Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11026–11030, November 1992
Biochemistry

Cornifin, a cross-linked envelope precursor in keratinocytes that is down-regulated by retinoids

KEITH W. MARVIN, MARGARET D. GEORGE, WATARU FUJIMOTO, NICHOLAS A. SAUNDERS, SUSAN H. BERNACKI, AND ANTON M. JETTEN*

Cell Biology Section, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709

Communicated by Howard Green, August 3, 1992

ABSTRACT **In this study, we have characterized the cDNA clone SQ37 that was isolated previously from a rabbit squamous cell library. The gene encodes a 14-kDa protein that appears to function as a component of the cross-linked envelope in squamous differentiating cells. The protein, which has been named cornifin, has a high content of proline (31%), glutamine (20%), and cysteine (11%) and contains 13 repeats of an octapeptide (consensus sequence, EPCQPKVP) at its C terminus. SQ37 mRNA and protein are induced during squamous differentiation of rabbit tracheal (RbTE) cells and human epidermal keratinocytes. This induction is repressed by retinoids. Immunohistochemical studies reveal SQ37 immunoreactivity in fragmented cross-linked envelopes from squamous-differentiated RbTE cells and in the suprabasal layers of the epidermis. *In situ* hybridization analysis showed that the presence of SQ37 mRNA is restricted to the suprabasal layers. Treatment of RbTE cells with a Ca^{2+} ionophore induces cross-linking of the SQ37 protein into higher molecular weight complexes. This cross-linking reaction appears to be mediated by transglutaminase type I. Our observations suggest that the protein encoded by SQ37 participates in the assembly of the cross-linked envelope.**

Epithelial cells from many different tissues are able to undergo squamous differentiation. In the tracheobronchial epithelium this differentiation occurs during vitamin A deficiency and after toxic or mechanical injury (1, 2). In other tissues, including skin, squamous differentiation constitutes the normal pathway of differentiation (3). The existence of histologically distinct layers in squamous epithelia is indicative of a multistage process of differentiation (3, 4). This view is supported by observations that each of these stages can be defined by the expression of specific biochemical and molecular markers (5–8). The formation of the cross-linked envelope is a characteristic feature during later stages of differentiation (9–11). The highly insoluble cross-linked envelope consists of a layer of covalently linked protein assembled just beneath the plasma membrane. Transglutaminases catalyze the formation of ε-(γ-glutamyl)lysine isopeptide bonds between cross-linked envelope precursors (11–15). Several proteins have been implicated in the formation of the cross-linked envelope, including involucrin and loricrin, which have been studied in detail (11, 16–22). The genes for these proteins have been cloned and sequenced (22–24).

Previously, this laboratory has reported (25) the isolation of several cDNA clones, including SQ37, that encode mRNAs abundantly expressed in differentiating squamous RbTE cells. In the present study, the DNA and protein sequences of cDNA clone SQ37 were analyzed. The predicted coding region of SQ37 shows high homology with that of the human gene for the small proline-rich protein 1 (spr-1) (26, 27). However, no function has been described for either gene product. Evidence presented in this study is consistent with the hypothesis that the SQ37 gene product participates in the formation of the cross-linked envelope. We have named this cross-linked envelope protein cornifin.

EXPERIMENTAL PROCEDURES

Cell Culture and Materials. RbTE cells and normal human epidermal keratinocytes (NHEKs) were obtained and cultured as described (15). Retinoic acid and the calcium ionophore Ro 2-2985 were obtained from Hoffmann–La Roche.

Cross-Linked Envelope Formation. Squamous cornifying cultures of RbTE cells were washed in phosphate-buffered saline (PBS) and cells were scraped in 2% (wt/vol) SDS/20 mM dithiothreitol (9). The envelopes were fragmented by pushing the suspension repeatedly through a 25-gauge needle. The suspension was then heated at 100°C for 30 min and cross-linked envelopes were collected by centrifugation and washed three times in PBS.

Transglutaminase Assay. Assay of transglutaminase type I activity was performed as described (15). Labeling of putative substrates of transglutaminase with dansylcadaverine was carried out as described (11, 18).

Sequencing. Sequencing of SQ37 (25) was carried out in both directions by the dideoxynucleotide chain-termination method with a Sequenase kit (United States Biochemical)(28). The DNA and deduced protein sequences were analyzed by the GCG sequence analysis software package (29). Primer extension was carried out as described (30).

Preparation of Antisera and Immunoblot Analysis. The peptides PKVPEPCQPKVPEPCQPKVPQPCQ (SQ37A) and KQPCTLPPQLQQHQVK (SQ37B) were synthesized and purified by the Protein Chemistry Laboratory (University of North Carolina, Chapel Hill). The peptides were cross-linked to bovine serum albumin with glutaraldehyde and injected subcutaneously into New Zealand White rabbits as described (31). Immunization boosts were given at 4-week intervals. Cellular proteins and conditioned medium from cultured RbTE cells were examined by immunoblot analysis as described (32). Immunoprecipitation using protein A-agarose was performed as described (31).

Immunohistochemical Staining. Tissues were fixed for 3 hr in fresh 4% (wt/vol) paraformaldehyde in PBS. Deparaffinized sections were incubated for 60 min with blocking solution [7.5% (wt/vol) nonfat dry milk powder/1% bovine serum albumin in PBS] and then for 60 min with a 1:1000 dilution of SQ37A or SQ37B antiserum or preimmune serum in blocking buffer. Subsequently, tissue sections were incubated for 60 min with biotinylated goat anti-rabbit IgG (The Jackson Laboratory) and then with streptavidin-horseradish

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NHEK, normal human epidermal keratinocyte; spr-1, small proline-rich protein 1.

*To whom reprint requests should be addressed.

peroxidase. Immunoreactivity was visualized using diaminobenzidine (31).

Northern Blot Analysis. Northern blot analysis was performed as described (33). Northern blots were probed with the *Eco*RI-excised 660-base-pair insert of SQ37-1, a sister clone of SQ37 in pBluescript SK−, or with a 1.12-kilobase *Pst* I fragment of the cDNA clone pGAD-28 (34), which contains an insert encoding chicken glyceraldehyde-3-phosphate dehydrogenase. Probes were labeled with [α-^{32}P]dCTP (≈3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) by using a random-priming kit (BRL–Life Science Technologies). After hybridization (24–72 hr at 42°C), blots were washed as described (33).

***In Situ* Hybridization.** Biopsy specimens of rabbit lip, tongue, and esophagus were fixed with 4% paraformaldehyde, dehydrated, and then embedded in paraffin. Ribonucleotide probes were synthesized with uridine 5′-[α-[^{35}S]thio]triphosphate (Amersham) from the full-length coding region of SQ37-1. *In situ* hybridization was performed using sense and antisense probes as described (35).

RESULTS

Previously, we reported the isolation of several cDNA clones, including SQ37, encoding mRNAs abundantly expressed in squamous-differentiated RbTE cells (25). In the present study, the insert of clone SQ37 was sequenced in both directions. The cDNA sequence is shown in Fig. 1. A putative initiation codon was evident 44 bases from the 5′ end of the cDNA. The open reading frame terminates with a stop codon 378 bases downstream from the initiation codon and is followed 273 bases further by an ATTAAA polyadenylylation signal near the 3′ end of the sequence. Primer extension revealed that the SQ37 mRNA extends for another 21 bases at the 5′ end. Based on the deduced amino acid sequence, SQ37 encodes a 14-kDa protein (Fig. 1) that contains 13 repeats of the consensus octapeptide EPCQPKVP at its C terminus and has a high content of proline (31%), glutamine (20%), lysine (13%), and cysteine (11%).

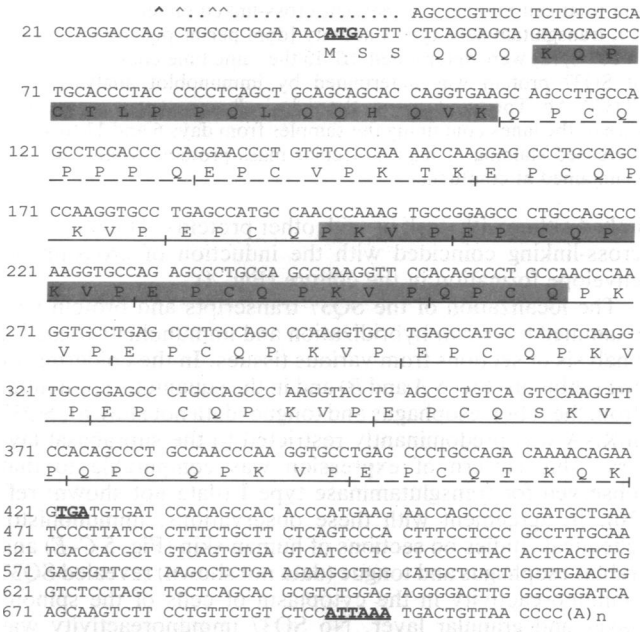

FIG. 1. Nucleotide and deduced amino acid sequence of cornifin (SQ37). The putative initiation codon (ATG), termination codon (TGA), and the polyadenylylation signal (ATTAAA) are indicated in boldface type. The deduced amino acid sequence is shown in the single letter code. The shaded amino acid sequence indicates the two peptides, designated SQ37A (amino acids 51–74) and SQ37B (amino acids 7–22), used to raise antibodies. ^, Termini of the primer extension reaction; ∧, predominant terminus; solid and dashed lines, repeats that are highly (88–100% identical) and moderately (≈50% identical) homologous to the consensus sequence EPCQPKVP, respectively.

The expression of SQ37 appeared to be limited to squamous-differentiating cells. This was indicated by observations showing that SQ37 hybridized to a 0.8-kilobase mRNA in squamous-differentiated RbTE cells and several squamous-differentiated tissues such as rabbit tongue and esophagus and human skin but not to RNA from rabbit brain, liver, kidney, muscle, and normal trachea (Fig. 2*A*). In addition, SQ37 hybridized to a 0.8-kilobase mRNA in cultured squamous-differentiated NHEKs but not in undifferentiated cells (Fig. 2*B*). Retinoic acid, which has been shown to downregulate the expression of several squamous-cell markers (1, 7, 15), also suppressed the induction of SQ37 mRNA in NHEKs (Fig. 2*B*).

To study the cellular distribution and function of this protein, antisera SQ37A-Ab and SQ37B-Ab were raised to the repeated sequence at the C terminus (SQ37A) and a unique N-terminal peptide (SQ37B) (Fig. 1). Total cellular proteins from squamous-differentiated RbTE cells were examined by immunoblot analysis (Fig. 3). Under nonreducing conditions, these antibodies reacted with a 15-kDa protein whereas under reducing conditions they recognized a protein with an apparent molecular mass of ≈23 kDa. This change in electrophoretic mobility may relate to alterations in the conformation of this protein and may be due to the reduction of intramolecular rather than intermolecular disulfide bridges. The specificity of the immunoreactivity was shown by competitive blocking of the protein–antisera interaction

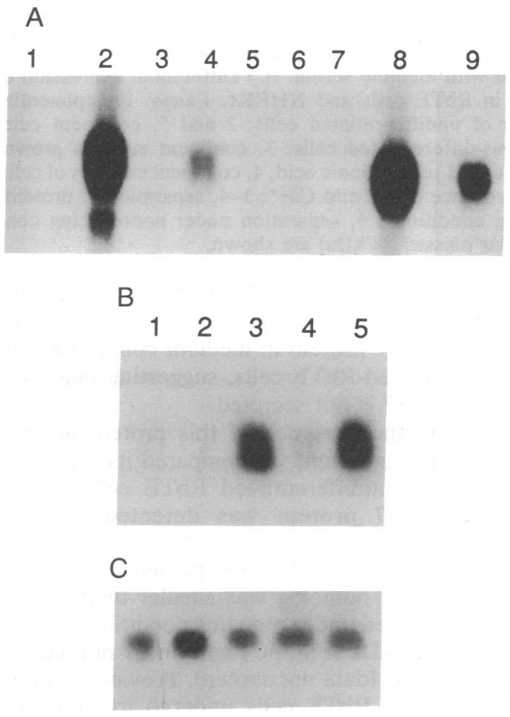

FIG. 2. Northern blot analysis of cornifin mRNA. Total RNA (30 μg) prepared from various tissues or cultured cells were fractionated, blotted to Nytran, and hybridized to ^{32}P-labeled probes for SQ37-1 (*A* and *B*) or glyceraldehyde-3-phosphate dehydrogenase (*C*). (*A*) RNA was from the following sources. Lanes: 1, rabbit liver; 2, tongue; 3, muscle; 4, esophagus; 5, kidney; 6, brain; 7, trachea; 8, cultured squamous-differentiated RbTE cells; 9, human skin. (*B* and *C*) RNA, from cultured NHEKs were analyzed. Lanes: 1, undifferentiated cells; 2, retinoic acid-treated (100 nM, 48 hr) cells; 3, confluent squamous-differentiated cells; 4, confluent retinoic acid-treated (100 nM, 48 hr) cells; 5, confluent Ca^{2+}-treated (1.8 instead of 0.15 mM for 48 hr) cells.

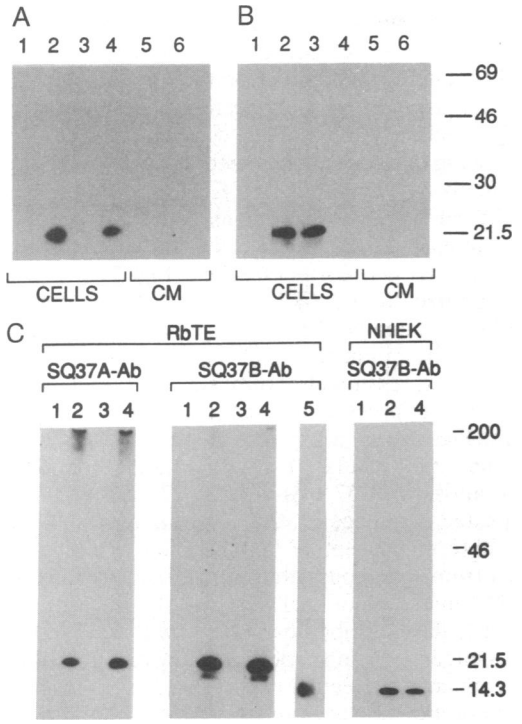

FIG. 3. Immunoblot analysis of SQ37 protein expression. (*A* and *B*) Proteins from squamous-differentiated RbTE cells were analyzed on an immunoblot using SQ37A-Ab (*A*) or SQ37B-Ab (*B*). Lanes: 1, preimmune rabbit serum; 2, immune serum; 3, immune serum plus peptide SQ37A (1 μg/ml); 4, immune serum plus peptide SQ37B (1 μg/ml); 5, medium conditioned (CM) by squamous-differentiated RbTE cells analyzed with preimmune serum; 6, same medium analyzed with immune serum. (*C*) Differential expression of SQ37 protein in RbTE cells and NHEKs. Lanes: 1, exponential-phase cultures of undifferentiated cells; 2 and 5, confluent cultures of squamous-differentiated cells; 3, confluent cultures grown in the presence of (1 μM) retinoic acid; 4, confluent cultures of cells grown in the presence of 1.8 mM Ca^{2+}; 1–4, separation of proteins under reducing conditions; 5, separation under nonreducing conditions. Molecular masses (in kDa) are shown.

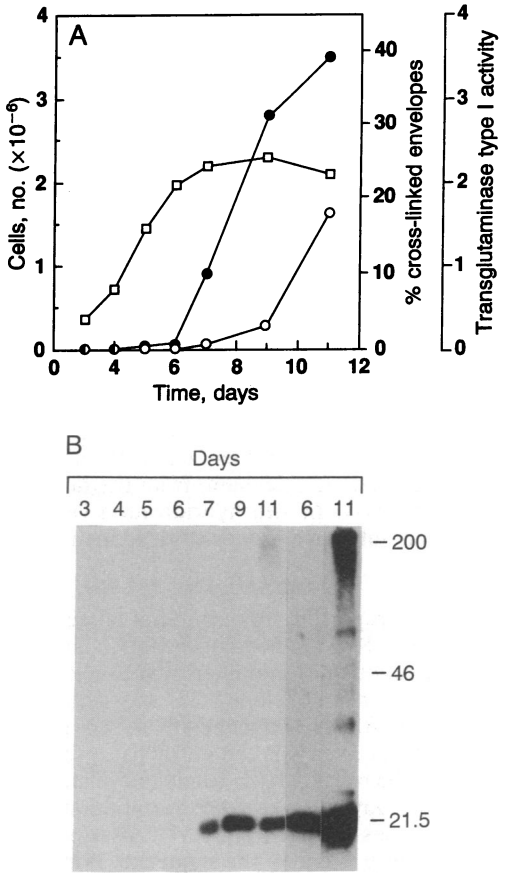

FIG. 4. Induction of SQ37 protein expression in relation to squamous cell differentiation. (*A*) RbTE cells were plated at 4×10^5 cells per 60-mm dish and at the times indicated cells were collected and total cell number (□), percent cross-linked envelope formation (○), transglutaminase type I activity [dpm per hr per mg of protein ($\times 10^{-4}$)] (●) were determined. (*B*) In the same time course, the level of SQ37 protein was determined by immunoblot analysis with SQ37A-Ab. The two lanes on the right in *B* represent an overexposure of the lanes containing the samples from days 6 and 11 to show increased staining of high molecular mass proteins when cells are maintained at confluence.

with homologous peptide and the absence of immunoreactivity with preimmune serum (Fig. 3*A*). The SQ37 antisera did not react with any protein in medium conditioned by squamous-differentiated RbTE cells, suggesting that the protein encoded by SQ37 is not secreted.

To examine the presence of this protein in relation to squamous differentiation, we compared its presence in differentiated and undifferentiated RbTE cells and NHEKs (Fig. 3*C*). SQ37 protein was detected in squamous-differentiated cells but not in undifferentiated cells and its induction was suppressed by the presence of retinoic acid. The human SQ37 homolog was smaller than that from the rabbit and migrated under reducing conditions with a molecular mass of 15 kDa (Fig. 3*C*) and under nonreducing conditions at 10 kDa (data not shown). Previous studies (1, 15) have shown that RbTE cells undergo irreversible growth arrest and start to express several squamous-cell markers after cultures reach confluence. Fig. 4 shows the expression of SQ37 protein in relation to the onset of squamous differentiation. SQ37 protein was dramatically increased after cultures reached confluence. The time course of this induction was very similar to that reported previously for transglutaminase type I (Fig. 4 and ref. 15). Initially, the SQ37 antisera detected only a 23-kDa protein in squamous cells but at later times the antisera reacted with several proteins of higher molecular mass and in particular with proteins >300 kDa. These results suggest that cornifin may become cross-linked either with itself or with other proteins. The increased cross-linking coincided with the induction of cross-linked envelope formation in the culture (Fig. 4).

The localization of the SQ37 transcripts and protein was examined by *in situ* hybridization and immunohistochemical analysis of sections from various tissues. In the epidermis of the rabbit lip (Fig. 5 *A* and *B*) and in the squamous epithelium from the rabbit esophagus and tongue (data not shown), SQ37 mRNA was predominantly restricted to the suprabasal layers. This pattern of expression was comparable to that observed for transglutaminase type I (data not shown; ref. 36). In agreement with these observations, immunohistochemical studies on sections of human skin (Fig. 5 *C–E*) and rabbit esophagus and tongue (data not shown) revealed SQ37 immunoreactivity in the cytoplasm of cells of the spinous layer and granular layer. No SQ37 immunoreactivity was observed in cells of the basal and cornified layers. In the granular layer, the localization of the immunoreactivity changed from the cytoplasm to the cell periphery. Fragments of cross-linked envelopes prepared from cultured RbTE cells were immunoreactive with SQ37A-Ab (Fig. 5*F*) but not with preimmune serum (Fig. 5*G*). These results are in agreement with the hypothesis that during squamous differentiation SQ37 becomes assembled into the cross-linked envelope.

Transglutaminases catalyze the formation of interpeptide bonds between constituents of the cross-linked envelope.

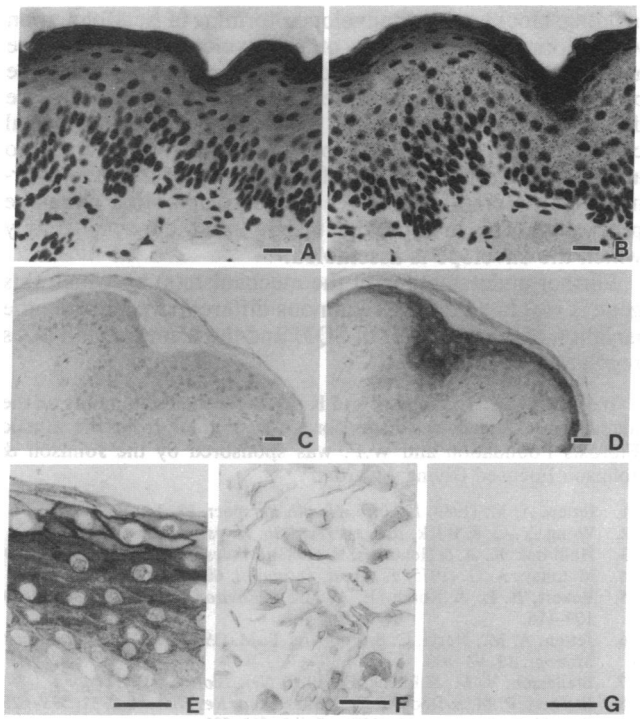

FIG. 5. Localization of SQ37 mRNA and protein expression by *in situ* hybridization (*A* and *B*) and immunohistochemical staining (*C–G*). *In situ* hybridization of sections of epidermis from rabbit lip using ^{35}S-labeled sense (*A*) or antisense (*B*) SQ37-1 probes. Sections of human epidermis were stained with preimmune serum (*C*) and SQ37B-Ab (*D* and *E*). Cross-linked envelopes isolated from squamous cultures of RbTE cells were stained with SQ37A-Ab (*F*) or with preimmune serum (*G*). (Bars = 50 μm.)

The amino acid composition of SQ37 suggested that it might be a substrate for transglutaminase. Addition of a Ca^{2+} ionophore has been used to activate transglutaminase type I and induce cross-linking of envelope precursors such as involucrin (11, 17). To study the cross-linking of SQ37, we treated confluent cultures of RbTE cells with the Ca^{2+} ionophore Ro 2-2985 (25 μg/ml) for 3 hr before cells were harvested and proteins were examined by immunoblot analysis. SQ37B-Ab recognized a 23-kDa protein in total cellular and soluble extracts of squamous-differentiated RbTE cells but not in particulate extracts (Fig. 6*A*). In extracts from cells treated with the Ca^{2+} ionophore, the immunoreactivity with the antibody shifted to high molecular mass proteins at the very top of the gel. The decrease in total immunoreactivity suggests a loss of epitopes probably due to the cross-linking of SQ37 into high molecular mass complexes. Transglutaminase-catalyzed cross-linking of proteins requires Ca^{2+} and is inhibited by the presence of amines that act as competitive inhibitors. Inclusion of EDTA (5 mM) or putrescine (1 mM) in the medium prevented the cross-linking of SQ37 (data not shown). When whole cell extracts were incubated with dansylcadaverine and proteins were separated by polyacrylamide gel electrophoresis, three main fluorescent bands, migrating at 22, 27, and 33 kDa were observed in RbTE extracts and three fluorescent bands, migrating at 14, 15, and 110 kDa, were observed in NHEK extracts (Fig. 6*B*). Immunoprecipation with SQ37B-Ab identified the 22-kDa and the 15-kDa dansylated proteins as rabbit and human cornifin, respectively. The dansylated protein of 27 kDa also reacted with SQ37B-Ab and may be cornifin cross-linked with itself or another protein. The 110-kDa dansylated protein in human extracts is probably involucrin (17, 23). These findings are in agreement with the hypothesis that transglutaminase is the enzyme that catalyzes the cross-linking of SQ37.

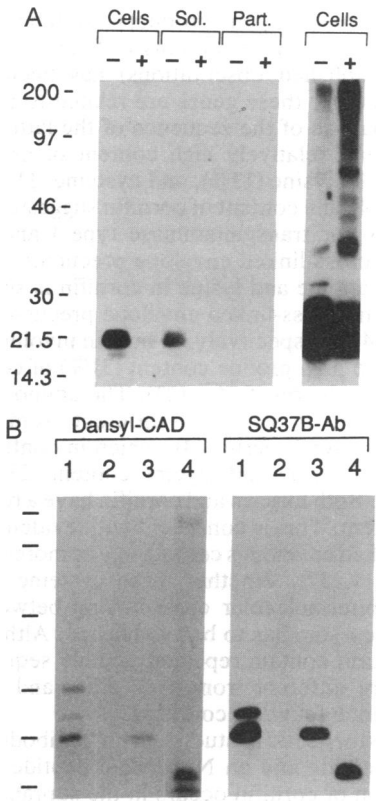

FIG. 6. Transglutaminase-induced cross-linking of SQ37 protein. (*A*) Confluent cultures of squamous-differentiated RbTE cells were treated with the calcium ionophore Ro 2-2985 (25 μg/ml) in dimethyl sulfoxide (lanes +) or dimethyl sulfoxide alone (lanes −) for 3 hr. Proteins from total cellular extract (Cells) and soluble (Sol.) and particulate (Part.) fractions were examined by immunoblot analysis using antiserum SQ37B-Ab. The two lanes on the right are an overexposure of the two lanes on the left to show increased staining of high molecular mass proteins after treatment with Ca^{2+} ionophore. (*B*) Squamous-differentiating RbTE cells and NHEKs were lysed by freezing–thawing in the presence of 1 mM EDTA. Cellular extracts were incubated at 37°C for 3 hr in a solution of 0.02 M borate buffer (pH 9.0), 10 mM $CaCl_2$, and dansylcadaverine (1 mg/ml). Lanes: 1, proteins from dansylated RbTE extracts; 2, dansylated RbTE proteins immunoprecipitated by preimmune serum; 3, dansylated RbTE proteins immunoprecipitated by SQ37B-Ab; 4, dansylated proteins from NHEK extracts. Proteins were separated by polyacrylamide gel electrophoresis and dansylated proteins were visualized with UV radiation (Dansyl-CAD); proteins were then examined by immunoblot analysis with SQ37B-Ab and immunoreactive proteins were identified by autoradiography (SQ37B-Ab).

DISCUSSION

In this study, we have characterized a cDNA (SQ37) encoding a cross-linked envelope precursor that we have named cornifin. The amino acid composition of cornifin, its cellular localization, its ability to become covalently cross-linked, and its presence in cross-linked envelopes support our conclusion regarding its function.

Comparison of the amino acid sequence of cornifin with that of the human small proline-rich protein spr-1 (26) suggests that SQ37 is either the rabbit homolog of spr-1 or a gene very closely related to spr-1. The 30 amino acids at its N terminus are 97% identical with that of spr-1, and each protein contains the sequence TKQK at its C terminus. Both proteins contain a tandem repeat that differs by only one amino acid. Cornifin contains 13 repeats of the consensus octapeptide EPCQPKVP whereas spr-1 has only 6 repeats of the consensus sequence EPCHPKVP (26). The difference in electrophoretic mobility between rabbit and human cornifin (Fig. 3*C*) is in agreement with the difference in molecular

mass of SQ37 and spr-1 gene products. No function for what appears to be a family of proteins (ref. 26, K. W. M. and A. M. J., unpublished observations) has been previously described. Whether these genes are related to pancornulins (19) awaits analysis of the sequence of the latter protein.

Cornifin has a relatively high content of proline (31%), glutamine (20%), lysine (13%), and cysteine (11%). The high glutamine and lysine content of cornifin suggested that it may be a substrate for transglutaminase type I and, therefore, function as a cross-linked envelope precursor. The relative content of glutamine and lysine in cornifin is similar to that reported for the cross-linked envelope precursor involucrin (≈26% and 7.4%, respectively, in human involucrin). On the other hand, the high proline content (31%) of cornifin is not shared with involucrin (5.7%) (23). The amino acid composition of cornifin is also very different from that of the envelope precursor loricrin (24), which in contrast to cornifin, has a high serine and glycine content (23% and 47%, respectively). Both loricrin and cornifin have a relatively high cysteine content. This is consistent with evidence indicating that cross-linked envelopes contain one or more cysteine-rich components (4, 37). Whether these cysteine residues are involved in intermolecular cross-linking between different envelope precursors has to be established. Although involucrin and loricrin contain repeated peptide sequences, these sequences are different from each other and from the repeated sequence found in cornifin.

Immunohistochemical studies with antibodies against a C-terminal peptide and an N-terminal peptide indicate that the expression of cornifin occurs in the suprabasal layers of several squamous differentiating tissues including epidermis, esophagus, tongue, and cultured squamous tracheobronchial and epidermal cells. Cornifin is initially localized in the cytoplasm of cells in the upper spinous and lower granular layers of human skin and then accumulates at the periphery of cells in the upper granular layer. This pattern of distribution is similar to that reported for involucrin (38). No SQ37 immunoreactivity was observed in the cornified layer. This may be due to the loss of antigenicity of cornifin when it is assembled with other proteins such as loricrin into the mature cross-linked envelope. A similar loss of immunoreactivity has been observed with certain anti-loricrin and anti-involucrin antisera (22, 38). SQ37 antisera also recognized epitopes in fragmented cross-linked envelopes isolated from RbTE cultures. This finding is consistent with the hypothesis that cornifin becomes part of the cross-linked envelope. This is supported further by experiments showing that cornifin becomes cross-linked either with itself or with other proteins at a time at which RbTE cells form cross-linked envelopes (Figs. 4 and 6). Likewise, transglutaminase type I is expressed during squamous cell differentiation at a time at which cornifin mRNA and protein are also induced (14, 15; Fig. 3). The Ca^{2+} dependence of the cross-linking and the inhibition by amines such as putrescine are consistent with a transglutaminase-catalyzed reaction. Furthermore, like other envelope precursors, cornifin can be covalently linked to dansylcadaverine when squamous cell extracts are incubated with this dansylated amine. These findings are in agreement with the hypothesis that cornifin is a substrate for transglutaminase type I. However, final proof must come from the identification of $\varepsilon(\gamma$-glutamyl)lysine transpeptide bonds involving cornifin protein.

The assembly of the cross-linked envelope is a complex process and likely to occur in several steps. The observations showing that involucrin, loricrin, and cornifin are induced at different times during squamous differentiation support the hypothesis that the envelope is formed in several stages involving different precursor proteins and different transglutaminases (5, 14–24, 39). Involucrin and cornifin may be early building blocks of the envelope, forming a scaffold upon which loricrin and perhaps other envelope precursors are assembled. The extent to which each cross-linked envelope precursor protein is integrated into the envelope may be different for each tissue and may determine the physical characteristics of the envelope. It would be of interest to analyze the structure of cornifin and to determine the intermolecular interactions between cornifin and other envelope precursors. This may provide insight into the mechanism by which the envelope is assembled.

Further understanding of the mechanism(s) by which this gene is regulated during squamous differentiation awaits the isolation of the promoter of SQ37 and the identification of its regulatory elements.

We thank Drs. T. Vollberg and K. Mills for their comments on the manuscript. S.H.B. was sponsored by a grant from the Cystic Fibrosis Foundation and W.F. was sponsored by the Johnson & Johnson Focused Giving Award.

1. Jetten, A. M. (1989) *Environ. Health Perspect.* **80**, 149–160.
2. Wong, Y.-C. & Buck, R. C. (1971) *Lab. Invest.* **24**, 55–66.
3. Holbrook, K. A. & Hennings, H. (1983) *J. Invest. Dermatol.* **81**, 125–130.
4. Matoltsy, A. G. (1975) *J. Invest. Dermatol.* **65**, 127–142.
5. Eckert, R. L. & Rorke, E. A. (1989) *Environ. Health Perspect.* **80**, 109–116.
6. Jetten, A. M., Nervi, C. & Vollberg, T. M. (1992) *J. Natl. Cancer Inst. Monogr.* **13**, 93–100.
7. Stellmach, V. M. & Fuchs, E. (1989) *New Biol.* **1**, 305–317.
8. Steinert, P. M. & Roop, D. R. (1988) *Annu. Rev. Biochem.* **57**, 593–625.
9. Sun, T.-T. & Green, H. (1976) *Cell* **9**, 511–521.
10. Rice, R. H. & Green, H. (1977) *Cell* **11**, 417–422.
11. Rice, R. H. & Green, H. (1979) *Cell* **18**, 681–694.
12. Abernethy, J. L., Hill, R. L. & Goldsmith, L. A. (1977) *J. Biol. Chem.* **252**, 1837–1839.
13. Yuspa, S. H., Ben, T. & Steinert, P. M. (1982) *J. Biol. Chem.* **257**, 9906–9908.
14. Thacher, S. M. & Rice, R. H. (1985) *Cell* **40**, 685–695.
15. Jetten, A. M. & Shirley, J. E. (1986) *J. Biol. Chem.* **261**, 15097–15101.
16. Simon, M. & Green, H. (1984) *Cell* **36**, 827–834.
17. Simon, M. & Green, H. (1985) *Cell* **40**, 677–683.
18. Baden, H. P., Kubilus, J., Phillips, S. B., Kvedar, J. C. & Tahan, S. R. (1987) *Biochim. Biophys. Acta* **925**, 63–73.
19. Phillips, S. B., Kubilus, J., Grassi, A. M., Goldaber, M. L. & Baden, H. P. (1990) *Comp. Biochem. Physiol. B: Comp. Biochem.* **95**, 781–788.
20. Michel, S., Schmidt, R., Robinson, S. M., Shroot, B. & Reichert, U. (1987) *J. Invest. Dermatol.* **88**, 301–305.
21. Zettergren, J. G., Peterson, L. L. & Wuepper, K. D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 238–242.
22. Mehrel, T., Hohl, D., Rothnagel, J. A., Longley, M. A., Bundman, D., Cheng, C., Lichti, U., Bisher, M. E., Steven, A. C., Steinert, P. M., Yuspa, S. H. & Roop, D. R. (1990) *Cell* **61**, 1103–1112.
23. Eckert, R. L. & Green, H. (1986) *Cell* **46**, 583–589.
24. Hohl, D., Mehrel, T., Lichti, U., Turner, M. L., Roop, D. R. & Steinert, P. M. (1991) *J. Biol. Chem.* **266**, 6626–6636.
25. Smits, H. L., Floyd, E. E. & Jetten, A. M. (1987) *Mol. Cell. Biol.* **7**, 4017–4023.
26. Kartasova, T. & van de Putte, P. (1988) *Mol. Cell. Biol.* **8**, 2195–2203.
27. Gibbs, S., Lohman, F., Teubel, W., van de Putte, P. & Backendorf, C. (1990) *Nucleic Acids Res.* **18**, 4401–4407.
28. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
29. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
30. Phelps, W. C. & Howley, P. M. (1987) *J. Virol.* **61**, 1630–1638.
31. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
32. Jetten, A. M., Bernacki, S. H., Floyd, E. E., Saunders, N. A., Pieniazek, J. & Lotan, R. (1992) *Cell Growth Differ.* **3**, 549–556.
33. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
34. Dugaiczyk, A. D., Haron, J. A., Stone, E. M., Dennison, O. E., Rothblum, K. N. & Schwartz, R. J. (1983) *Biochemistry* **22**, 1605–1613.
35. Noji, S., Takahashi, N., Nohno, T., Koyama, E., Yamaai, T., Muramatsu, M. & Taniguchi, S. (1990) *Acta Histochem. Cytochem.* **23**, 353–366.
36. Michel, S., Bernerd, F., Jetten, A. M., Floyd, E. E., Shroot, B. & Reichert, U. (1992) *J. Invest. Dermatol.* **98**, 364–368.
37. Tezuka, T. & Masae, T. (1987) *J. Invest. Dermatol.* **78**, 150–154.
38. Watt, F. M., Boukamp, P., Hornung, J. & Fusenig, N. E. (1987) *Arch. Dermatol. Res.* **279**, 335–340.
39. Nagae, S., Lichti, U., DeLuca, L. M. & Yuspa, S. H. (1987) *J. Invest. Dermatol.* **89**, 51–58.