Journal of Cellular Biochemistry 57:647–654 (1995)

Post-Translational Abnormality of the Type II Cyclic AMP-Dependent Protein Kinase in Psoriasis: Modulation by Retinoic Acid

Sylvie Tournier, Pascale Gerbaud, Wayne B. Anderson, Suzane M. Lohmann, Danièle Evain-Brion, and Françoise Raynaud

Laboratoire de Physiopathologie du Développement, 75230 Paris Cedex 05, France (S.T., P.G., D.E.-B., (F.R.); Labor für Klinische Biochemie, Medizinische Universitätsklinik, 8700 Würzburg, Germany (S.M.L.); Laboratory of Cellular Oncology, National Cancer Institute, National Institute of Health, Bethesda, Maryland 20892 (W.B.A.)

Abstract Previously, we have reported a decrease in the binding of a cAMP analog to the regulatory subunits of cAMP-dependent protein kinase (cAMP-PK), as well as a decrease in cAMP-PK activities, in psoriatic cells. Retinoic acid (RA) treatment of these cells can induce an increase in cAMP-PK toward normal levels. To better define the effect of retinoic acid on the cAMP-PK system in psoriatic fibroblasts, Western blot analysis using an RIIα specific antibody and in vivo phosphorylation experiments were carried out to determine possible changes in the RII regulatory subunit. Our results indicate a decrease in the binding of the cAMP analog 8-azido-[^{32}P]-cAMP with no change in the level of RII protein in psoriatic fibroblasts. In addition, by two-dimensional gel electrophoresis we observed the presence of a phosphorylated form of RII unique to psoriatic cells which is suppressed by RA treatment. This study suggests an altered posttranslational modification of the cAMP-PKII in psoriatic fibroblasts which can be reversed by exposure of these cells to RA. © 1995 Wiley-Liss, Inc.

Key words: cAMP-PKII, retinoic acid, psoriasis, phosphorylation, RIIα

Psoriatic lesions offer a model to study benign cell hyperproliferation with abnormal cell differentiation. The predominant role of the dermis in the appearance of the epidermal manifestation of the disease suggests a key role for fibroblasts in the pathogeny of the disease [Saiag et al., 1985].

Cyclic AMP (cAMP) has been implicated as a modulator of cellular growth and differentiation in a variety of cell types. With a few exceptions, most of the biological effects of cAMP are mediated through activation of the cAMP-dependent protein kinases (cAMP-PK), which in mammalian cells consists of several types of holoenzymes derived from four different genes encoding for regulatory subunits (R) designated RIα, RIβ, RIIα, and RIIβ [Scott et al., 1987; Jahnsen et al., 1986; Lee et al., 1983; Clegg et al., 1988] and three slightly differing α, β, and γ forms of the catalytic (C) subunits [Uhler et al., 1986; Showers and Maurer, 1986; Beebe et al., 1989]. The type II regulatory subunit (RII) has been shown to undergo autophosphorylation at serine^{95} of the primary sequence. RII has also been shown to be phosphorylated in vitro by glycogen synthase kinase 3 and in vivo by casein kinase II, but at different sites [Carmichael et al., 1982; Hemmings et al., 1982]. More recently, Keryer et al. [1991] have shown that the RII regulatory subunit is also phosphorylated in vivo by the p34^{cdc2} mitotic kinase in HeLa cells.

We previously demonstrated that the proliferation of psoriatic fibroblasts in culture is not modulated by cAMP [Evain-Brion et al., 1985], which apparently is related to a deficiency of cAMP-PK in these cells [Evain-Brion et al., 1986]. cAMP-dependent protein kinase activity is decreased in psoriatic fibroblasts, and exposure of these cells to retinoic acid induces an

Abbreviations used: cAMP-PK, cAMP-dependent protein kinase; RA, retinoic acid; R, regulatory subunit of the cAMP-PK; PAGE, polyacrylamide gel electrophoresis.

Received March 19, 1993; revised June 29, 1994; accepted September 13, 1994.

Address reprint requests to Françoise Raynaud, Laboratoire de Physiopathologie du Développement, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05, France.

© 1995 Wiley-Liss, Inc.

increase in cAMP-PK activity to levels found in normal human fibroblasts [Raynaud et al., 1987, 1989]. In addition, recent studies have demonstrated that a synthetic retinoid (acitretin) used in the treatment of this disease acts very rapidly (within minutes) to elevate 8-azido-[^{32}P]-cAMP binding in erythrocytes isolated from psoriatic patients [Raynaud et al., 1993].

Different reports indicate a specific role for the two types of cAMP-PK in the regulation of cellular functions. Specific cellular localization [Nigg et al., 1985; De Camilli et al., 1986] and association of type II holoenzyme with the p34^{cdc2} kinase [Tournier et al., 1991] suggests a key role for this kinase in the regulation of the cell cycle. Due to the hyperproliferation of psoriatic fibroblasts in monolayer culture and the predominant decrease in type II cAMP-PK activity [Evain-Brion et al., 1986], it is of interest to define the type of abnormality observed in the cAMP-PKII in psoriatic cells and the effect of retinoic acid on this enzyme.

MATERIALS AND METHODS

Materials

Dulbecco modified Eagle medium (DMEM) and glutamine were from ICN Biomedicals (Costa Mesa, CA), fetal calf serum from Seromed (Munich, Germany), molecular weight standards from Bio-Rad, cAMP and protease inhibitors from Sigma. All other chemicals used were of analytical grade and purchased from Sigma, Merck (Darmstadt, Germany) or Bio-Rad (Richmond, CA).

Fibroblast Culture

Human fibroblasts were isolated from normal and untreated adult psoriatic patients (lesional or unlesional skin) by enzymatic digestion of small pieces (4 mm diameter) of punch biopsies from subjects as previously described [Evain-Brion et al., 1986]. Cells were grown on 100 mm culture dishes in a 5% CO_2 humidified atmosphere at 37°C in culture medium (DMEM, 10% fetal calf serum, penicillin [50 U/ml] and streptomycin [50 μg/ml]) which was changed every 3 days. The cells reached confluency within seven days. Subconfluent, growing populations of cells (10^6 cells/dish) were used in the experiments described.

Photoaffinity Labeling With 8-azido-[^{32}P]-cAMP

RI and RII were photoaffinity labeled as previously described [Walter et al., 1977] in a reaction mixture (80 μl) of 10 mM Mes, pH 6.2/10 mM MgCl2/1.0 μM 8-azido-[^{32}P]-cAMP (50 Ci/mmol) containing 100 μg of cytosolic protein. Ten micromoles cAMP was included in some samples for the determination of nonspecific labeling. Mixtures were incubated for 60 min in the dark at 4°C and then irradiated for 10 min with a UV lamp. The samples were then heated at 100°C for 2 min in Laemmli sample buffer. The samples were analyzed by SDS-PAGE on a 10% gel according to Laemmli [1970]. The gels were dried and autoradiographed at −80°C using Cronex 4 Dupont medical X-ray film.

Antibodies

The polyclonal antibody against rat heart RIIα was prepared as reported previously [De Camilli et al., 1986].

Immunoblotting

Human fibroblasts were incubated for 48 h in the presence and absence of 1 μM RA. Aliquots (150 μg protein) of cytosol prepared from normal and psoriatic fibroblasts were heated at 100°C for 2 min in electrophoresis sample buffer, and SDS-PAGE analysis of each sample was performed on a 10% gel. Proteins were transferred to a immobilon PVDF transfer membrane (Millipore) using a semi dry blotting system (Bio-Rad). The blots were probed with the RIIα antibody and specific bands detected with ^{125}I-protein A (Amersham) as previously described [Tournier et al., 1991].

In Vivo Phosphorylation and Immunoprecipitation

Cells plated onto a 60 mm cell culture dish were incubated in serum- and phosphate-free medium for 13 h with 200 μCi/ml of ^{32}Pi (10 mCi/ml Amersham) at 37°C. The following procedures were then performed at 0–4°C. After incubation, cells were washed three times with ice-cold PBS containing 10 mM NaF and then harvested by scraping into lysis buffer A (50 mM Tris-HCl, pH 7.5/150 mM NaCl/1% Triton X-100/0.1% SDS/1 mM sodium vanadate/10 mM NaF, and aprotinin, leupeptin, antipain, soybean trypsin inhibitor, benzamidine [each 20 μg/ml]). The cell extract was then centrifugated at 13,000*g* for 20 min and protein concentrations were determined in the supernatant by fluorometric assay using bovine serum albumin as the standard [Bohlen et al., 1973]. The deter-

gent solubilized fibroblast extract (500 μg of protein) was immunoprecipitated with RIIα antibody as previously described [Tournier et al., 1991]. For two-dimensional gel electrophoresis, samples were solubilized in isoelectric focusing lysis buffer. Two-dimensional electrophoresis was performed as previously described [Tournier et al., 1991] with a 10% acrylamide gel in the second dimension. The gels were dried and autoradiographed at −80°C using Cronex 4 Dupont medical X-ray film.

In Vivo ^{35}S Methionine Labeling and Immunoprecipitation

Cells plated onto a 100 mm cell culture dish were incubated in serum- and methionine-free medium for 18 h with 100 μCi/ml of 35 methionine (1,054 mCi/ml Amersham) at 37°C. The procedures for cell lysis, immunoprecipitation of RIIα, (100 μg of protein) and two dimensional gel electrophoresis were performed at 0–4°C as described in the above paragraph.

Densitometric Scanning of Autoradiographs

Densitometric scanning of autoradiographs was obtained from a nonsaturated autoradiographs of Western blot and 8-azido-[^{32}P]-cAMP binding experiments. The experiments were carried out for all normal and psoriatic subjects at the same time and each gel contained the same internal control. The results are expressed as relative units of optical density.

RESULTS

The cAMP-dependent protein kinase regulatory subunits present in cytosols prepared from normal and psoriatic human fibroblasts were specifically labeled with 8-azido-[^{32}P]-cAMP. In Figure 1A, normal human fibroblasts were photoaffinity labeled in the presence or absence (lanes a and b, respectively) of 10 μM unlabeled cAMP to demonstrate specifically labeled bands. A decrease in the ability of the regulatory subunits RI and RII to bind the 8-azido-[^{32}P]-cAMP was observed in psoriatic fibroblasts (Fig. 1A, lane d) compared to normal fibroblasts (Fig. 1A, lane b). This decrease in the ability of the regulatory subunits of cAMP-PK to bind the cAMP analog was more pronounced with the type II regulatory subunit. Retinoic acid treatment (1 μM RA for 48 h) increases the binding of 8-azido-[^{32}P]-cAMP to the regulatory subunits RI and RII in psoriatic fibroblasts (Fig. 1A, lane e) but

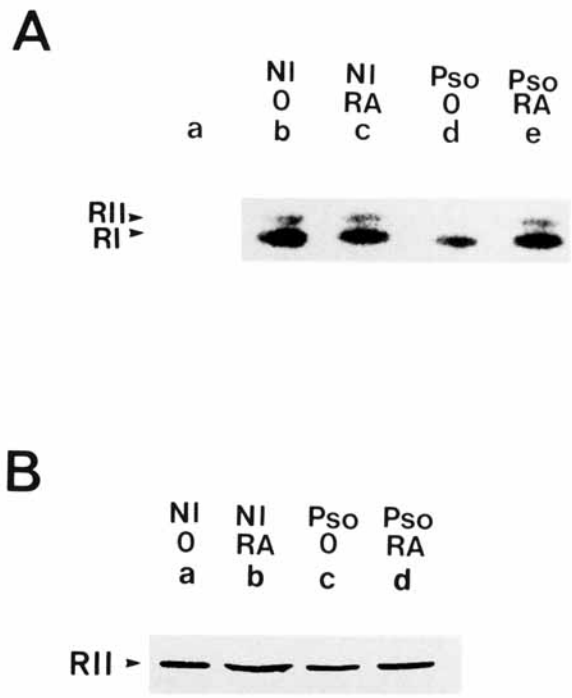

Fig. 1. Immunoblot analysis of 8-azido-[^{32}P]-cAMP binding to RII subunit of PKA protein from cytosols of normal and psoriatic human fibroblasts. **A:** Cytosols of human fibroblasts were specifically labeled with 8-azido-[^{32}P]-cAMP as described in materials and methods and samples were analysed on a 10% SDS-PAGE gel prior to autoradiography. Identical samples of 100 μg of fibroblast cytosol proteins from normal (NI) (**lanes a–c**) and psoriatic (Pso) (**lanes d,e**) subjects treated with (lanes c,e) or without (lanes a,b,d) of 1 μM RA for 48 h were photoaffinity labeled in the absence (lanes b–e) and presence (lane a) of 10 μM unlabeled cAMP to demonstrate specifically labeled bands. A 12 h film exposure is shown. **B:** Cytosols (150 μg protein) prepared from normal (NI) (**lanes a,b**) and psoriatic (Pso) (**lanes c,d**) fibroblasts treated with (lanes b,d) or without (lanes a,c) of 1 μM RA for 48 h were subjected to SDS-PAGE on a 10% gel and then transferred to an Immobilon PVDF membrane. The membrane was incubated with anti-RII a antiserum and immunoblot analysis was carried out as described in materials and methods.

had no effect on the binding of 8-azido[^{32}P]cAMP to RI and RII of normal fibroblasts (Fig. 1A, lane c).

To determine if the decrease in binding of 8-azido-[^{32}P]-cAMP to the RII regulatory subunit might result from a decrease in the amount of RII protein in psoriatic fibroblasts compared to normal fibroblasts, Western blot analysis was carried out using anti-RII specific antibody. No significant difference was detected in the amount of the RII regulatory subunit present in cytosols prepared from normal (Fig. 1B, lane a) and psoriatic (Fig. 1B, lane c) subjects. Further, retinoic acid treatment of normal (Fig. 1B, lane

b) and psoriatic (Fig. 1B, lane d) fibroblasts did not modify the amount of RII protein. The amount of RII subunit protein (Western blot analysis) and the amount of 8-azido-[^{32}P]-cAMP binding on RII present in fibroblast cultures established from skin biopsies of 8 normal subjects and 9 psoriatic patients (9 primary cultures from involved psoriatic plaques and 5 primary cultures from uninvolved skin of the same patients) were quantitated. As shown in Figure 2A and as previously described [Raynaud, 1987], quantitation by densitometric scanning of autoradiographs showed a significant decrease in the 8-azido-[^{32}P]-cAMP binding to RII between normal and psoriatic fibroblasts ($P < 0.001$) as well as a significant increase in the 8-azido-[^{32}P]-cAMP binding to RII in psoriatic fibroblasts after retinoic acid treatment ($P < 0.001$). All results are expressed as the mean ± SEM of relative units: NL (0) = 4.26 ± 0.65; NL (RA) = 3.95 ± 0.85; Pso.IN (O) = 1.47 ± 1.08; Pso.IN (RA) = 4.34 ± 0.80; Pso.UNI (O) = 1.47 ± 0.80; Pso.UNI (RA) = 4.21 ± 1.16. In contrast, as shown in Figure 2B, quantitation by densitometric scanning of autoradiographs showed no significant differences between the amount of RII regulatory subunit protein present in normal and psoriatic fibroblasts incubated in the presence and absence of retinoic acid. All results are expressed as the mean ± SEM of relative units: NL (0) = 5.39 ± 0.97; NL (RA) = 6.45 ± 1.25; Pso.IN (O) = 4.92 ± 0.63; Pso.IN (RA) = 4.62 ± 0.65 SEM; Pso.UNI (0) = 5.82 ± 0.60; Pso.UNI (RA) = 4.28 ± 0.75. These results indicate that the decrease observed in the labeling of the RII regulatory subunit by the 8-azido-[32P]-cAMP in psoriatic fibroblasts as compared to normal is related to an alteration in the ability of the regulatory subunit to bind the cAMP analog rather than to a change in the level of RII protein. Retinoic acid treatment was able to restore normal binding of the cAMP analog in psoriatic fibroblasts. To confirm this result, normal and psoriatic cells were labeled in vivo with ^{35}S methionine followed by immunoprecipitation using an antibody specific for the RIIα subunit. As shown in Figure 3, after analysis of the ^{35}S labeled immunoprecipitates by two dimensional SDS-PAGE, we observed a similar expression of two isoforms of RII in normal and psoriatic fibroblasts (spot 1: Mr = 54, pI = 5.0, spot 2 Mr = 56, pI = 5.0).

Furthermore, in order to rule out a possible abnormally expressed RIIβ isoenzyme in psoriatic fibroblast, we performed specific immunoblotting using an anti-RIIβ antibody. Results with this antibody showed no difference in expression of the RIIβ protein between normal and psoriatic fibroblasts (data not shown).

To further examine the possibility of an altered post-translational modification of the type II regulatory subunit in psoriatic fibroblasts, we carried out in vivo labeling of human fibroblasts with ^{32}P orthophosphate, followed by immunoprecipitation with an antibody specific for the RIIa subunit. As shown in Figure 4, after analysis of the ^{32}P-labelled immunoprecipitates by two-dimensional SDS-PAGE, we observed one phosphorylated form of RII in normal fibroblasts (spot 1: Fig. 4a, Mr = 54kDa; pI = 5.1) and two phosphorylated forms of RII in psoriatic fibroblasts prepared from uninvolved (4c) and involved (4e) skin (spot 1: Mr = 54 kDa, pI = 5.1; spot 2: Mr = 56kDa, pI = 5). These spots were not detected in psoriatic or normal material immunoprecipitated by a preimmune serum (Fig. 4g). These results clearly indicate an alteration in the post-translational modification of the RII subunit in psoriatic fibroblasts. The second phosphorylated form (spot 2) of RII present in psoriatic fibroblasts completely disappeared following retinoic acid treatment of uninvolved (Fig. 4d) and involved (Fig. 4f) psoriatic fibroblasts. In contrast, the spot 1 phosphorylated form of RII found in both normal and psoriatic fibroblasts is not significantly modified by retinoic acid treatment of these cells (Fig. 4b,d,f). These results demonstrate that the phosphorylated form(s) of RII can be modulated by RA treatment in psoriatic fibroblasts.

DISCUSSION

In this study we show that the decreased amount of 8-azido-[^{32}P]-cAMP labeling observed with the type II regulatory subunit of PKA present in psoriatic fibroblasts is related to a decreased ability of this regulatory subunit to bind the cAMP analog and not to a decrease in the amount of RII protein present in the psoriatic cell. This modification of cAMP binding to the RII subunit in psoriatic fibroblasts appears to correlate with an altered phosphorylated form of RII noted in these cells.

The type II regulatory subunit can be phosphorylated either by the catalytic subunit of the cAMP-PK or by other protein kinases [Carmichael et al., 1982]. The presence of phosphate in the autophosphorylation site of the type II regu-

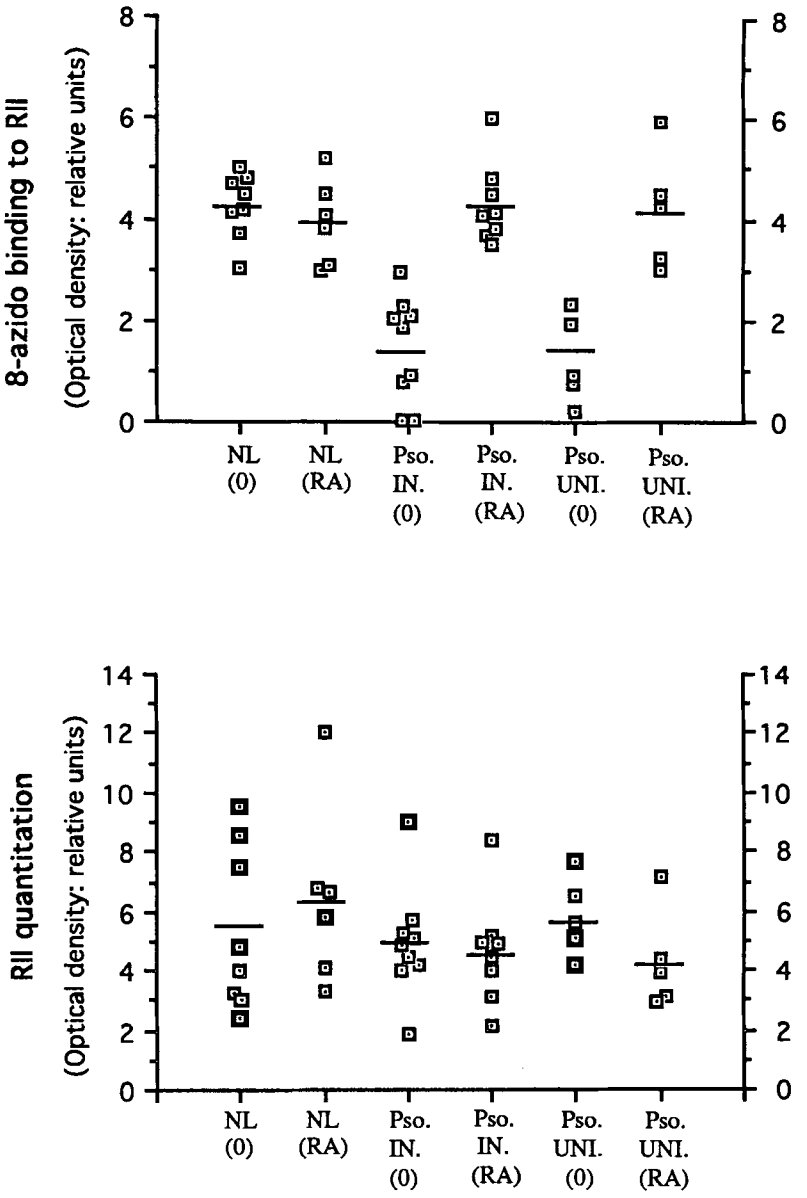

Fig. 2. Quantitation of RII protein levels and 8-azido-[^{32}P]-cAMP binding to RII present in cytosols from normal and psoriatic fibroblasts. Cultured fibroblasts were established from skin biopsies obtained from normal (NL: 8 subjects) and psoriatic (Pso.IN: 9 subjects for involved skin [IN]; Pso. UNI: 5 subjects for uninvolved skin [UNI]) subjects as described. **A:** Cytosol proteins (150 μg) from normal (NL) and psoriatic fibroblasts (Pso.IN and Pso.UNI) treated with (RA) or without (0) retinoic acid were specifically labeled with 8-azido-[^{32}P]-cAMP as described in Materials and Methods and samples were analysed on a 10% SDS-PAGE gel prior to autoradiography. The amount of 8-azido-[^{32}P]-cAMP binding to RII was quantitated by densitometric scanning of the autoradiographs. Results are expressed as relative units of optical density. A statistical comparison was done using Student's *t*-test. A significant decrease was observed in fibroblasts from psoriatic subjects compared to normal ($P < 0.001$) as well as a significant increase in psoriatic fibroblasts after treatment by retinoic acid (NL[0]: 8 subjects, mean 4.26 relative units ± 0.65 SEM; NL [RA]: 6 subjects, mean 3.95 relative units 0.85 SEM; Pso.IN.[0]: 9 patients, mean 1.47 relative units ± 1.08 SEM; Pso.IN.[RA]: 8 patients, mean 4.34 relative units ± 0.80 SEM; Pso.UNI.[0]: 5 patients, mean 1.47 relative units ± 0.80 SEM; Pso.UNI.[RA]: 5 patients, mean 4.21 relative units ± 1.16 SEM). **B:** Cytosols (150 μg) from normal (NL) and psoriatic fibroblasts (Pso.IN and Pso.UNI) incubated in the presence (RA) or absence (0) of 1 mM RA for 48 h were subjected to SDS-PAGE on a 10% gel and then transferred to an immobilon PVDF membrane. The membrane was incubated with anti-RIIα antiserum and immunoblot analysis was carried out. The amount of RII protein was quantitated by densitometric scanning of the autoradiographs of the immunoblots. Results are expressed as relative units of optical density. Statistical comparison was done using Student's *t*-test. No significant differences were observed between normal and psoriatic subjects with or without retinoic acid treatment (NL[0]: 8 subjects, mean 5.39 relative units ± 0.97 SEM; NL[RA]: 6 subjects, mean 6.45 relative units ± 1.25 SEM; Pso.IN.[0]: 9 patients, mean 4.92 relative units ± 0.63 SEM; Pso.IN[RA]: 8 patients, mean 4.62 relative units ± 0.65 SEM; Pso.UNI.[0]: 5 patients, mean 5.82 relative units ± 0.60 SEM; Pso.UNI.[RA]: 5 patients, mean 4.28 relative units ± 0.75 SEM).

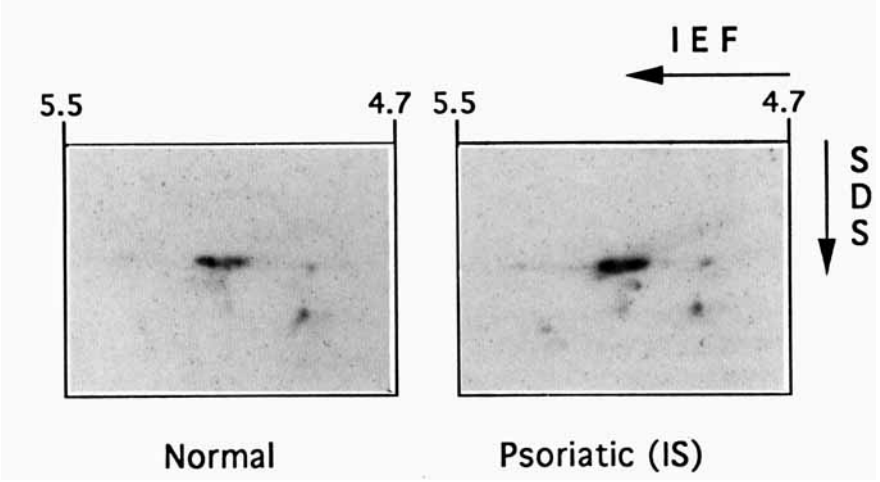

Fig. 3. Immunoprecipitation of [^{35}S] labeled RII regulatory subunit from extracts prepared from normal and psoriatic human fibroblasts. The cells were metabolically labeled with ^{35}S-methionine, the labeled cells were lysed, and RII protein was immunoprecipitated as described in Materials and Methods. The immunoprecipitates were solubilised in SDS sample buffer and separated by two dimensional gel electrophoresis. The ^{35}S labeled RII proteins were detected by autoradiography.

latory subunit increases the dissociation constant for the regulatory and catalytic subunit complex and slows the rate of reassociation. A possible role for the phosphorylation of RII by casein kinase II or by glycogen synthase kinase 3 has not been established. However, Keryer et al. [1991, 1993] have shown that phosphorylation of the cAMP-PKII by $p34^{cdc2}$ results in a decrease in cAMP-binding to the RII regulatory subunit.

Therefore, it was of interest to determine possible differences in the phosphorylation state(s) of RII present in normal and psoriatic fibroblasts. We found two phosphorylated forms of RII (spot 1 and spot 2) in psoriatic fibroblasts and only one (spot 1) in normal fibroblasts. The form specifically observed in psoriatic fibroblasts (spot 2) differed both in its apparent molecular weight and isoelectric point from the spot 1 form of RII found in both normal and psoriatic fibroblasts. This spot 2 phosphorylated form might correspond to a further modified state of RIIα which results in a decrease in 8-azido-cAMP binding.

The molecular mechanisms responsible for the effects of retinoic acid and its synthetic derivatives have not been fully elucidated. An important insight has been provided by the discovery of three retinoic acid receptors (RARs), α, β, γ, and the RXR receptors [Petkovich et al., 1987; Giguere et al., 1987; Mangelsdorf et al., 1990; Leid et al., 1992). The RARs are believed to function in a manner analogous to other members of the steroid/thyroid hormone receptor family, with which they have a high degree of sequence similarity. Retinoic acid receptor α is expressed predominantly in human dermal fibroblasts [Redfern and Todd, 1992]. However, the mechanism of action of retinoic acid to modulate cAMP-dependent protein kinase remains to be elucidated. We previously demonstrated that retinoic acid treatment of psoriatic cells induced an elevation in cAMP-PK activity [Raynaud et al., 1987, 1989]. This could be related either to an increase in cAMP-PK levels or to a modification of the enzyme to influence cAMP binding and phosphotransferase activity. In this study we show that retinoic acid treatment increases the ability of the type II regulatory subunit to bind 8-azido[^{32}P]cAMP without modifying the amount of RII protein. This indicates that retinoic acid is able to modify the type II cAMP-dependent protein kinase at a post-translational level. Conceivably, this might be due to a covalent modification by retinoylation (retinoic acid acylation), which has been shown to occur in a variety of eukaryotic cell lines [Renstrom and DeLuca, 1989]. Indeed, we recently demonstrated that as in HL60 cells [Takahashi et al., 1991], RII is retinoylated in human fibroblasts and that this retinoylation is increased in psoriatic fibroblasts (unpublished data). However, as shown in this study, retinoic acid also is able to induce the modification of type II cAMP-dependent protein kinase at another post-translational level by altering a phosphorylated form of the RII regulatory subunit present in psoriatic fibroblasts. In conclusion, there appears to be an

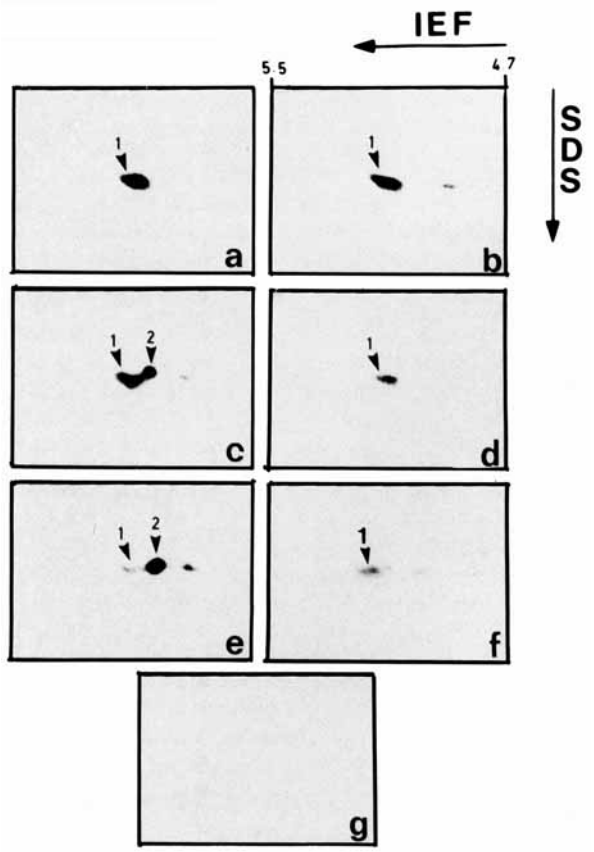

Fig. 4. In vivo phosphorylation of the RII regulatory subunit present in normal and psoriatic human fibroblasts. In vivo labeling of human fibroblasts with 32Pi was followed by immunoprecipitation with specified antibody and analysis of the immunoprecipitates by SDS-PAGE and autoradiography. **a,b:** Normal human fibroblasts incubated in the presence (b) or absence (a) of 1 μM retinoic acid, before in vivo labeling with ^{32}Pi were immunoprecipitated with the anti-RII α antibody. **c,d:** Uninvolved psoriatic fibroblasts incubated in the presence (d) or absence (c) of 1 μM retinoic acid before in vivo labeling with ^{32}Pi were immunoprecipitated with the anti-RII α antibody. **e,f:** Involved psoriatic fibroblasts incubated in the presence (f) or absence (e) of retinoic acid, before in vivo labeling with ^{32}Pi were immunoprecipitated with the anti-RII a antibody. **g:** Normal and psoriatic fibroblasts were immunoprecipitated with a pre-immune serum after in vivo labeling with ^{32}Pi.

altered form of RII present in psoriatic cells characterized by a decrease in the ability to bind 8-azido-[^{32}P]cAMP and by modification of RII to a form detected as spot 2 by two-dimensional gel electrophoresis. These alterations noted in the RII present in psoriatic fibroblasts are reversed by retinoic acid treatment of the cells.

ACKNOWLEDGMENTS

This work was supported by a grant from l'Association pour la Recherche contre le Cancer.

REFERENCES

Beebe SJ, Oyen O, Sandberg M, Froysa A, Hannson V, Jahnsen T (1989): Molecular cloning of a unique, tissue-specific protein kinase (Cγ) from human testis-representing a third isoform for the catalytic subunit of cAMP-dependent protein kinase. Mol Endocrinol 4:465–475.

Bohlen P, Stein S, Dairman W, Unfriend S (1973): Fluorimetric assay of proteins in nanogram range. Arch Biochem Biophys 155:213–220.

Carmichael DF, Geahlen RL, Allen SM, Krebs EG (1982): Type II regulatory subunit of cAMP-dependent protein kinase. J Biol Chem 257:10440–10448.

Clegg CH, Cadd GG, McKnight GS (1988): Genetic characterization of a brain specific form of the type I regulatory subunit of cAMP-dependent protein kinase. Proc Natl Acad Sci U S A 85:3703–3707.

De Camilli P, Moretti M, Denis Donini S, Walter U, Lohmann SM (1986): Heterogeneous distribution of the cAMP receptor protein RII in the nervous system: Evidence for its intracellular accumulation on microtubules, microtubule-organizing centers, and in the area of the golgi complex. J Cell Biol 103:189–203.

Evain-Brion D, Raynaud F, Plet A, Laurent P, Leduc B (1985): Effect of retinoids on cAMP-mediated events in human psoriatic fibroblasts. In Saurat JH (ed): "Retinoids: New Trends in Research and Therapy." Basel: Karger, pp 46–55.

Evain-Brion D, Raynaud F, Plet A, Laurent P, Leduc B, Anderson WB (1986): Deficiency of cyclic AMP-dependent protein kinases in human psoriasis. Proc Natl Acad Sci U S A 83:5272–5276.

Giguere V, Ong ES, Segui P, Evans RM (1987): Identification of a receptor for the morphogen retinoic acid. Nature 330:624–629.

Hemmings BA, Aitken A, Cohen P, Rymond M, Hofmann F (1982): Phosphorylation of the type-II regulatory subunit of cyclic-AMP-dependent protein kinase by glycogen synthase kinase 3 and glycogen synthase kinase 5. Eur J Biochem 127:473–481.

Jahnsen T, Hedin L, Kidd VJ, Beattie WG, Lohmann SM, Walter U, Durica J, Schulz TZ, Schiltz E, Browner M, Lawrence CB, Goldman D, Ratoosh SL, Richards JS (1986): Molecular cloning, cDNA structure and regulation of the regulatory subunit of type II cAMP-dependent protein kinase from rat ovarian granulosa cells. J Biol Chem 261:12352–12361.

Keryer G, Cavadore JC, Bornens M (1991): Mitotic phosphorylation of the regulatory subunit of cAMP-dependent protein kinase type II by cdc2 kinase. J Cell Biol 115(Abstr): 1644.

Keryer G, Zhijun L, Cavadore JC, Erlichman J, Bornens M (1993): Phosphorylation of the regulatory subunit (RIIβ) of type IIβ cAMP-dependent protein kinase by cyclin B/P34cdc2 impairs its binding to MAP2. Proc Natl Acad Sci U S A 90:5418–5422.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Lee DC, Carmichael DF, Krebs EG, McKnight GS (1983): Isolation of a cDNA clone for the type I regulatory subunit of bovine cAMP-dependent protein kinase. Proc Natl Acad Sci U S A 80:3608–3612.

Leid M, Kastner P, Lyons R, Nakshatri H, Saunders M, Zacharewski T, Chen JY, Staub A, Garnier J, Mader S, Chambon P (1992): Purification, cloning and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. Cell 68:377–395.

Mangelsdorf DJ, Ong ES, Dyck JA, Evans RM (1990): Nuclear receptor that identifies a novel retinoic acid response pathway. Nature 345:224–229.

Nigg EA, Schäfer G, Hilz H, Eppenberger HM (1985): cAMP dependent protein kinase type II is associated with the Golgi complex and with centrosomes. Cell 41:1039–1051.

Petkovich M, Brand NJ, Kurst A, Chambon P (1987): A human retinoic acid receptor which belongs to the family of nuclear receptors. Nature 330:144–150.

Raynaud F, Leduc C, Anderson WB, Evain-Brion D (1987): Retinoid treatment of human psoriatic fibroblasts induces an increase in cyclic AMP-dependent protein kinase activity. J Invest Dermatol 89:105–110.

Raynaud F, Gerbaud P, Enjolras O, Gorin I, Donnadieu M, Anderson WB, Evain-Brion D (1989): A cAMP binding abnormality in psoriasis. Lancet i:1153–1156.

Raynaud F, Gerbaud P, Bouloc A, Gorin I, Anderson WB, Evain-Brion D (1993): Rapid effect of treatment of psoriatic erythrocytes with the synthetic retinoid acitretin to increase 8-azido cyclic AMP binding to the RI regulatory subunit. J Invest Dermatol 100:77–81.

Redfern CP, Todd C (1992): Retinoic acid receptor expression in human skin keratinocytes and dermal fibroblasts in vitro. J Cell Sci 102:113–121.

Renstrom B, DeLuca HF (1989): Incorporation of retinoic acid into proteins via retinoyl-CoA. Biochim Biophys Acta 998:69–74.

Saiag P, Coulomb B, Lebreton C, Bell E, Dubertret L (1985): Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model in vitro. Science 230:669–672.

Scott JD, Glaccum MB, Zoller MJ, Ulher MD, Helfman DM, McKnight GS, Krebs EG (1987): The molecular cloning of a type II regulatory subunit of the cAMP-dependent protein kinase from rat skeletal muscle and mouse brain. Proc Natl Acad Sci U S A 84:5192–5196.

Showers MO, Maurer RA (1986): A cloned bovine cDNA encodes an alternate form of the catalytic subunit of cAMP-dependent protein kinase. J Biol Chem 261:16288–16291.

Takahashi N, Liapi C, Anderson WB, Breitman TR (1991): Retinoylation of the cAMP-binding regulatory subunits of the type I and type II cAMP-dependent protein kinases in HL60 cells. Arch Biochem Biophys 290:293–302.

Tournier S, Raynaud F, Gerbaud P, Lohmann SM, Dorée M, Evain-Brion D (1991): Association of type II cAMP-dependent protein kinase with p34cdc2 protein kinase in human fibroblasts. J Biol Chem 266:19018–19022.

Uhler MD, Chrivia JC, McKnight GS (1986): Evidence for a second isoform of the catalytic subunit of cAMP-dependent protein kinase. J Biol Chem 261:15360–15363.

Walter U, Uno J, Liu AYC, Greengard P (1977): Identification, characterization and quantitative measurement of cyclic AMP receptor proteins in cytosol of various tissues using photoaffinity ligand. J Biol Chem 252:6494–6500.