN were determined by immunoblot and immunoprecipitation analysis, respectively, as described under "Experimental Procedures."

DISCUSSION

We have identified FN as a molecule whose biosynthesis is down-regulated by RA in normal, but not in Ha-ras-transformed NIH-3T3 cells. The inhibition of FN biosynthesis by RA is specific, as neither collagen type IV nor laminin were affected by RA, and is RA dose- and time-dependent. Two lines of evidence suggest that RA acts on FN at a post-transcriptional level. First, the rate of newly synthesized intracellular FN is reduced by RA treatment, an event not due to an increased FN turnover rate. Second, RA did not alter the levels of FN transcripts, consistent with the absence of RARE or RXRE in the promoter region of the FN gene (37, 38). Effects of RA in other cell systems have been reported. FN mRNA and protein levels were increased in primary hepatocytes from vitamin A-deficient rats, while RA treatment caused a reduction of FN mRNA and protein levels (39). In C3H10T1/2 fibroblast cells, a complex RA-dependent regulation of FN was observed as the cell surface levels increased, while intracellular FN and FN mRNA decreased after RA (19).

RA generally controls gene expression at the transcriptional level. However, lipoprotein lipase enzyme expression in 3T3-L1 adipocytes was down-regulated, but mRNA levels were not affected by RA treatment (40). RA induction of differentiation of F9 cells is achieved by controlling the expression of various genes. Early responsive genes are thought to be transcriptionally regulated, while late responsive genes may be controlled both at the transcriptional and post-transcriptional levels (41). Laminin biosynthesis is switched on, while synthesis and secretion of FN is switched off by RA treatment of F9 cells (42–44).

Transformation of NIH-3T3 cells with an activated ras protein caused RA to be expressed to lower levels than in normal cells, while RA induction of RARγ was absent. In addition to specific mutations in the RARα gene (5), alteration of
action of RA on the intracellular FN. We conclude that RARα down-modulation by RA is likely mediated by RXRs, since RA reduces RARα levels, even when we blocked RA-mediated signaling pathways by the overexpression of the RAR dominant negative gene, RARα403. In addition the RXR-selective retinoid, SR11237, is as powerful as RA in down-regulating RARα protein.

In our RARα overexpression studies we isolated a clone, 13A, which appeared to be insensitive to RA in that the two gene products, FN and RARα, were not responsive to RA. The mechanism of the observed RA resistance is not clear. We showed that action of RA on FN is mediated by RAR and is dependent on RAR/RAR heterodimer, while RARα inhibition by RA is likely mediated by RXR/RXR homodimer. The fact that RA resistance is observed for both gene products argues against a defect localized entirely on the RAR signaling pathway. If this were the case, RARα protein expression should have remained sensitive to RA effects. Introduction of an activated ras alters the responsiveness to RA and the level of expression of RARs. Manipulation of RAR protein levels was utilized in an attempt to correlate these two observations.

Overexpression of the RARα gene in Ha-ras NIH-3T3 cells was not sufficient to overcome the block in FN and RARα (data not shown) responsiveness. Failure to regain RA sensitivity after constitutively expressing RARβ in lung cancer cells has been reported (45). RA-dependent induction of a luciferase reporter gene fused to the RARE of the RARβ promoter was equally or more efficient in Ha-ras NIH-3T3 than in normal cells (data not shown). Caution should be used in evaluating the physiological relevance of reporter gene assays, because the RARE is taken out of the context of its natural promoter. Swisshelm et al. (50) showed that when a 1.5-kilobase region of the RARβ2 promoter was used in reporter gene constructs, instead of the RARE, suppression of RA-induced activation was detected in human MCF-7 breast cancer cells. The observation that β-RARE-tk-LUC can be activated in Ha-ras NIH-3T3 cells suggests that necessary factors for activating βRARE are functional. The level of retinoid receptor expression often does not correlate with RA-responsiveness. RARα was expressed in most leukemia cells whether or not they were responsive to RA (51–53). In melanomas, the level of RARβ and RARγ were similar in RA-sensitive and -resistant cells (54). These findings and the observed failure of RARα overexpression to confer RA responsiveness to Ha-ras cells suggests that other factors are required to mediate RA action. These factors may be missing or not functional after ras transformation.

Acknowledgments—We thank Dr. Steven J. Collins for the retroviral construct LXRα403SN and for reviewing the manuscript prior to submission. We are also indebted to Drs. Keiko Ozato and Saverio Minucci for their gift of constructs containing the β-RARE-tk-luciferase reporter gene. We thank Dr. Pierre Chambon for the pSG5-RAR construct and the mouse-polynucleotide RAR antibodies.

REFERENCES

1. De Luca, L. M. (1991) FASEB J. 5, 2924–2933
2. Lotan, R. (1980) Biochim. Biophys. Acta 605, 33–91
3. Penncrck, S. M., Lucas, D. A., and Grippo, J. F. (1994) Leukemia 8, S1–S10
4. Chamber, P. (1994) Celi Biol. 5, 115–125
5. Bollag, W., and Holdener, E. E. (1992) Ann. Oncol. 3, 513–526
6. Smard, J., Sugarman, J., Glass, C., and Lipsick, J. (1995) Mol. Cell. Biol. 15, 2474–2481
7. Talmage, D. A., and Lackey, R. S. (1992) Oncogene 7, 1837–1845
8. Talmage, D. A., and Listerud, M. (1994) Oncogene 9, 3557–3563
9. Cox, L., Lotz, J., Muller, W., and Garte, S. J. (1991) Cancer Res. Clin. Oncol. 112, 102–118
10. Kosa, K., and De Luca, L. M. (1993) Biochem. Biophys. Res. Commun. 196, 1025–1033
11. Gogas, J., Ehrlich, T., Cohen, O., Wishniak, O., Tainsky, M. A., Segal, S., Ray-Arizman, B., and Isakov, N. (1992) Int. J. Cancer 50, 329–335
12. Kreider, B. L., and Rovera, G. (1992) Oncogene 7, 135–140
13. Buettner, R., Yin, S. O., Hong, Y. S., Bocchini, E., and Tainsky, M. A. (1991) Mol. Cell. Biol. 11, 3873–3883
14. Hynes, R. O. (1990) Fibronedin, Springer Verlag, New York
15. Hynes, R. O., and Yamada, K. M. (1982) J. Cell Biol. 95, 369–377
16. Chandler, L. A., and Bourgeois, S. (1991) Cell Growth & Diff. 2, 379–384
17. Chandler, L. A., Ehretsmann, C. P., and Bourgeois, S. (1994) Mol. Cell. Biol. 14, 3085–3093
18. Bollag, G., and McCormick, F. (1991) Annu. Rev. Cell Biol. 7, 601–632
19. Scita, G., and Wolf, G. (1994) Mol. Cell. Biol. 14, 3085–3093
20. Bollag, G., and McCormick, F. (1991) Annu. Rev. Cell Biol. 7, 601–632
21. Scita, G., and Wolf, G. (1994) Biochim. Biophys. Acta 1223, 29–35
22. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) Anal. Biochem. 150, 76–85
23. Gaub, M. P., Rochette-Egly, C., Lutz, Y., Ali, S., Matthes, H., Scheuer, I., and Chambon, P. (1992) Exp. Cell Res. 201, 335–346
24. Ali, M., Torian, B. E., and Vedeckis, W. V. (1992) Biochem. Biophys. Res. Commun. 182, 1032–1039
25. Brand, N. J., Petkovich, M., and Chambon, P. (1990) Nucleic Acids Res. 18, 6799–6806
26. Southern, E. M., and Berg, P. (1982) J. Mol. Appl. Genet. 1, 327–341
27. Petkovich, M., Brand, N. J., Krust, A., and Chambon, P. (1989) Nature 339, 714–717
28. Zelent, A., Krust, A., Petkovich, M., Kastner, P., and Chambon, P. (1989) Nucleic Acids Res. 18, 444–450
29. Tsai, S., Bartelmez, S., Heyman, R., Damm, K., Evans, R., and Collins, S. J. (1992) Genes & Dev. 6, 2258–2269
30. Damms, K., Heyman, R. A., Umesono, K., and Evans, R. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2989–2993
31. Minucci, S., Zand, D. J., Dey, A., Marks, M. S., Nakata, T., Grapito, J. F., and Ozato, K. (1994) Mcl. Cell. Biol. 14, 360–372
32. MacGregor, G. R., and Caskey, C. T. (1989) Nucleic Acids Res. 17, 2365
33. Olden, K., and Yamada, K. M. (1977) Cell 11, 957–969
34. Tsou, H. C., Lee, X., Si, S. P., and Peacocke, M. (1994) Exp. Cell Res. 211, 74–81
35. Dean, D. C., Boulus, C. L., and Bourgeois, S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1876–1880
36. Nakajima, T., Nakamura, T., Tsuchiya, S., Nakada, S., and Oda, K. (1992) Mol. Cell. Biol. 12, 2837–2846
37. Kim, H. Y., and Wolf, G. (1987) J. Biol. Chem. 262, 365–371
38. Kamei, Y., Kawada, T., Fujita, A., Etinose, J., Noe, L., and Sugimoto, E. (1992) Biochem. Int. 26, 923–934
39. The, H. D., Marchio, A., Tiollais, P., and Dejean, A. (1995) EMBO J. 14, 429–433
40. de The, H., Vivanco-Ruiz, M., Tiollais, P., Stunnenberg, H., and Dejean, A. (1990) Nature 343, 177–180
41. Swisshelm, K., Ryan, K., Lee, X., Tsou, H. C., Peacocke, M., and Sagar, R. (1995) Cell Growth & Diff. 5, 133–143
42. Gallacher, R. E., Said, F. P., Pua, J., Pappenhausen, P. R., Pajetta, E., and Wiernik, P. H. (1989) Leukemia 3, 789–795
43. Largement, C., Dietter, K., Corral, J., C., Hack, F. M., and Lawrence, H. J. (1989) Blood 74, 99–102
44. Wang, C., Curtiss, J. E., Minden, M. D., and McCulloch, E. A. (1989) Leukemia 3, 264–269
45. Clifford, J. L., Petkovich, M., Chambon, P., and Lotan, R. (1990) Mol. Endocrinol. 4, 1546–1553
Retinoic Acid Down-regulation of Fibronectin and Retinoic Acid Receptor Proteins in NIH-3T3 Cells: BLOCK OF THIS RESPONSE BY ras TRANSFORMATION
Giorgio Scita, Nadine Darwiche, Eileen Greenwald, Miriam Rosenberg, Katerina Politi and Luigi M. De Luca

*J. Biol. Chem.* 1996, 271:6502-6508.
doi: 10.1074/jbc.271.11.6502

Access the most updated version of this article at [http://www.jbc.org/content/271/11/6502](http://www.jbc.org/content/271/11/6502)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 16 of which can be accessed free at [http://www.jbc.org/content/271/11/6502.full.html#ref-list-1](http://www.jbc.org/content/271/11/6502.full.html#ref-list-1)