Retinoic Acid Stimulation of Human Dermal Fibroblast Proliferation Is Dependent on Suboptimal Extracellular Ca²⁺ Concentration

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Human dermal fibroblasts failed to proliferate when cultured in medium containing 0.15 mmol/l (millimolar) Ca²⁺ (keratinocyte growth medium [KGM]) but did when the external Ca²⁺ concentration was raised to 1.4 mmol/l. All-trans retinoic acid (retinoic acid) stimulated proliferation in KGM but did not further stimulate growth in Ca²⁺-supplemented KGM. The ability of retinoic acid to stimulate proliferation was inhibited in KGM prepared without Ca²⁺ or prepared with 0.03 mmol/l Ca²⁺ and in KGM treated with 1 mmol/l ethylene-glycol-bis-(β-aminoethyl ether)N,N'-tetra acetic acid. Using ⁴⁵Ca²⁺ to measure Ca²⁺ influx and efflux, it was found that retinoic acid minimally increased Ca²⁺ uptake into fibroblasts. In contrast, retinoic acid treatment of fibroblasts that had been pre-equilibrated for 1 day with ⁴⁵Ca²⁺ inhibited release of intracellular Ca²⁺ into the extracellular fluid. Retinoic acid also stimulated ³⁵S-methionine incorporation into trichloroacetic acid-precipitable material but in contrast to its effect on proliferation, stimulation of ³⁵S-methionine incorporation occurred in both high-Ca²⁺ and low-Ca²⁺ medium. These data indicate that retinoic acid stimulation of proliferation, but not protein synthesis, is dependent on the concentration of Ca²⁺ in the extracellular environment. (Am J Pathol 1990, 136: 1275–1281)

Topical administration of all-trans retinoic acid (retinoic acid) improves several features of photoaged skin, including fine and deep wrinkles, texture, and color.[1,2] Similar changes are seen in the skin of hairless mice after topical retinoic acid treatment.[3,4] Histologic examination of retinoic acid-treated skin reveals epidermal thickening with

increased keratinocyte proliferation, thickening of the granular layer, and increased deposition of alcian blue-staining (GAG-like) material.[1,3] Dermal features include increased numbers of fibroblasts, evidence of fibroblast activation, and formation of new extracellular matrix.[1,3–5] In contrast to these effects after topical administration, oral retinoid (etretinate) treatment in psoriasis acts to inhibit proliferation.[6,7] These seemingly parodoxic effects of retinoids are also observed *in vitro* where retinoids can either stimulate or inhibit fibroblast and keratinocyte proliferation, depending on the source of the cells and the conditions of maintenance.[8–14] The cellular basis for these diverse effects of retinoids is not fully understood.

In the present study, we demonstrate that the ability of retinoic acid to stimulate human dermal fibroblast proliferation is dependent on the concentration of Ca²⁺ in the extracellular environment. Retinoic acid stimulated the growth of fibroblasts in culture medium containing 0.15 mmol/l Ca²⁺. Fibroblasts maintained in this concentration of Ca²⁺ did not proliferate in the absence of retinoic acid. In contrast to its stimulating effect in the presence of suboptimal external Ca²⁺, retinoic acid failed to further stimulate proliferation of cells that were maintained in the presence of a concentration of Ca²⁺ (1.4 mmol/l) that supported proliferation without retinoic acid.

Materials and Methods

Cells

Human dermal (neonatal foreskin) fibroblasts were used in this study. They were obtained as primary cultures and stored in liquid N₂ at passage 2 to 4. The fibroblasts were grown in monolayer culture using minimal essential medium of Eagle with Earle's salts (MEM) containing nonessential amino acids, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 10% fetal bovine serum as culture me-

dium. Cell growth was at 37°C and 5% CO_2. Cells were subcultured with 0.25% trypsin/0.02% EDTA as required. Experiments were conducted on cells through passage 20. In experiments the cells were cultured in keratinocyte growth medium (KGM) (Clonetics, Inc., San Diego, CA). Keratinocyte growth medium is a low Ca^{2+} (0.15 mmol/l), serum-free medium (MCDB 153) containing epidermal growth factor, insulin, hydrocortisone, ethanolamine, phosphoethanolamine, and bovine pituitary extract as growth-promoting supplements. Fibroblast growth in KGM was compared to growth in KGM supplemented with 1.4 mmol/l Ca^{2+}. In some experiments KGM prepared without added Ca^{2+} or prepared with 0.03 mmol/l Ca^{2+} (Clonetics, Inc.) or Ca^{2+}-chelated KGM were used.

Reagents

Retinoic acid (type XX) was obtained from Sigma Chemical Co. (St. Louis, MO). Stock solutions were prepared in dimethyl sulfoxide (DMSO) (5 mg/ml) and stored frozen and protected from light. Working solutions were prepared in the appropriate culture medium at the time of use. When cells were treated with all-trans retinoic acid, they were protected from light during the incubation period. The final concentration of DMSO in the cultures treated with 1.0 μg/ml of retinoic acid (highest concentration used) was 0.02%. This concentration of DMSO had no detectable effect by itself on cell growth or on Ca^{2+} metabolism.

The Ca^{2+} ionophore A23187 was obtained from Sigma Chemical Co. It was prepared as a 100 μmol/l (micromolar) solution in DMSO and diluted in the appropriate culture medium at the time of use.

Proliferation Assay

Fibroblast proliferation was measured by seeding 35-mm (diameter) culture dishes with 1 × 10^5 cells per dish in MEM supplemented with 10% fetal bovine serum. One to two hours later the cells were washed and incubated in KGM (containing 0.15 mmol/l Ca^{2+}) or in KGM supplemented with 1.4 mmol/l Ca^{2+}. Cells in each group were left without further treatment or treated as described in Results. The cultures were then incubated at 37°C and 5% CO_2 for 24, 48, or 72 hours. After incubation, the cells were harvested with trypsin and counted using an electronic particle counter. Before counting, the trypsinized cells were examined under phase-contrast microscopy to confirm that they were in single-cell suspension. To evaluate proliferation data statistically, the cell number at time zero (1 × 10^5 cells) was subtracted from the values ob-

tained at the end of the incubation period. The remaining values were then compared using Student's *t*-test.

35S-methionine Incorporation

^{35}S-methionine incorporation into protein was measured by seeding 35-mm (diameter) culture dishes with 1 × 10^5 cells per dish in MEM supplemented with 10% fetal bovine serum. One to two hours later the cells were washed, incubated in KGM, and treated as described in Results. One day later each dish was incubated with 1 μCi of ^{35}S-methionine for 1 hour. At the end of this incubation period, the cells were washed twice in phosphate-buffered saline (PBS) and lysed in a solution of PBS containing 1% Triton X-100, 0.5% deoxycholate and 0.1% sodium dodecylsulfate (PBS-TDS). Lysates were precipitated with 10% (final) trichloroacetic acid (TCA) and washed twice in TCA. A sample of the washed precipitate was resuspended in aquous scintillation fluid and counted for total ^{35}S-methionine incorporation.

45Ca2+ Uptake and Release Studies

^{45}CaCl$_2$ was obtained from ICN (Irvine, CA). For ^{45}Ca^{2+} uptake studies, fibroblasts were plated at 2.5 × 10^5 cells/dish in MEM supplemented with 10% fetal bovine serum. After the cells had a chance to attach and spread, they were washed three times in KGM and incubated in KGM with or without 1 μg/ml of retinoic acid. One μCi of ^{45}Ca^{2+} was added to each dish and the cells were then incubated at 37°C and 5% CO_2. At various times later, the culture fluid was removed and the cells werer rapidly washed three times with cold KGM. The final wash contained 5 mmol/l EGTA. The cells were lysed in PBS-TDS and the total amount of ^{45}Ca^{2+} taken up by the cells determined by β-scintillation counting. In some experiments the cells were pre-equilibrated with KGM for 1 day and then treated with 1 μg/ml of retinoic acid and 1 μCi of ^{45}Ca^{2+}. In other studies, the cells were pre-equilibrated with both KGM and ^{45}Ca^{2+} and then treated with 1 μg/ml of retinoic acid.

To measure ^{45}Ca^{2+} release from prelabeled cells, the cells were plated at 2.5 × 10^5 cells/dish exactly as described above. They were incubated for 1 day in KGM containing 1 μCi of ^{45}Ca^{2+} to load the cells. One μg/ml of retinoic acid was then added directly to the wells without first removing nonincorporated ^{45}Ca^{2+}. One hour after addition of retinoic acid, the cells were washed three times with cold KGM (with or without retiffoic acid) to remove nonincorporated ^{45}Ca^{2+}. The cells were then incubated for various periods of time in KGM with or without retinoic acid. ^{45}Ca^{2+} released into the culture fluid was determined by β-scintillation counting.

Table 1. *Effect of Retinoic Acid on Human Dermal Fibroblast Proliferation in KGM and in KGM Supplemented with 1.4 mmol/l Ca^{2+}*

Retinoic acid	KGM	Number of cells × 10^5		
		(*P* value)	KGM + 1.4 mM Ca^{2+}	(*P* value)
None	1.37 ± 0.04		3.23 ± 0.49	
0.5 μg/ml	2.24 ± 0.52	*P* < 0.001	2.93 ± 0.76	NS
1.0 μg/ml	1.89 ± 0.48	*P* < 0.01	2.40 ± 0.72	*P* < 0.05

Cells were plated at 1 × 10^5 cells/dish on day zero as described in Materials and Methods and incubated for 2 days. Values shown represent average number of cells/dish on day 2 ± standard deviations based on four samples/data point in a single experiment. The experiment was repeated three times with similar results. Statistical significance levels were determined using the Student's *t*-test. NS, not significant at the *P* < 0.05 level.

Results

Effects of Retinoic Acid on Fibroblast Proliferation in KGM and KGM Supplemented with 1.4 mmol/l Ca2+

Fibroblasts were incubated for 2 days in KGM (0.15 mmol/l Ca^{2+}) or in KGM supplemented with 1.4 mmol/l Ca^{2+}. In KGM alone, proliferation was almost completely inhibited while in KGM supplemented with 1.4 mmol/l Ca^{2+}, proliferation occurred (Table 1). Retinoic acid (0.5 to 1.0 μg/ml) significantly stimulated proliferation in KGM but did not further stimulate cell growth in KGM supplemented with 1.4 mmol/l Ca^{2+} (Table 1). The data shown in Table 1 were obtained at a single time point (2 days after treatment), but in addition, a complete time-response study was performed with retinoic acid. There were only minimal differences between control and retinoic acid-treated cells after 1 day (usually less than 5%). Differences were observed by day 2 and were even greater after 3 days. Dose-response studies indicated that retinoic acid was maximally effective at 0.5 to 1.0 μg/ml (1.7 to 3.4 × 10^{-6} mol/l [molar]) but was less effective at higher and lower concentrations. We routinely observed no stimulation at 0.01 μg/ml of retinoic acid and only minimal stimulation at 0.1 μg/ml. At concentrations of 2.5 and 5.0 μg/ml, proliferation rates were already decreased almost to the values obtained in the absence of retinoic acid. At the highest concentration used (5.0 μg/ml) there was an increase in trypan blue-staining cells, indicative of toxicity. This was not observed at any of the lower concentrations examined (not shown). In regard to the lower concentrations, it should be noted that the cells were exposed to a low concentration of retinoic acid (approximately 5 × 10^{-9} mol/l) in the fetal bovine serum-containing medium before the start of the experiment. Whether chronic exposure to low levels of retinoic acid influenced the subsequent response of the cells to higher concentrations was not investigated.

Role of Extracellular Ca2+ in Retinoic Acid-stimulated Fibroblast Proliferation

The ability of retinoic acid to stimulate fibroblast proliferation in medium containing 0.15 mmol/l Ca^{2+} but not in medium containing 1.4 mmol/l Ca^{2+} suggested a role for extracellular Ca^{2+} in this process. To examine this possibility, we modulated the availability of extracellular Ca^{2+} to the cells in a number of ways and examined the ability of retinoic acid to stimulate proliferation under these conditions. These data are shown in Table 2 and Figure 1. The data in Table 2 show that the ability of retinoic acid to stimulate fibroblast proliferation was inhibited in medium containing 0.03 mmol/l Ca^{2+} and in medium containing only traces of Ca^{2+} (*i.e.*, in KGM prepared without added Ca^{2+}). Similarly, chelation of exogenous Ca^{2+} with 1 mmol/l EGTA also inhibited retinoic acid-induced proliferation (Table 2). In additional studies, the Ca^{2+} inophore A23187 was examined for effects on fibroblast proliferation in KGM and in KGM supplemented with 1.4 mmol/l Ca^{2+}. In the presence of Ca^{2+}-supplemented KGM, A23187 inhibited proliferation at all concentrations examined (0.005 to 1.0 μmol/l) (Figure 1). However, in KGM, low concentrations of A23187 stimulated proliferation (Figure 1).

Effects of Retinoic Acid on 45Ca2+ Uptake and Release from Fibroblasts

Because retinoic acid stimulation of fibroblast proliferation in KGM was sensitive to modulation of extracellular Ca^{2+} levels, we considered the possibility that retinoic acid facilitated the uptake of Ca^{2+} from the extracellular environment. To examine this, we measured uptake of ^{45}Ca^{2+} by control and retinoic acid-treated (1.0 μg/ml) cells. As can be seen in Figure 2A, however, ^{45}Ca^{2+} entry into non–pre-equilibrated cells was only minimally stimulated by pretreatment for 1 day with retinoic acid. In other studies, ^{45}Ca^{2+} entry into fibroblasts was measured immediately after retinoic acid treatment. Similar results were obtained to those shown in Figure 2A. Retinoic acid (1.0 μg/ml) treatment only minimally increased the rate of ^{45}Ca^{2+} uptake. Finally, the effects of retinoic acid (1.0 μg/ml) were examined on the uptake of ^{45}Ca^{2+} by cells that had been pre-equilibrated for 1 day with KGM containing ^{45}Ca^{2+}. Again, there was only a slight increase in ^{45}Ca^{2+} uptake after retinoic acid treatment (not shown).

Table 2. *Inhibition of Retinoic Acid-induced Proliferation in KGM Prepared with Reduced Ca^{2+} Levels and in the Presence of EGTA*

Treatment	Number of cells × 10^5	(P value)
KGM (0.15 mM Ca^{2+}) + 0.5 µg/ml retinoic acid	1.12 ± 0.08 2.00 ± 0.05	P < 0.001
KGM (0.03 mM Ca^{2+}) + 0.5 µg/ml retinoic acid	1.02 ± 0.06 1.29 ± 0.09	NS
KGM (without Ca^{2+}) + 0.5 µg/ml retinoic acid	1.16 ± 0.02 1.26 ± 0.11	NS
KGM (0.15 mM Ca^{2+} + 1 mM EGTA) + 0.5 µg/ml retinoic acid	0.89 ± 0.09 0.87 ± 0.16	NS

Cells were plated at 1 × 10^5 cells/dish on day zero and treated as described in Materials and Methods for 2 days. KGM without Ca^{2+} was prepared without exogenous Ca^{2+} but contains traces of Ca^{2+}. Values shown represent average number of cells/dish on day 2 ± standard deviations based on five samples/data point in two separate experiments. Statistical significance levels were determined using the Student's *t*-test. NS, not significant at the P < 0.05 level.

In contrast to the lack of substantial stimulation of $^{45}Ca^{2+}$ uptake, treatment of prelabeled cells with retinoic acid (1.0 µg/ml) inhibited $^{45}Ca^{2+}$ release into the extracellular fluid. For this, cells prelabeled with $^{45}Ca^{2+}$ were treated with retinoic acid and the cumulative amount of $^{45}Ca^{2+}$ released into the culture medium measured at various times later as described in Materials and Methods. Significant inhibition was observed as early as 1 to 2 hours after retinoic acid treatment (Figure 2B). Differences between control and retinoic acid-treated cells were still visible as late as 6 hours after treatment (not shown). After six hours, the amount of $^{45}Ca^{2+}$ remaining with the cells was approximately two times higher in the retinoic acid-treated cells than in control cells (17% *versus* 8%).

Effects of Retinoic Acid on Fibroblast Incorporation of 35S-methionine into TCA-precipitable Material

We next examined the effects of retinoic acid on incorporation of ^{35}S-methionine into TCA-precipitable material.

Cells were maintained in KGM or KGM supplemented with 1.4 mmol/l Ca^{2+} and treated for 1 day with various concentrations of retinoic acid. The cells were then incubated for another hour with ^{35}S-methionine (1 µCi/dish) and the amount of radioactivity incorporated into TCA-precipitable material was determined. Figure 3 indicates that the same concentrations of retinoic acid that stimulated proliferation in KGM also stimulated protein synthesis. At optimal concentrations, ^{35}S-methionine incorporation into TCA-precipitable material was increased approximately four times over the amount incorporated under control conditions. Virtually identical results were obtained in KGM supplemented with 1.4 mmol/l Ca^{2+}: retinoic acid at 0.5 and 1.0 µg/ml stimulated ^{35}S-methionine into TCA-precipitable material by two to three times of that incorporated by untreated fibroblasts (Figure 3).

Discussion

Results from the present study indicate that retinoic acid-stimulation of human dermal fibroblast proliferation re-
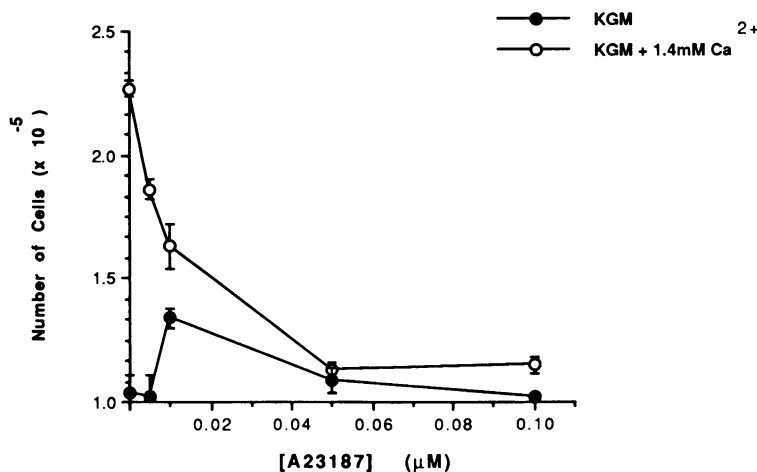

Figure 1. *Effects of A23187 on fibroblast proliferation in KGM and in KGM supplemented with 1.4 mmol/l Ca^{2+}. Cells were plated at 1 × 10^5 cells/dish on day zero as described in Materials and Methods and incubated for 2 days. Values shown represent average number of cells/dish on day 2 ± standard deviations based on six samples/data point in three independent experiments.*

Figure 2. A: *Effect of retinoic acid on fibroblast uptake of $^{45}Ca^{2+}$ from the culture medium. The experiment was carried out as described in Materials and Methods. The values represent average counts per minute of $^{45}Ca^{2+}$ taken up by 1×10^5 cells ± standard deviations based on triplicate samples per data point in a single experiment. The experiment was repeated three times with similar results.* B: *Effect of retinoic acid on release of $^{45}Ca^{2+}$ from prelabeled fibroblasts. The experiment was carried out as described in Materials and Methods. The values represent cumulative average counts per minute of $^{45}Ca^{2+}$ released by 1×10^5 cells into the culture medium ± standard deviations based on triplicate samples per data point in a single experiment. The experiment was repeated three times with similar results.*
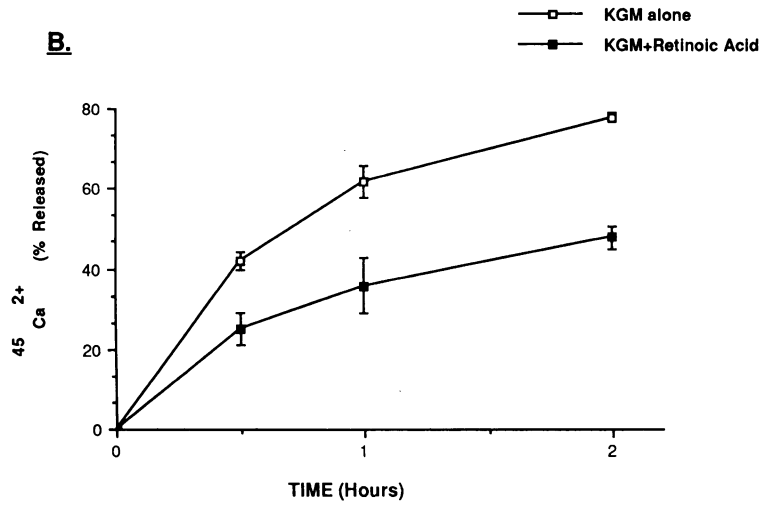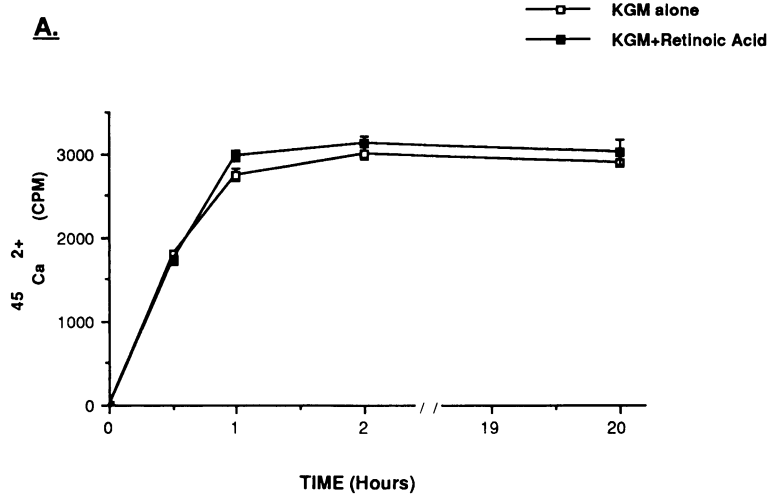
A.

— □ — KGM alone
— ■ — KGM+Retinoic Acid

B.

— □ — KGM alone
— ■ — KGM+Retinoic Acid

Figure 3. *Stimulation of ^{35}S-methionine incorporation into TCA-precipitable material in 0.15 mmol/l and 1.4 mmol/l Ca^{2+}. Fibroblasts were cultured in KGM and in KGM supplemented with 1.4 mmol/l Ca^{2+} with and without 0.5 µg/ml and 1.0 µg/ml retinoic acid. Values represent average counts per minute (CPM) of ^{35}S-methionine incorporated into 1×10^4 cells during a 1-hour pulse ± differences between individual values and averages based on duplicate samples/data point in a single experiment. The experiment was repeated two times with similar results.*

■ No Retinoic Acid
▓ 0.5 µg/ml Retinoic Acid
▨ 1.0 µg/ml Retinoic Acid
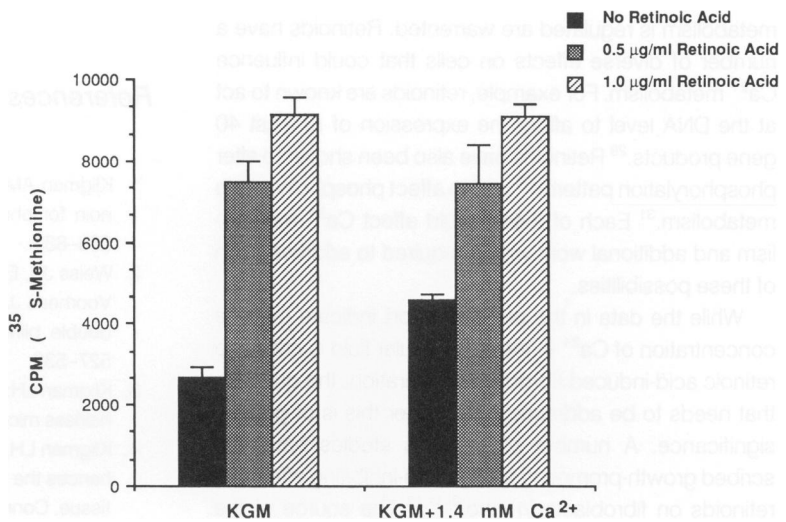
quires availability of extracellular Ca^{2+} but only occurs when the concentration of extracellular Ca^{2+} is suboptimal for proliferation in the absence of retinoic acid. The evidence for this includes the findings that retinoic acid-induced proliferation occurred in KGM containing 0.15 mmol/l Ca^{2+} but was inhibited in culture medium prepared without added Ca^{2+} and in culture medium containing the Ca^{2+} chelator EGTA. In addition, retinoic acid also failed to further stimulate proliferation of cells maintained in the presence of a concentration of external Ca^{2+} (1.4 mmol/l) that supported proliferation without retinoic acid. In past studies it has been found that stimulation of fibroblast proliferation by platelet-derived growth factor (PDGF) also required suboptimal Ca^{2+} concentrations—at least when the fibroblasts were maintained in low-density, serum-free culture.[16] Under the conditions of our experiments, the cells were plated at relatively low density (approximately 2×10^4 cells/cm^2) and under these conditions, the action of retinoic acid may be similar to that described for PDGF. It should be noted, however, that PDGF can also stimulate mitosis of density-inhibited fibroblasts and this occurs in the presence of high as well as low external Ca^{2+} concentrations.[16] We have not seen similar effects with retinoic acid (Varani J, unpublished observation).

Several previous studies have focused on the regulation of fibroblast proliferation and on the role of Ca^{2+} in this process. It has been shown that fibroblast proliferation occurs at external Ca^{2+} concentrations of 0.5 to 1.5 mmol/l but ceases at concentrations less than 0.5 mmol/l.[17-19] In addition, it has been shown that the stimulation of mitosis in a number of different cell types, including fibroblasts, is associated with a rise in intracellular free Ca^{2+}.[16,20-27] However, this has not been found universally[28] and, in any case, it is difficult to determine if changes in intracellular free Ca^{2+} are responsible for the induction of proliferation or the result. This issue has yet to be addressed in the case of retinoic acid. Regardless of its role in proliferation, efforts to understand how Ca^{2+} metabolism is regulated are warrented. Retinoids have a number of diverse effects on cells that could influence Ca^{2+} metabolism. For example, retinoids are known to act at the DNA level to affect the expression of at least 40 gene products.[29] Retinoids have also been shown to alter phosphorylation patterns[30] and to affect phosphoinositide metabolism.[31] Each of these could affect Ca^{2+} metabolism and additional work will be required to address each of these possibilities.

While the data in the present report indicate that the concentration of Ca^{2+} in the extracellular fluid is critical to retinoic acid-induced fibroblast proliferation, the question that needs to be addressed is whether this is of general significance. A number of previous studies have described growth-promoting and growth-inhibiting effects of retinoids on fibroblasts *in vitro*.[8-11,13] The source of the

cells, their age in culture, and the conditions of maintenance have all been postulated to affect responsiveness to retinoids. Retinoic acid has also been suggested to facilitate fibroblast proliferation *in vivo*.[3,4] Additional studies are needed to determine if the concentration of extracellular Ca^{2+} is critical to retinoic acid-induced proliferation under any of these conditions.

Retinoids have also been shown to either stimulate or inhibit keratinocyte proliferation. In general, rapidly proliferating keratinocytes, such as those found in psoriatic plaques and those cultured *in vitro* under conditions that maintain the undifferentiated state (for example, low-passage cells grown in KGM), are inhibited by retinoids[6,7,14] while keratinocytes that are not rapidly growing, such as those maintained in culture in the absence of growth factors, are stimulated.[14] It is interesting, in light of the present data, that optimal proliferation of normal, undifferentiated human epidermal keratinocytes *in vitro* occurs when extracellular Ca^{2+} levels are low (*i.e.*, 0.05 to 0.15 mmol/l).[32] Proliferation of these cells is inhibited at higher Ca^{2+} levels (*i.e.*, 1.4 mmol/l).[32] If retinoic acid functions primarily by increasing Ca^{2+} availability, then growth inhibition of keratinocytes could be the biologic consequence of the same molecular events that in fibroblasts stimulate growth. It should be noted that our recent study showed that, while retinoic acid was able to stimulate proliferation of keratinocytes maintained in growth factor-deficient medium, it did not overcome the inhibition of proliferation occurring in the presence of elevated extracellular Ca^{2+}.[14]

Whatever role Ca^{2+} plays in retinoic acid-induced changes in proliferation, this is unlikely to fully explain how retinoic acid influences fibroblastic cells. Retinoic acid-induced protein synthesis occurred in the presence of high external Ca^{2+} as well as low external Ca^{2+}. Because production of new proteins (extracellular matrix components) is one of the features of retinoic acid-treated skin,[3-5] this may occur independently of alterations in proliferation.

References

1. Kligman AM, Grove GL, Hirose R, Leyden JJ: Topical tretinoin for photoaged skin. J Am Acad Dermatol 1986, 15: 836–839
2. Weiss JS, Ellis CN, Headington JT, Tincoff T, Hamilton TA, Voorhees JJ: Topical tretinoin improves photoaged skin: A double blind, vehicle-controlled study. JAMA 1988, 259: 527–532
3. Kligman LH: Effects of all-trans retinoic acid on the dermis of hairless mice. J Am Acad Dermatol 1986, 15:779–785
4. Kligman LH, Duo CH, Kligman AM: Topical retinoic acid enhances the repair of ultraviolet damaged dermal connective tissue. Connect Tissue Res 1984, 12:139–150

5. Zelickson AS, Mottaz JH, Weiss JS, Ellis CN, Voorhees JJ: Topical tretinoin in photoaging: An ultrastructural study. J Cutan Aging & Cosmetic Dermatol 1988, 1:41–47

6. Orfanos CE, Runne U: Tissue changes in psoriatic plaques after oral administration of retinoid. Dermatologica 1978, 157 (Suppl 1) 19–25

7. Williams ML, Elias PM: Nature of skin fragility in patients receiving retinoids for systemic effect. Arch Derm 1981, 117: 611–619

8. Lacroix A, Anderson GDL, Lippman ME: Retinoids and cultured human fibroblasts. Exp Cell Res 1980, 130:339–344

9. Harper RA, Burgoon T: Differential effects of retinoic acid on the growth of normal fibroblast-like cells *in vitro* from human, swine and rabbit skin. Cell Bio Int Rep 1982, 6:163–170

10. Demetriou AA, Levenson SM, Rettura G, Seifter E: Vitamin A and retinoic acid: Induced fibroblast differentiation in vitro. Surgery 1985, 98:931–934

11. Priestly GC: Proliferation and glycosaminoglycan secretion in fibroblasts from psoriatic skin: Differential response to retinoids. Br J Dermatol 1987, 117:575–583

12. Elias PM, Fritsch P, Lampe M, Williams M, Brown B, Nemanic MK, Grayson S: Retinoid effects on epidermal structure, differentiation and permeability. Lab Invest 1981, 44:531–540

13. Stanulis-Praeger BM, Gilchrest BA. Effect of donar age and prior sun exposure on growth inhibition of cultured human dermal fibroblasts by all-trans retinoic acid. J Cell Physiol 1989, 139:116–124

14. Varani J, Nickoloff BJ, Dixit VM, Mitra RS, Voorhees JJ: All-trans retinoic acid stimulates growth of adult human keratinocytes cultured in growth factor-deficient medium, inhibits thrombospondin and fibronectin, and reduces adhesion. J Invest Derm 1989, 93:449–454

15. Grynkiewicz G, Poenie M, Tsien TY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985, 260:3440–3450

16. Betsholtz C, Westermark B: Growth factor-induced proliferation of human fibroblasts in serum-free culture depends on cell density and extracellular calcium concentration. J Cell Physiol 1984, 118:203–210

17. Boynton AL, Whitfield JF, Isaacs RJ, Morton HJ: Control of 3T3 cell proliferation by calcium. In Vitro 1974, 10:12–17

18. Boynton AL, Whitfield JF, Isaacs RJ, Tremblay R: The control of human WI-38 proliferation by extracellular calcium and its elimination by SV-40 virus-induced proliferative transformation. J Cell Physiol 1977, 92:241–248

19. Whitfield JF, MacManus JP, Rixon RH, Boynton AL, Youdale T, Swierenga S: The positive control of cell proliferation by the interplay of calcium ions and cyclic nucleotides; a review. In Vitro 1976, 12:1–18

20. Moolenaar WH, Tertoolen LGJ, de Laat SW: Growth factors immediately raise cytoplasmic free Ca^{2+} in human fibroblasts. J Biol Chem 1984, 259:8066–8069

21. Magnaldo I, L'Allemain G, Charmlard JC, Moenner M, Barritauet I, Pouyssegur J: The mitogenic signaling pathway of fibroblast growth factor is not mediated through polyinositide hydrolysis and protein kinase C activation in hamster fibroblasts. J Biol Chem 1986, 261:16,916–16,922

22. Fukuo K, Morimoto S, Kaji K, Koh E, Hironaka T, Morita R, Kim S, Onishi T: Association of increased intracellular free Ca^{2+} by platelet-derived growth factor with mitogenesis but not proteoglycan synthesis in chondrocytes—Effect of suramin. Cell Calcium 1989, 10:29–35

23. Tsien RY, Pozzan T, Rink TJ: T-cell mitogens cause early change in cytoplasmic free Ca^{2+} and membrane potential in lymphocytes. Nature 1982, 295:68–71

24. Poenie M, Alderton J, Tsien RY, Steinhardt RA: Changes of free calcium levels with stages of cell division cycle. Nature 1985, 315:147–149

25. Hesbeth TR, Moore JP, Morris JDH, Taylor MV, Rogers J: A common signal of calcium and pH signals in the mitogenic stimulation of eukaryotic cells. Nature 1985, 313:481–484

26. Moolenaar WH, Aerts RJ, Tertoolen LGJ, de Laat SW: The epidermal growth factor calcium signal in A431 cells. J Biol Chem 1986, 261:279–284

27. Hepler JR, Nakahata N, Lovenberg TW, DiGuiseppi J, Herman B: Epidermal growth factor stimulates a rapid accumulation of inosital (1,4,5)-triphosphate and a rise in cytosolic calcium mobilized from intracellular stores in A431 cells. J Biol Chem 1987, 262:2951–2956

28. Praeger FC, Reinlib L, Donowitz M, Gilchrest BA: [Ca^{2+}]ᵢ independent mitogenesis in cultured human fibroblasts revealed by single cell microfluorimetry. Biochem Biophys Res Commun 1989, 159:862–870

29. Chytil F, Sherman DR: How do retinoids work? Dermatologica 1987, 175:8–12

30. Spearman TN, Fontana JA, Butcher FR, Durham JP: Effect of retinoic acid on the phosphorylation of endogenous proteins in HL-60 cells. J Cell Physiol 1989, 138:349–357

31. Lanciotti M, Longone P, Cornaglia-Ferraris P, Ponzoni M: Retinoic acid inhibits phosphatidyl-inositol turnover only in RA-sensitive while not in RA-resistant human neuroblastoma cells. Biochem Biophys Res Commun 1989, 161:284–289

32. Hennings H, Michael D, Cheng C, Steinert P, Holbrook K, Yuspa SH: Calcium regulation of growth and differentiation of mouse epidermal cells in culture. Cell 1980, 19:245–259