MOLECULAR AND CELLULAR BIOLOGY, May 1988, p. 2204–2210
0270-7306/88/052204-07$02.00/0

Vol. 8, No. 5

Novel Protein in Human Epidermal Keratinocytes: Regulation of Expression during Differentiation

TONJA KARTASOVA,[1]†* GOOS N. P. VAN MUIJEN,[2] HILDE VAN PELT-HEERSCHAP,[1] AND PIETER VAN DE PUTTE

Laboratories of Molecular Genetics and Biochemistry, State University of Leiden, 2333 AA Leiden,[1] and Department of Pathology, University Medical Center, 2333 AL Leiden,[2] the Netherlands

Received 16 September 1987/Accepted 12 January 1988

Recently, two groups of cDNA clones have been isolated from human epidermal keratinocytes; the clones correspond to genes whose expression is stimulated by exposure of the cells to UV light or treatment with 4-nitroquinoline 1-oxide or 12-*O*-tetradecanoylphorbol 13-acetate (T. Kartasova and P. van de Putte, Mol. Cell. Biol. 8:2195–2203, 1988). The proteins predicted by the nucleotide sequence of both groups of cDNAs are small (8 to 10 kilodaltons), are exceptionally rich in proline, glutamine, and cysteine, and contain repeating elements with a common sequence, PK*PEPC. These proteins were designated sprI and sprII (small, proline rich). Here we describe the characterization of the sprIa protein, which is encoded by one of the group 1 cDNAs. The expression of this protein during keratinocyte differentiation in vitro and the distribution of the sprIa protein in some human tissues was studied by using a specific rabbit antiserum directed against a synthetic polypeptide corresponding to the 30 amino acids of the C-terminal region of the sprIa gene product. The results indicate that the expression of the sprIa protein is stimulated during keratinocyte differentiation both in vitro and in vivo.

Human epidermal keratinocytes derive from the uppermost cell layers of the skin, where they form a multilayer cell system. When keratinocytes leave the dividing basal cell layer, they start to differentiate into large, finally anucleated, keratinized cells. Grown in vitro under standard conditions, keratinocytes undergo differentiation as well, resembling the in vivo situation (11). Low calcium concentrations (<0.1 mM) in the culture medium have been shown to inhibit the process of differentiation so that the cells keep dividing without differentiating into a multilayer colony (5, 15). When the calcium concentration is raised to the standard value of 1.8 mM, cells are induced to differentiate; morphological changes can be seen already within 5 min after the switch (5). Twenty four hours later a multilayer of differentiated cells is formed. Therefore keratinocytes represent an attractive model system for the study of changes in gene expression during cell differentiation. This system was used to analyze the expression of a protein (sprIa) whose corresponding cDNA has been recently isolated as belonging to a family of related sequences whose expression is stimulated at the mRNA level by treatment of cultured human epidermal keratinocytes with UV light, 4-nitroquinoline 1-oxide, or 12-*O*-tetradecanoylphorbol 13-acetate (7). The sprIa protein, which possesses a repeated structure, is characterized, and its expression is investigated in relation to the differentiation of keratinocytes.

MATERIALS AND METHODS

Cell culture. A primary culture of epidermal keratinocytes derived from human foreskin was established as described by Reinwald and Green (11) with the modifications of Ponec et al. (10). Keratinocytes were grown in the presence of a feeder layer of gamma-irradiated 3T3 cells in a 3:1 mixture of Dulbecco-Vogt medium and Ham F12 medium supplemented with 0.4 μg of hydrocortisone per ml, 10^{-10} M cholera toxin (Schwarz/Mann, Orangeburg, N.Y.), 10 ng of epidermal growth factor (EGF) (Collaborative Research, Inc., Waltham, Mass.), and 5% fetal calf serum. For experiments cells from passages 2 through 4 were used. Medium containing a low calcium concentration was prepared as a 3:1 mixture of calcium-free Dulbecco-Vogt medium and standard Ham F12 medium supplemented with 5% chelex-treated fetal calf serum (2) and hydrocortisone, cholera toxin, and EGF as above. The final calcium concentration was 0.06 mM as determined by flame photometry. The switch in calcium concentration in the medium from high to low and vice versa was done as follows. Cells were grown first at standard calcium concentration (1.8 mM) until confluency. Then the calcium concentration was dropped to 0.01 mM (calcium-free Dulbecco-Vogt medium with 5% chelex-treated fetal calf serum and standard concentrations of hydrocortisone, cholera toxin, and EGF) for 2 days and then raised to 0.06 mM for 1 day. The monolayers of basal cells were freed from differentiating cells by vigorous aspiration with a pipette. Differentiating cells were collected from the medium by centrifugation. Fresh medium containing the standard (1.8 mM) calcium concentration was added to the monolayers of basal cells; 2, 4, 6, 12, and 24 h after this switch, cells were trypsinized, collected by centrifugation, and stored at −20°C. Control cells were kept in the medium containing low calcium concentration (0.06 mM).

Expression of 15B cDNA in reticulocyte lysate. The sequence of the cDNA insert of clone 15B between the *Eco*RI and *Hin*dIII sites (see Fig. 2) was cloned just downstream of the SP6 promoter in the SP65 vector (Promega Biotec Co., Madison, Wis.). The plasmid linearized by *Hin*dIII digestion was used as a template for in vitro RNA synthesis by SP6

* Corresponding author.
† Present address: Building 37, Room 3B25, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, MD 20892.

polymerase as described previously (3, 4). After synthesis the DNA template was removed from the reaction mixture by treatment with DNase (5 ng/ml, RNase free) for 30 min at 37°C. RNA was extracted with phenol and, after concentration by alcohol precipitation, was used for in vitro protein synthesis in a rabbit reticulocyte lysate (14) (generous gift of Lyda Neeleman, Department of Biochemistry, University of Leiden). [^{35}S]methionine (specific activity, 1,000 Ci/mmol) and [^{3}H]proline (specific activity, 32.2 Ci/mmol) were used at concentrations of 1.5 μCi/μl. Reactions were carried out for 1 h at 30°C and terminated by freezing at −20°C.

RNA isolation and Northern blot analysis. All procedures concerning poly(A)$^+$ RNA isolation and Northern (RNA) blot analysis were as described previously (6).

Peptide synthesis. The peptide corresponding to 30 amino acids of the C-terminal region of the sprIa protein was synthesized by the solid-phase method of Barany and Merrifield (1). The protecting groups were removed by HF treatment, and the synthetic peptide was purified on a G-25 column.

Immunization. A New Zealand White rabbit was immunized subcutaneously with 0.5 mg of the synthetic peptide dissolved in 0.5 ml of phosphate-buffered saline (PBS) containing 0.5 ml of incomplete Freund adjuvant. The animal was boosted 2, 4, 6, and 8 weeks after the first immunization by subcutaneous injections, and antiserum was drawn 9 weeks after the first immunization.

Enzyme-linked immunosorbent assay. Wells of a microdilution plate were coated overnight at 37°C with 1 μg of the peptide in 50 mM sodium carbonate buffer (pH 9.6). After the wells were rinsed with the same buffer, they were incubated for 1 h at 37°C with buffer A (0.2% Na_2HPO_4, 0.07% NaH_2PO_4, 0.82% NaCl, 0.05% Tween 20 [pH 7.4]). Then the synthetic peptide-specific antiserum diluted in buffer A was added to the wells, and the plate was incubated for 2 h at 37°C. As a control, preimmune serum from the same rabbit was used. After extensive rinsing with PBS, goat anti-rabbit immunoglobulin conjugated to horseradish peroxidase was added to the wells, and the plate was incubated for 1 hour at 37°C. After extensive rinsing with PBS, 5-aminosalicylic acid (0.8 mg/ml) in 10 mM sodium phosphate buffer (pH 6.0) containing 0.01% H_2O_2 was added as a substrate to visualize the reaction.

SDS-PAGE and immunoblotting. After trypsinization, keratinocytes grown in vitro were collected by centrifugation. Cell pellets were lysed in a loading buffer containing 10 mM H_3PO_4, 2.5% sodium dodecyl sulfate (SDS), 20% β-mercaptoethanol, and 8 M urea and sonicated for 1 s to reduce viscosity. Reticulocyte lysate translation mixtures were diluted by the loading buffer in a 1:1 ratio. Samples were subjected directly, i.e., without boiling, to SDS–12% polyacrylamide gel electrophoresis (PAGE) (7). After SDS-PAGE the proteins were transferred to nitrocellulose for 2 h at a constant current (0.2 A) in a Tris-glycine buffer (pH 8.3) containing 20% methanol (12). Blots were baked overnight at 65°C. After incubation for 1 h at 37°C with PBS containing 0.5% gelatin, blots were transferred to the PBS solution containing 0.5% gelatin, 5% normal human serum, and a 1:30 dilution of the peptide-specific antiserum and incubated with shaking for 1.5 to 2 h at 37°C. As a control, preimmune serum from the same rabbit was used. In competition experiments the synthetic peptide was added together with the synthetic peptide-specific antiserum. A concentration of 1.25 μg/ml appeared to be most efficient in the inhibition of the immunoreaction. After extensive rinsing with PBS containing 0.05% Tween 20, swine anti-rabbit immunoglobulin conjugated to horseradish peroxidase was added in a 1:400 dilution in PBS containing 5% human serum and 0.5% gelatin and incubated for 1.5 to 2 h at 37°C. Blots were extensively rinsed with PBS containing 0.05% Tween 20 and then with PBS alone and finally incubated for 10 to 20 min with chloronaphthol (3 mg/ml of methanol; Sigma Chemical Co., St. Louis, Mo.) diluted with 100 ml of PBS containing 0.03% H_2O_2.

Coupling of polypeptide to CNBr-activated Sepharose 4B. A 3-mg sample of the synthetic peptide was dissolved in 5 ml of coupling buffer containing 0.1 M H_3BO_3, 25 mM $Na_2B_4O_7$, and 75 mM NaCl. Then 3 g of CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) was added, and the preparation was incubated for 15 min in 1 mM HCl, washed extensively with 2 liters of 1 mM HCl–0.5 liter of coupling buffer, and incubated with the synthetic peptide for 3 h at room temperature with shaking. Then 100 ml of blocking agent–1 M ethanolamine (pH 8.0) was added, and incubation was continued for 2 h. The peptide-bound Sepharose was extensively washed alternately with coupling buffer containing 1 M NaCl and sodium acetate buffer (pH 4.0) containing 1 M NaCl. Sepharose was suspended in 10 mM Tris hydrochloride buffer (pH 7.6) containing 150 mM NaCl (TBS) and 20 mM NaN_3 and stored at 4°C until use.

Affinity chromatography on peptide-bound Sepharose. The synthetic peptide-specific antiserum (5 ml) was incubated with 10 ml of the synthetic peptide-bound Sepharose for 2 h at room temperature in a TBS solution with shaking. The Sepharose suspension was transferred to a Buchner funnel, and unreacted serum was collected. The Sepharose was extensively washed (see above) with the following solutions: 1 liter of TBS, 250 ml of TBS containing 1 M NaCl, and again with 0.5 liter of TBS. Bound antibodies were eluted from the Sepharose with 0.1 M glycine hydrochloride (pH 2.6) and collected in fractions of 2.5 ml containing 150 μl of 2 M Tris hydrochloride (pH 9.0). The optical density at 280 nm of eluted peak fractions varied from 0.062 to 0.071. Five fractions were collected. Purified antibodies were used at 1:2 dilutions in Western blot (immunoblot) analysis and in indirect immunoperoxidase staining of sections of some normal human tissues.

Human tissues. Human tissues were obtained from abortion material of a 15-week-old fetus and from autopsy of a 48-year-old adult. Tissues were deep-frozen in OCT-compound and stored at −70°C.

Preparation of cytoskeletal fractions. Cytoskeletal fractions of cultured keratinocytes were collected by centrifugation after extraction with 10 mM Tris hydrochloride (pH 7.4) containing 1.5 M KCl and 1% Triton X-100.

Immunohistochemical staining. Indirect immunoperoxidase staining of frozen sections was done by the method of Nakane and Pierce (9) as described previously (13).

RESULTS

In the accompanying paper we describe the isolation of two groups of related cDNA clones, corresponding to genes whose expression in human epidermal keratinocytes is stimulated by exposure to UV light and by treatment with 4-nitroquinoline 1-oxide or 12-*O*-tetradecanoylphorbol 13-acetate (7). The proteins encoded by these genes represent a family of small (89- to 72-amino-acid) polypeptides that are extremely rich in proline, glutamine, and cysteine and contain repeating elements. These proteins were designated sprI and sprII (small, proline rich). The identification and characterization of one of the sprI proteins, sprIa, encoded by

FIG. 1. Amino acid sequence deduced from the cDNA sequence of clone 15B. Symbol: ▭, repeating elements. The 30 C-terminal amino acids chosen for peptide synthesis are underlined.

clone 15B from group 1 cDNAs is presented in this paper. The primary structure of the sprIa protein, including the repeating elements, is shown in Fig. 1.

In vitro synthesis of the sprIa protein. As an approach to gain more knowledge about this peculiar protein, a part of the insert of cDNA clone 15B containing the complete open reading frame for the protein was subcloned into the pSP65 plasmid (Fig. 2), followed by in vitro transcription with SP6 RNA polymerase. The newly synthesized RNA served as a template for direct in vitro protein synthesis in a rabbit reticulocyte lysate system. When [^{35}S]methionine was used as a radiolabeled precursor in protein synthesis, no incorporation of radioactivity above background was found. However, when [^{35}S]methionine was substituted with [^{3}H]proline, incorporation of label in trichloroacetic acid-insoluble material could be easily detected. Since no [^{35}S]methionine was incorporated during in vitro protein synthesis, the first methionine, which is the only one in the amino acid sequence of the sprIa protein, is probably removed from the final amino acid sequence. Whether this happens also in vivo remains to be determined.

Upon analysis on a one-dimensional polyacrylamide gel under denaturating conditions (SDS-PAGE), the in vitro-synthesized sprIa protein migrated as a single band of 18 kilodaltons (kDa), but only when the boiling step, which is generally used for protein denaturation just before loading on gels, was omitted (Fig. 3, lane a). Boiling of the sample apparently led to the aggregation of the sprIa protein, since in this case it migrated as a smear in the region of 43 kDa (Fig. 3, lane b).

Preparation and characterization of antibodies. To identify the sprIa protein in vivo and its intracellular localization and to study its expression in different human tissues, polyclonal antibodies were raised in a rabbit against an in vitro-synthesized polypeptide of 30 amino acids corresponding to the C terminus of the sprIa protein (Fig. 1). The immunoreactivity of the antiserum was tested by enzyme-linked immunosorbent assay with the synthetic peptide used for immunization as the antigen. The titer of the antiserum against the peptide in the enzyme-linked immunosorbent assay was 10,000 (data not shown). The in vitro-synthesized sprIa protein was specifically detected by the antiserum on Western blots (data not shown).

The antiserum was used to probe a Western blot containing proteins from keratinocytes grown under standard conditions. Boiling of the protein samples before loading on gel was omitted in all experiments described below, except for some special cases which are indicated. Two major bands (50 and 18 kDa) and two minor bands (28 and 21 kDa) were detected (Fig. 4, lane a); no protein band smaller than 18 kDa was present. Controls with preimmune serum did not show any immunoreaction (data not shown). The two major proteins could be separated by extraction of cellular proteins with a hypertonic solution by which the cytoskeleton is separated from other cell components. The 50-kDa protein appeared to be exclusively present in the cytoskeletal fraction (Fig. 4, lane b), whereas the 18-kDa protein was found only in the soluble fraction (Fig. 4, lane c).

The specificity of the antiserum was checked in a competition experiment with the synthetic peptide. In the presence of the synthetic peptide, the antibody reaction with the 18-kDa protein and with two minor bands of 21 and 28 kDa was inhibited completely (Fig. 4, lane d); the reaction with the 50-kDa protein was not affected. To purify the antibodies

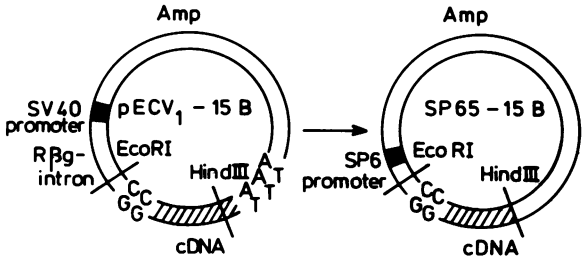

FIG. 2. Cloning of the cDNA insert of clone 15B in the SP65 vector.

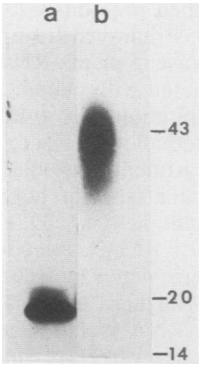

FIG. 3. Analysis of the [^{3}H]proline-labeled, in vitro-synthesized sprIa protein on 12% polyacrylamide–SDS gel. Samples were loaded directly on gel (a) or boiled for 10 min (b). Numbers indicate molecular size markers (in kDa).

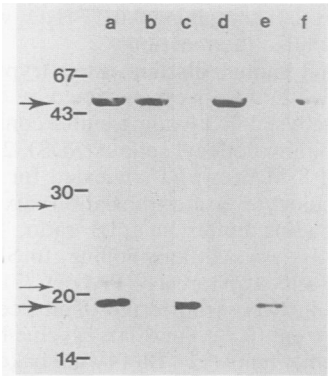

FIG. 4. Western blot analysis of proteins from cultured epidermal keratinocytes probed with the peptide specific antiserum. Samples were directly loaded on gels. Two major and two minor positively reacting bands are indicated by large and small arrows, respectively. Lanes: immunoreaction with total cellular proteins (a), separation of cytoskeletal (b) and cytosolic (c) proteins by extraction with 1.5 M KCl in the presence of 1% Triton X-100, selective inhibition of the immunoreaction with total protein fraction by addition of the peptide (d), and separation by chromatography on the peptide-bound Sepharose of two antibody pools reacting specifically with the peptide (e) and nonreacting (f). Numbers indicate molecular size markers (in kDa).

specifically reacting with the synthetic peptide, this peptide was coupled to CNBr-activated Sepharose. The antiserum was passed through the column containing the synthetic peptide-bound Sepharose. Antibodies which bound specifically to the synthetic peptide as well as those which did not bind were tested on Western blots. The fraction which bound to the synthetic peptide-bound Sepharose contained antibodies specifically reacting only with the 18-kDa protein (Fig. 4, lanes e and f). The fraction which did not bind to the Sepharose column contained antibodies specific only for the 50-kDa protein. Both fractions showed no signal with the 21- and 28-kDa proteins, probably because of the low titer of purified antibodies.

Analysis of the expression of the sprIa protein in cultured keratinocytes. The presence of the sprIa protein in differentiating and proliferating keratinocytes was analyzed with total antiserum by Western blotting (Fig. 5, lanes a and b, respectively). The separation of differentiating and proliferating cells is described in Materials and Methods. The concentration of the 18-kDa protein was much higher in differentiating keratinocytes than in proliferating cells. The 21- and 28-kDa proteins were detected only in differentiating cells. Boiling of the sample just before loading on the gel resulted in a significant loss of the 18-kDa protein (compare lanes a′ and a″ in Fig. 5).

To define more precisely the mechanism of regulation of the expression of the sprIa protein during differentiation, keratinocyte cultures were switched from low calcium (0.06 mM) to standard calcium concentration (1.8 mM) to stimulate differentiation. At 2, 4, 6, and 24 h after the switch total cellular proteins were analyzed by Western blotting. Already 2 h after switching, the 18-kDa protein was detected in amounts larger than those in control cells cultured with low calcium (Fig. 6; compare lanes a and b with lanes c through f). The 21- and 28-kDa proteins were not detected even 24 h after the switch.

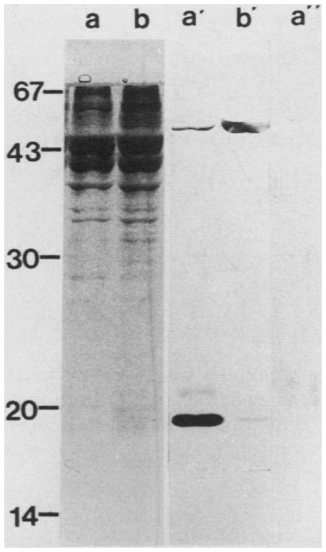

FIG. 5. Coomassie brilliant blue-stained gels (a, b) and corresponding immunoblots after reaction with the peptide-specific antiserum (a′, b′, a″). Analysis of protein from terminally differentiated (a, a′, a″) and basal (b, b′) cells. Cells were cultured under high-calcium concentration and then switched to low calcium concentration for 2 days. Basal cells were collected by trypsinization, and terminally differentiated cells were collected from the culture medium by centrifugation. Samples were loaded on gels directly (a, b, a′, b′) or after 10 min of boiling (a″). Numbers indicate molecular size markers (in kDa).

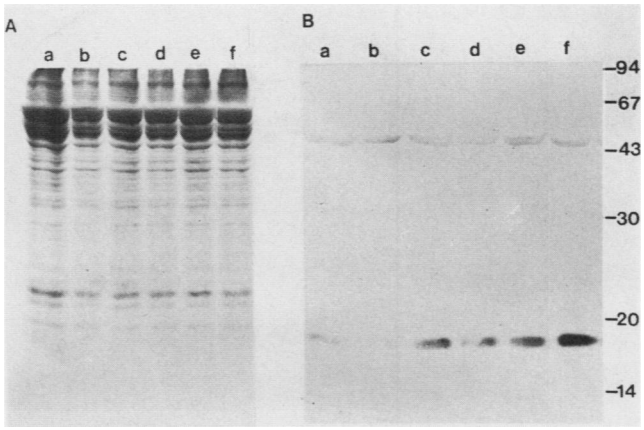

FIG. 6. One-dimensional gel electrophoresis of proteins from cultured keratinocytes stained with Coomassie brilliant blue (A) and the corresponding immunoblot after reaction with the peptide specific antiserum. Cells were cultured under low calcium concentration (a is the same as b, only twice the amount of protein was loaded in a) and switched to high calcium concentration for 2 (c), 4 (d), 6 (e), and 24 (f) h. Numbers indicate molecular size markers (in kDa).

To study at what level the expression of the sprIa protein is regulated, we isolated poly(A)$^+$ RNA from keratinocytes grown at the low calcium concentration and from cells 12 h after a switch from low to standard calcium concentration. No difference in the mRNA concentration was observed between keratinocytes cultured at low and standard calcium concentrations in Northern blot hybridizations (Fig. 7).

Tissue distribution of the sprIa protein. Antibodies purified by the synthetic peptide-Sepharose column and specifically reacting only with the 18-kDa protein (Fig. 4, lane e) were used in immunohistochemical staining of sections of some human tissues. In all experiments the absence of immunoreaction with preimmune serum was observed. In skin sections derived from foot sole, basal and two to three suprabasal cell layers were very weakly positive, and the stratum corneum was negative (Fig. 8A), whereas the stra-

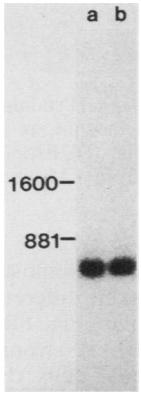

FIG. 7. Northern blot analysis of poly(A)$^+$ RNA isolated from keratinocytes grown at low calcium concentration (a) or 12 h after the switch to high calcium concentration (b) and hybridized with the ^{32}P-labeled 15B cDNA (total plasmid). Numbers indicate molecular size markers (in bases).

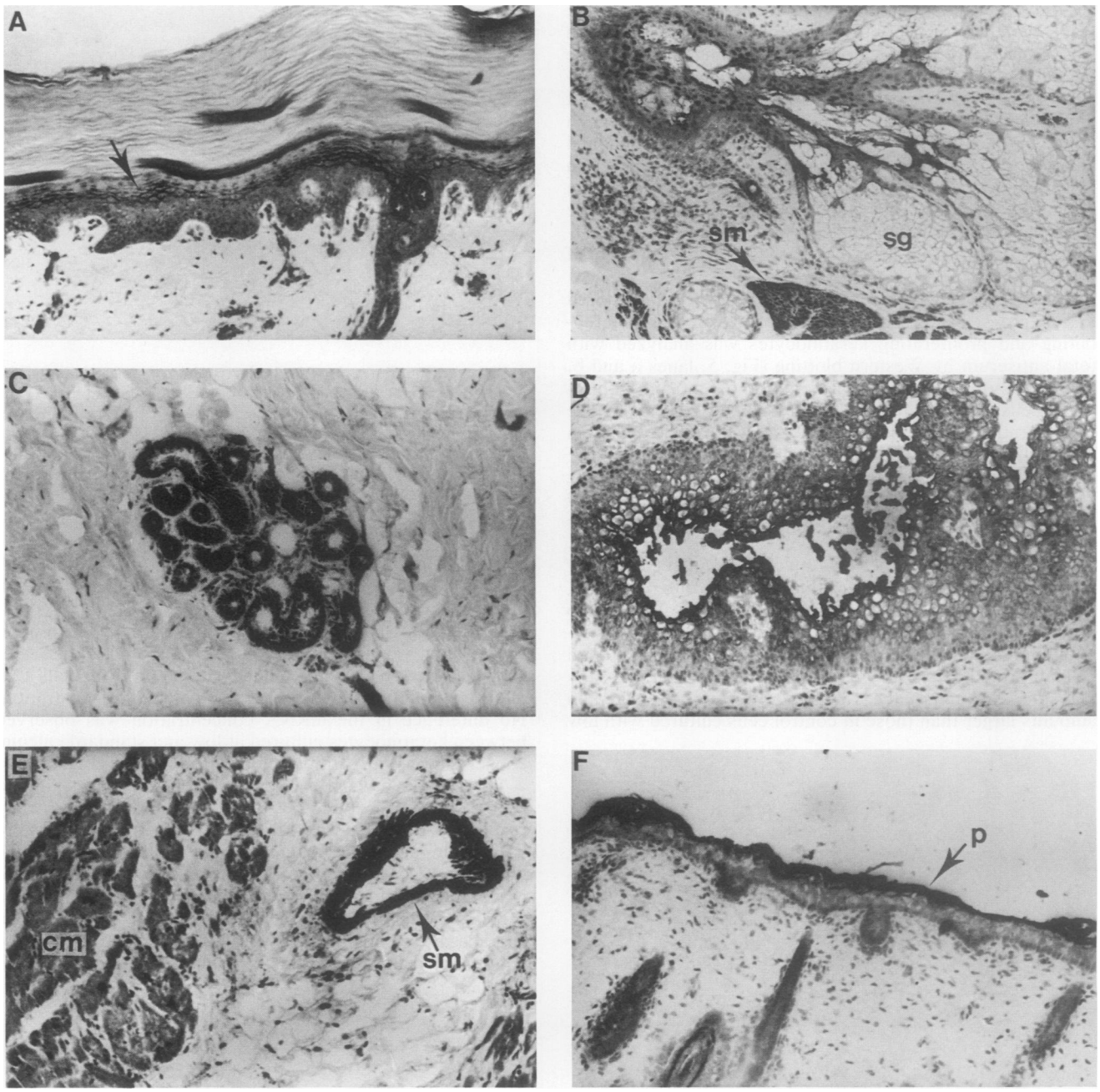

FIG. 8. Immunohistochemical staining of tissues from a 48-year-old adult. (A) Foot sole. Positively reacting suprabasal layers of stratum granulosum and stratum spinosum are indicated by an arrow. (B) Forehead skin. Sebaceous gland (sg) and smooth muscle cells (sm) are indicated. (C) Sweat glands. (D) Esophagus. (E) Heart. Cardiac muscle (cm) cells and smooth muscle (sm) cells of a blood vessel are indicated. (F) Skin tissue from a 15-week-old fetus. Periderm (p) is indicated.

tum granulosum and stratum spinosum were clearly positive. In sections from head skin, differentiated cells in hair follicles were clearly positive (Fig. 8B) and sebaceous glands were negative (Fig. 8B). A strong positive reaction was observed in myoepithelial cells (Fig. 8C) and in smooth muscle cells (Fig. 8B and E). In esophagus cells the positive reaction increased gradually from cell layers above the basal layer, which was negative, to the outermost layer (Fig. 8D). Cardiac muscle cells in heart sections were negative (Fig. 8E). Fifteen-week-old fetal epidermis was negative, but the periderm was clearly positive in its reaction with the specific antibodies (Fig. 8F).

DISCUSSION

The SP6 transcription system and a reticulocyte translation system were used to synthesize the sprIa protein in vitro. Analysis of the in vitro-synthesized sprIa protein on SDS-PAGE reveals two peculiarities. First, in contrast to most known proteins, the sprIa protein tends to aggregate rather than to denature upon boiling in loading buffer.

Second, the sprIa protein has a predicted molecular size of approximately 10 kDa but migrates on an SDS-PAGE gel as a 18-kDa protein. This remarkable migration can be due to the fact that the sprIa protein is hydrophylic and as consequence probably binds fewer SDS molecules than it should according to its molecular weight. Therefore it migrates slower on a polyacrylamide gel. The high proline content (29%) or dimerization of the protein can also be responsible for this abnormal migration.

The specificity of the antibodies directed against the synthetic peptide toward the sprIa protein is confirmed by the following results. The protein from cultured keratinocytes, which is recognized by the antibodies on Western blots, migrates similarly to the in vitro-synthesized sprIa protein and is also sensitive to boiling. Moreover, the antibodies react on Western blots with the in vitro-synthesized sprIa protein (data not shown). The two additional bands of 21 and 28 kDa detected in vivo but not in vitro can be due to posttranslational modification of the sprIa protein in vivo or to antibody reaction with sprIa-related proteins sharing antigenic determinants with the sprIa protein and the synthetic peptide. Immunoreaction of these two bands with specific antiserum is inhibited by addition of the synthetic peptide, indicating that these two bands indeed are related to the sprIa protein. The nature of the 50-kDa protein remains unknown.

The high proline content and the presence of repeats suggest a structural role for the sprIa protein (Fig. 1). However, during extraction with a hypertonic buffer the sprIa protein is not found in the cytoskeletal fraction. This makes it unlikely that the protein is involved in the network of cytoskeleton proteins such as cytokeratins and actins.

Induction of the sprIa protein synthesis after switching the calcium concentration suggests a correlation of the sprIa protein expression with keratinocyte differentiation. This conclusion is also supported by the fact that no positive immunoreaction was found on Western blots with total protein fractions from two nondifferentiating cell lines of epithelial origin: HeLa and A431 (data not shown). No change in the mRNA concentration could be detected on Northern blots 12 h after a switch from low (0.06 mM) to standard (1.8 mM) calcium concentration, suggesting that the regulation of the expression of the sprIa protein is at the level of mRNA translation or protein turnover. Since the sprIa RNA was induced by 12-*O*-tetradecanoylphorbol 13-acetate treatment (7), the lack of induction of the sprI RNA during a calcium switch indicates that these two agents may induce the keratinocyte differentiation via different pathways.

The data on immunohistological staining of sections of human skin confirm the conclusion, drawn from in vitro studies of the sprIa expression in cultured keratinocytes, that the expression of the sprIa protein is regulated during differentiation. The presence of the sprIa protein was demonstrated in cells of the stratum granulosum and stratum spinosum and in differentiated cells of hair follicles and esophagus but to a lesser extent in cells of the proliferating layers. The absence of a reaction in the stratum corneum may be due either to the absence of the sprIa protein in this cell layer or to degradation or selective masking of the sprIa protein which would prevent the immunoreaction with the antibodies, although the sprIa protein was not detected on a Western blot when an extract from stratum corneum was analyzed (data not shown). The positive immunoreaction of the specific antibodies with myoepithelial and smooth muscle cells (along with the cells of periderm) might be the consequence of the cross-reaction of these antibodies with myosin or a related protein, since by computer analysis a limited homology has been found between the sprIa protein and chicken light L1 chain of myosin (7).

The sprIa protein was identified via cDNA cloning as encoded by a gene whose expression is stimulated at the mRNA level by UV light, 4-nitroquinoline 1-oxide, and 12-*O*-tetradecanoylphorbol 13-acetate. However, a high steady-state level of the sprIa protein in cells of mixed keratinocyte population makes it difficult to estimate changes in the amounts of the sprIa protein before and after UV irradiation or treatment with 4-nitroquinoline 1-oxide or 12-*O*-tetradecanoylphorbol 13-acetate. Pulse-label experiments and immunospecific precipitation of the labeled sprIa protein are necessary to clarify this question.

In conclusion, the expression of the sprIa protein is stimulated during differentiation of keratinocytes probably via translational control or protein turnover. The role of this protein in the development of the cellular response to the UV irradiation and in the keratinocyte differentiation remains to be elucidated.

ACKNOWLEDGMENTS

We acknowledge J. Kempenaar, W. Haasnoot, L. Neeleman, and H. Verbeek for expert technical assistance, S. Warnaar and M. Ponec for helpful discussions, and B. J. C. Cornelissen and J. H. J. Hoeijmakers for critical reading of the manuscript.

This work was supported in part by the Foundation for Medical Scientific Research in the Netherlands and by the J. A. Cohen Institute for Radiopathology and Radiation Protection.

LITERATURE CITED

1. **Barany, G., and R. B. Merrifield.** 1980. Solid-phase peptide synthesis, p. 1–248. *In* E. Gross and J. Meienhofer (ed.); The peptides. Academic Press, Inc., New York.
2. **Brennan, J. K., J. Mansky, L. G. Roberts, and M. A. Lichtman.** 1975. Improved methods for reducing calcium and magnesium concentrations in tissue culture medium: application to studies of lymphoblast proliferation *in vitro*. In Vitro **11**:354–360.
3. **Butler, E. T., and M. J. Chamberlin.** 1982. Bacteriophage SP6-specific RNA polymerase. I. Isolation and characterization of the enzyme. J. Biol. Chem. **257**:5772–5778.
4. **Green, M. R., T. Maniatis, and D. A. Melton.** 1983. Splicing of *in vitro* globin transcripts in oocytes. Cell **32**:681–694.
5. **Hennings, H., D. Michael, C. Cheng, P. Steinert, K. Holbrook, and S. H. Yuspa.** 1980. Calcium regulation of growth and differentiation of mouse epidermal cells in culture. Cell **19**:245–254.
6. **Kartasova, T., B. J. C. Cornelissen, P. Belt, and P. van de Putte.** 1987. Effects of UV, 4-NQO and TPA on expression of genes in human epidermal keratinocytes. Nucleic Acids Res. **15**:5945–5962.
7. **Kartasova, T., and P. van de Putte.** 1988. Isolation, characterization, and UV-stimulated expression of two families of genes encoding polypeptides of related structure in human keratinocytes. Mol. Cell. Biol. **8**:2195–2203.
8. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
9. **Nakane, P. K., and G. B. Pierce.** 1966. Preparation and application for the localization of antigens. J. Histochem. Cytochem. **14**:929–931.
10. **Ponec, M., J. A. Kempenaar, and E. R. de Kloet.** 1981. Corticoids and cultured human epidermal keratinocytes. Specific intracellular binding and clinical efficacy. J. Invest. Dermatol. **76**:211–214.
11. **Reinwald, J. G., and H. Green.** 1975. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell **6**:331–344.

12. **Tobin, H., T. Staehelin, and J. Gordon.** 1983. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
13. **Van Muijen, G. N. P., D. J. Ruiter, C. van Leeuwen, F. Prins, K. Rietsma, and S. O. Warnaar.** 1984. Cytokeratin and neurofilament in lung carcinomas. Am. J. Pathol. **116**:363–369.
14. **Van Tol, R. G. L., and L. van Vloten-Doting.** 1979. Translation of alfalfa mosaic virus RNA 1 in the mRNA-dependent translation system from rabbit reticulocyte lysates. Eur. J. Biochem. **93**:461–468.
15. **Watt, F. M., and H. Green.** 1982. Stratification and terminal differentiation of cultured epidermal cells. Nature (London) **295**:343–436.