# Characterization of an 80-kD Membrane Glycoprotein (GP80) of Human Keratinocytes: A Marker for Commitment to Terminal Differentiation *In Vivo* and *In Vitro*

Michael P. Schön, Alain Limat,* Brigitte Hartmann,† and C. Eberhard Klein‡

Department of Dermatology, University Medical Center Steglitz, The Free University of Berlin, Berlin, Germany; *Department of Dermatology, University of Berne, Berne, Switzerland; †Department of Dermatology, University of Ulm, Ulm; and ‡Department of Dermatology, University of Würzburg, Würzburg, Germany

**We have characterized an 80-kD cell-surface glycoprotein (gp80) identified by monoclonal antibody BT 15, the expression of which is closely associated with a commitment to terminal squamous or follicular differentiation of keratinocytes in normal adult and fetal human epidermis. Maximum expression was found in the suprabasal layers, but basal cells located at the epidermal sulci were also clearly positive, in contrast to the virtually negative basal cells at the epidermal ridges. This protein was also present in benign hyperproliferative disorders of the epidermis (i.e., common warts, keratoacanthoma, psoriasis, and seborrhoic keratoses) with monoclonal antibody BT 15 preferentially staining suprabasal cells and some basal cells at the epidermal sulci. Gp80 was completely lacking in most basal cell carcinomas; the only exceptions were two cases of partially cornifying tumors that were strongly stained around keratotic pearls. In squamous cell carcinomas, gp80 was expressed in keratinized areas of the tumors.**

**In organotypic keratinocyte cultures that resemble the *in vivo* situation, gp80 was strongly expressed in the suprabasal layers. However, unlike known markers for terminal differentiation, gp80 was weakly expressed by basal cells. Synthesis rates of gp80 were high in keratinocyte cell suspensions freshly prepared from skin, and decreased in primary cultures and first and second subcultures (ratio 10:4:2:1). Elevated concentrations of the $Ca^{++}$ that increased stratification of cultured keratinocytes resulted in a two- to threefold increase of gp80 synthesis. Gp80 was not synthesized at detectable levels by the immortal keratinocyte cell line HaCaT; however, it was expressed in HaCaT cultures treated with mitomycin C, indicating an association with cessation of growth. Pulse-chase experiments revealed that gp80 is synthesized from a 55-kD precursor molecule, the maturation of which was prevented by treating cells with tunicamycin. Glycosidase digestion of BT 15 immunoprecipitates from untreated cells indicated that the predominant post-translational modification of the protein is N-linked glycosylation. Our data indicate that gp80 is a glycoprotein that is expressed by growth-arrested human keratinocytes or as part of the terminal differentiation program.** *J Invest Dermatol 105:418–425, 1995*

Differentiation of the human epidermis, with formation of a well-stratified squamous epithelium, requires complex changes in morphology, cellular functions, and underlying biochemical activity of the keratinocyte [1–4]. This is accompanied by expression of a large variety of different molecules mediating various cellular functions [1,5–17].

In this paper, we report the characterization of an 80-kD cell-surface glycoprotein (gp80) defined by monoclonal antibody BT 15 [5], which was generated by immunizing mice with cell extracts of the breast cancer cell line SK-BR-7. In previous studies, monoclonal antibody BT 15 was shown to react preferentially with suprabasal cell layers of the epidermis [5] and with the ductal portions of eccrine sweat glands [18] in normal human skin. It was our goal to characterize the expression of gp80 in normal and diseased human skin and to identify conditions regulating its synthesis *in vivo* and *in vitro*. First, a variety of specimens of adult and fetal normal skin, benign hyperproliferative disorders, and malignant tumors of the skin were examined immunohistochemically. Because these investigations revealed a striking association of gp80 expression with cells committed to terminal squamous and follicular differentiation, we compared its expression in organotypic cultures *in vitro* with expression of known markers of terminal differentiation. Thirdly, the regulation of synthesis of gp80 in normal and immortal keratinocytes was studied by means of radioimmunoprecipitation. In this way, levels of synthesis were compared in normal keratinocytes immediately after isolation from the epidermis and after continued culture. Also, the influence of

Manuscript received October 19, 1994; final revision received April 3, 1995; accepted for publication May 23, 1995.

This work was supported in part by a grant of the Deutsche Forschungsgemeinschaft to CE Klein (DFG KI 510/2-2).

Reprint requests to: Michael P. Schön, Department of Rheumatology and Immunology, Harvard Medical School, The Seeley G. Mudd Building, Room 517, 250 Longwood Avenue, Boston, MA 02115.

0022-202X/95/$09.50 • SSDI0022-202X(95)00262-J • 

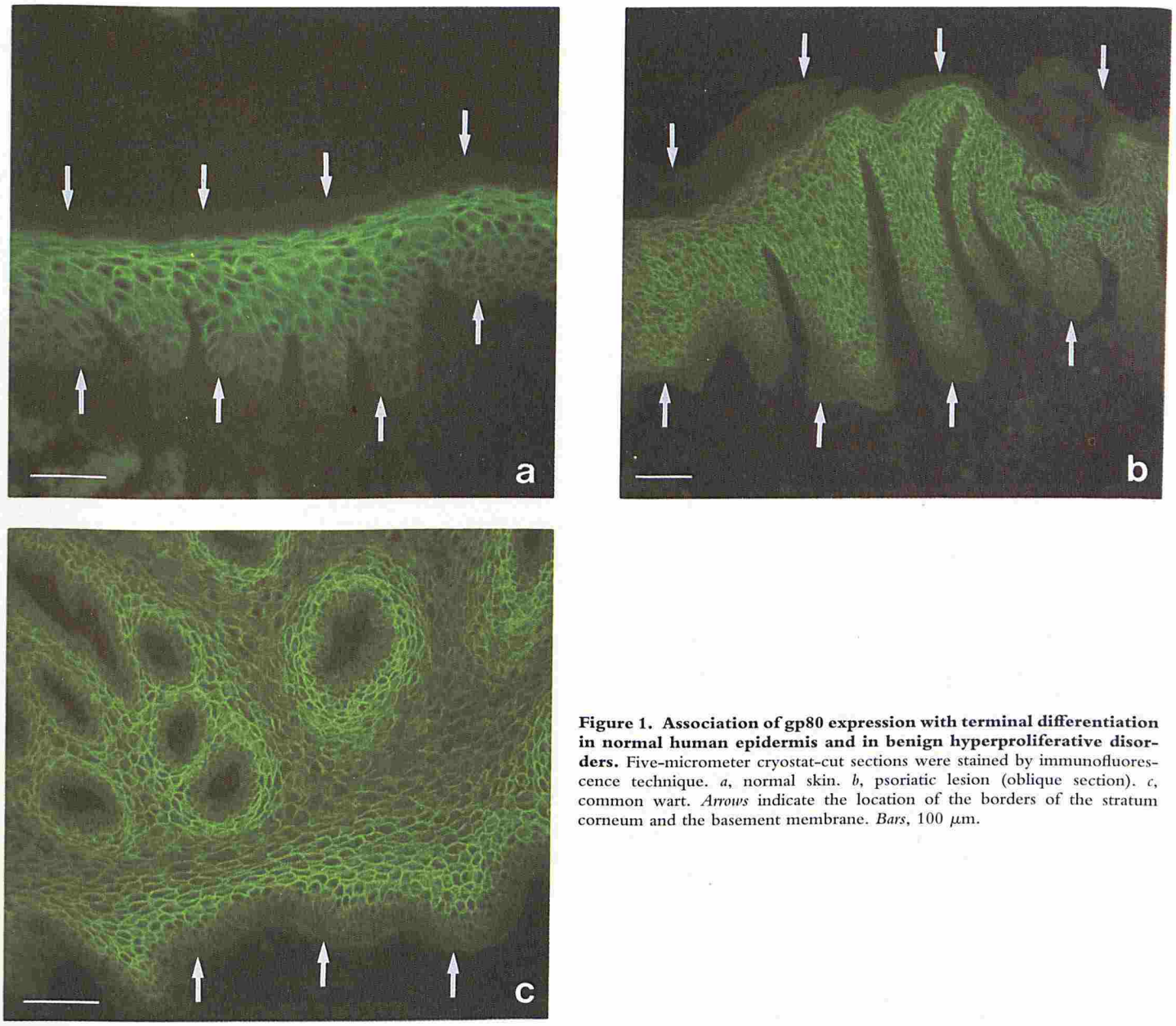


**Figure 1. Association of gp80 expression with terminal differentiation in normal human epidermis and in benign hyperproliferative disorders.** Five-micrometer cryostat-cut sections were stained by immunofluorescence technique. *a*, normal skin. *b*, psoriatic lesion (oblique section). *c*, common wart. *Arrows* indicate the location of the borders of the stratum corneum and the basement membrane. *Bars*, 100 μm.

different concentrations of $Ca^{++}$ on gp80 synthesis was examined. Gp80 synthesis in transformed keratinocytes was investigated with and without treatment with mitomycin C. Finally, the biochemical nature and the post-translational processing of gp80 was examined by pulse-chase experiments as well as by glycosylation studies using tunicamycin treatment and glycosidase digestion.

## MATERIALS AND METHODS

**Cells and Culture Conditions** Primary cultures of human keratinocytes were established using surgical specimens as described [14,19]. Briefly, full-thickness skin was freed of subcutaneous tissue and pieces of 2–3 mm were incubated with 0.1% trypsin in phosphate-buffered saline for 4 h at 37°C. Thereafter, the epidermis was easily peeled from the dermis and both sides were scraped to remove adherent basal keratinocytes. Basal keratinocytes were co-cultured with a feeder layer of postmitotic human dermal fibroblasts as described [20]. The cells were cultured in Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum, 100 U/l penicillin/streptomycin, 10 ng/ml L-glutamine (Biochrom, Berlin, Germany), 12 ng/ml epidermal growth factor, and 30 ng/ml choleratoxin (Sigma, Deisenhofen, Germany). Confluent cultures were obtained after 10–14 d. The influence of different $Ca^{++}$ concentrations on BT 15 synthesis was examined using cultures grown in keratinocyte serum-free medium supplemented with bovine pituitary extract and 20 ng/ml epidermal growth factor (Gibco, Eggenstein, Germany) containing 0.09, 0.30, 0.90, or 2.00 mM $Ca^{++}$.

HaCaT, a spontaneously immortalized human keratinocyte line [21], was the generous gift of N.E. Fusenig (German Cancer Research Center, Heidelberg, Germany). HaCaT cells were cultured in the same medium as normal keratinocytes (without epidermal growth factor and choleratoxin) and were used in a subconfluent state for all experiments. Non-proliferative HaCaT cultures were obtained by treatment with 2.5 or 8 μg/ml mitomycin C for 8 h.

For organotypic cultures, keratinocytes were seeded at $10^5$ cells/$cm^2$ onto collagen gels containing human dermal fibroblasts ($10^5$/ml) and raised to the air/liquid interface for 2 weeks as described [22]. Keratinocytes stratified well under these conditions and showed regular orthokeratosis.

**Monoclonal Antibodies and Antibody Preparation** Monoclonal antibody (MoAb) BT 15 (IgG 1) was generated by immunizing mice with the breast cancer cell line SK-BR-7 [5]. Ascites or hybridoma culture supernatant were used as the source of MoAb. Antibody purification was performed by precipitation of hybridoma supernatant with 0.5 M ammonium sulfate followed by affinity chromatography using a protein A–Sepharose column (Sigma). MoAb BT 15 was very poorly reactive in Western blots.

MoAb MH 99, which recognizes a 38-kD membrane glycoprotein the

expression of which is inversely correlated with keratotic differentiation [7,17,23,24], was used for comparison in the synthesis studies. MoAb KS 8.60 (keratin 10) was purchased from Sigma, and MoAbs specific for involucrin and filaggrin were purchased from BTI (Stoughton, MA). Normal mouse serum (Dako) was used as a control.

**Tissues and Immunohistochemistry** Biopsies or surgical specimens of normal and diseased skin and organotypic cultures of human keratinocytes were snap-frozen in liquid nitrogen; Cryostat tissue sections of 5 μm were cut, mounted on slides, and fixed in acetone at 4°C. Fetal skin was obtained from spontaneous abortions according to the regulations of the committee on medical ethics of the University of Ulm. Immunofluorescence and ABC (Vector laboratories, Burlingame, CA) stainings were performed as previously described [5,7]. For alkaline phosphatase–anti-alkaline phosphatase staining, the fixed sections were incubated with MoAb for 30 min at 20°C, washed twice in phosphate-buffered saline, and then incubated with rabbit–anti mouse immunoglobulin (Dianova, Hamburg, Germany) and the alkaline phosphatase–anti-alkaline phosphatase complex (Dako). To reinforce staining, the procedure was repeated two times. Thereafter, the slides were stained with naphthol AS-BI sodium phosphate (Sigma) and new fuchsin (Merck, Darmstadt, Germany) and, finally, counterstained with hematoxylin.

**Metabolic Labeling, Radioimmunoprecipitation, and Sodium Dodecylsulphate–Polyacrylamide Gel Electrophoresis (SDS-PAGE)** Subconfluent cultures of normal keratinocytes and HaCaT cells were metabolically labeled with 50 μCi/ml $^{35}$[S]methionine (Amersham, Braunschweig, Germany) for 6 h at 37°C and 5% $CO_2$. For pulse-chase experiments, the cell cultures were labeled with 100 μCi/ml $^{35}$[S]methionine for 15 min. Radioactive medium was replaced by non-radioactive medium in the chase periods. Immunoprecipitations were performed as described [24]. Briefly, glycoproteins were enriched from whole cell lysates by concanavalin-A (Con-A) affinity chromatography (Pharmacia, Freiburg, Germany) as described [7,24]. To compare glycoprotein synthesis, equal amounts of radioactivity of the Con-A bound fractions ($1.5 \times 10^6$ cpm) were immunoprecipitated. In other experiments (e.g., glycosylation studies with tunicamycin-treated cells), the proteins were immunoprecipitated directly from crude extracts ($3 \times 10^7$ cpm) without prior Con-A affinity chromatography. Samples were then incubated for 90 min at 4°C with 1 μg of MoAb, and immune complexes precipitated with protein A–sepharose (Pharmacia) coupled to rabbit anti-mouse immunoglobulin (Dako). The precipitates were washed and processed as described [7,17,24]. For fluorography, SDS-PAGE gels were immersed in 0.5 M sodium salicylate for 20 min at 20°C and then dried. Precipitated glycoproteins were quantitated by density scanning of the fluorographs.

**Glycosylation Studies** For inhibition of asparagine-(N-)–linked glycosylation of newly synthesized proteins, keratinocyte cultures were incubated for 4 h in regular medium supplemented with 1–5 μg/ml tunicamycin (Sigma) and then metabolically labeled for 4 h in the presence of 1–5 μg/ml tunicamycin. Thereafter, immunoprecipitation of the antigens was performed as described above.

Glycosidase digestion was carried out using immunopurified BT-15 antigen according to a previously described protocol [24]. The protein A–immune complexes were washed twice and incubated at 37°C with 0.04 U/ml neuraminidase (Sigma), 2 U/ml N-glycanase (glycopeptidase F from Boehringer Mannheim, Germany), or, after treatment with neuraminidase, with 0.02 U/ml O-glycanase (endo-alpha-N-acetylgalactosaminidase from Boehringer Mannheim). Glycosidase digestion was stopped with 2% SDS, and samples were processed by SDS-PAGE as described above.

## RESULTS

### Expression of gp80 *In Vivo* Is Associated with a Commitment to Terminal Differentiation of Human Keratinocytes

Expression of gp80 was examined in normal skin, in benign hyperproliferations, and in malignant disorders. Normal adult skin from various regions of the body showed strong expression in the suprabasal layers **(Fig 1*a*)**. However, a distinct apico-lateral staining with MoAb BT 15 was also observed in basal cells located at the epidermal sulci, as compared to the virtually absent reactivity with basal cells at the epidermal ridges. These observations were paralleled by a similar expression pattern in fetal skin; three specimens of twelfth and fourteenth gestational week, biopsied from both the scalp and the thigh region, showed no or very weak expression of gp80 in the basal layer, but strong reactivity in the suprabasal layers, i.e., stratum intermedium (data not shown). Interestingly, neither primary epithelial germs of fetal skin nor secondary epithelial germ buds arising in early anagen hair follicles expressed gp80 **(Fig 2)**. In terminal hair follicles, gp80 was expressed in the inner root sheath and in the inner portions of the outer root sheath.

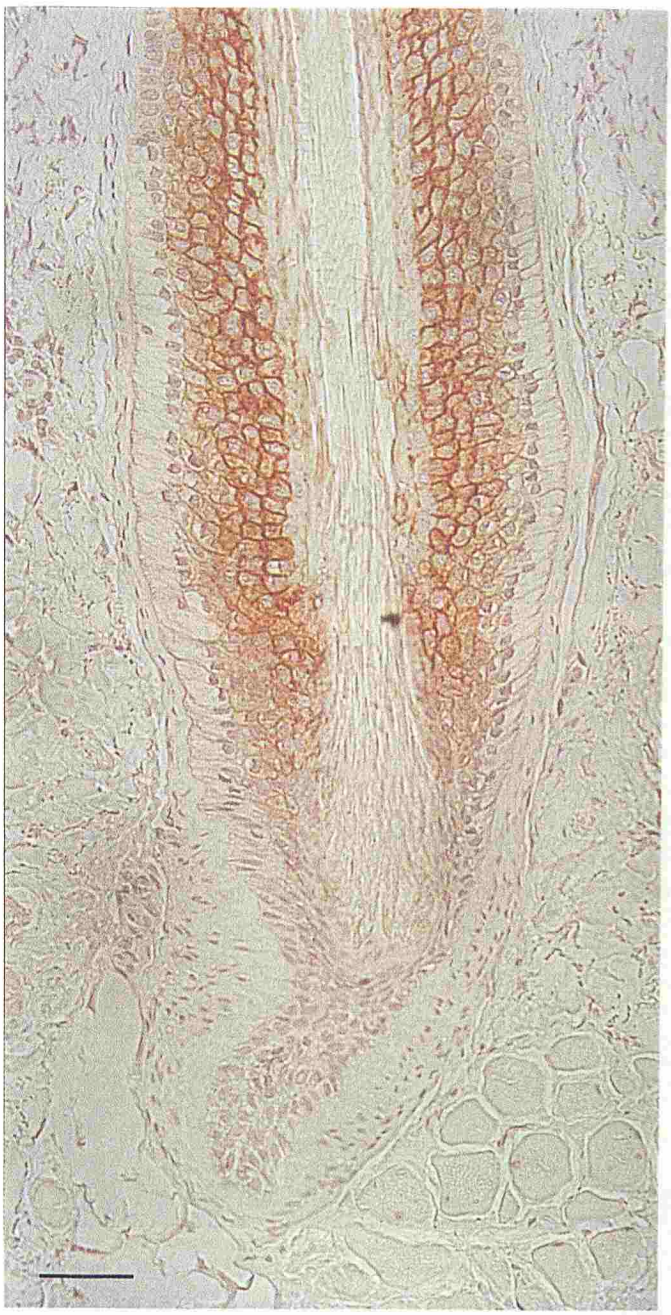

**Figure 2. Gp80 expression is lacking in an outgrowing epithelial germ bud of a terminal hair follicle.** A 5-μm longitudinal cryostat-cut section of a terminal follicle with a secondary epithelial germ bud was stained by the APAAP technique. *Bar*, 100 μm.

In two of two specimens of psoriatic epidermis **(Fig 1*b*)** as well as three of three common warts **(Fig 1*c*)** the basal layer keratinocytes located in the rete pegs did not stain for gp80, whereas suprabasal keratinocytes and basal cells located at the sulci were strongly reactive. Similar expression of gp80 was found in five of five keratoacanthomas and in four of four seborrhoic keratoses (data not shown).

We then examined 26 basal cell carcinomas for gp80 expression, among them two tumors with areas of keratinization **(Fig 3)**. Basal

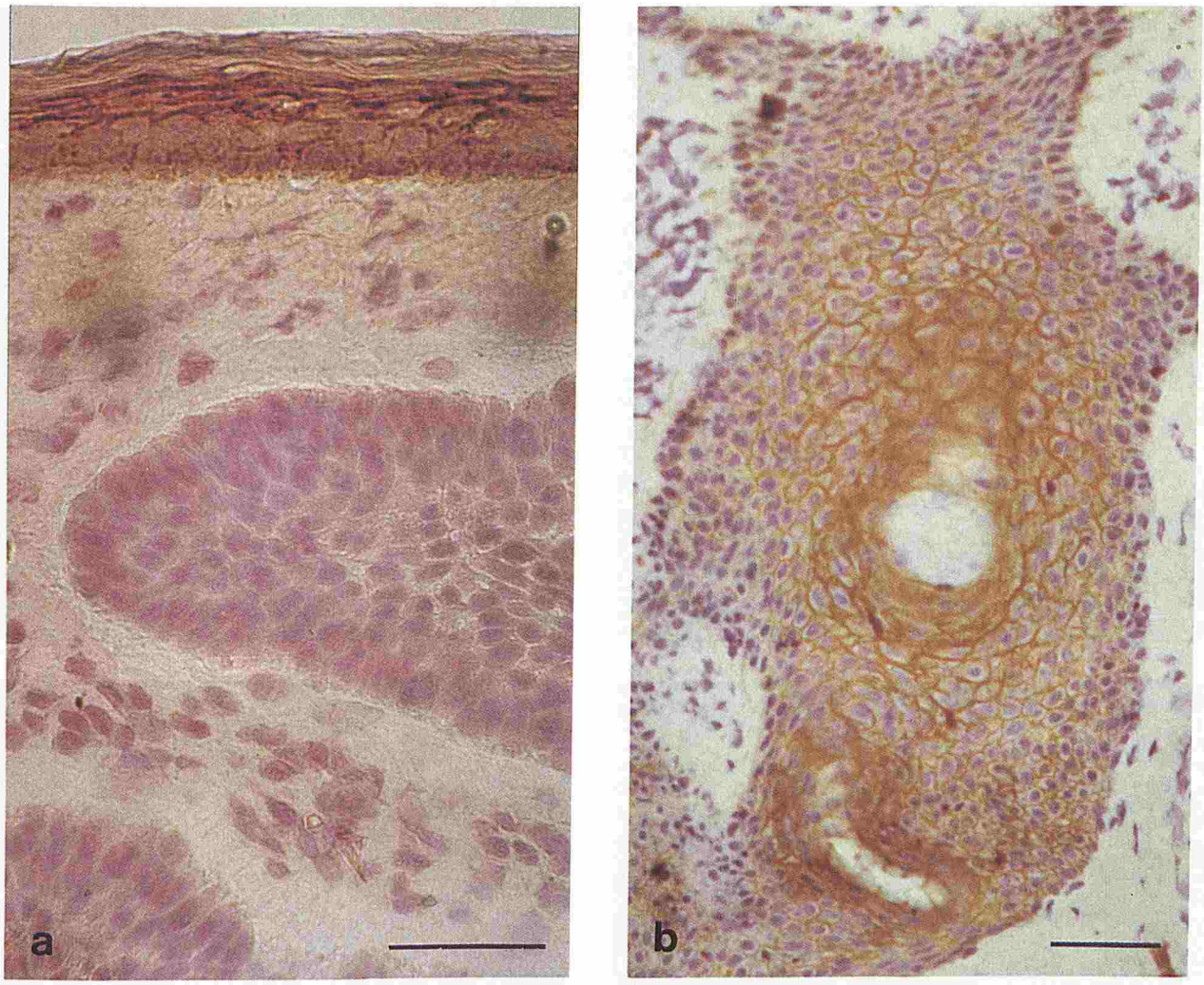


**Figure 3. Lack of gp80 expression in basal cell carcinomas (BCC) and induction in keratinizing areas.** Five-micrometer cryostat-cut sections were stained with MoAb BT 15 using the immunoperoxidase method. *a*, solid BCC with overlaying epidermis. *b*, cornifying BCC with horn pearls in the center of the tumor. *Bars*, 100 μm.

cell carcinomas were generally completely lacking BT 15 reactivity. In six of the tumors, however, up to 20% of the inner cells showed trace expression. Interestingly, in the two cases of keratinizing basal cell carcinomas, the cells around the horn pearls were strongly reactive. In four of four squamous cell carcinomas, 20–25% of the outermost (marginal) cells showed weak reactivity with MoAb BT 15 (data not shown). In three of the cases, almost all (90%) of the inner cells revealed strong expression; in the remaining case, the great majority (70%) were positive. Also in squamous cell carcinomas, BT 15 expression appeared associated with keratotic differentiation: more differentiated portions stained more strongly than did less differentiated portions.

**Gp80 Expression in Organotypic Cultures of Human Keratinocytes Resembles the *In Vivo* Expression** The intriguing distribution of gp80 *in vivo* led us to investigate its expression in keratinocytes cultured organotypically at the air/liquid interface on an artificial collagen/fibroblast dermis-equivalent. Keratinocytes stratified well and showed clear differentiation into basal, suprabasal, and horny layers resembling native tissue. Expression of gp80 was compared with known markers of epidermal terminal differentiation **(Fig 4)**. As expected, keratin 10 **(Fig 4*b*)**, involucrin **(Fig 4*c*)**, and filaggrin **(Fig 4*d*)** were found preferentially expressed in the suprabasal layers of the artificial epidermis demonstrating the development of a well-differentiated and orderly structured tissue in the organotypic culture system. Gp80 showed preferential expression in the suprabasal layers **(Fig 4*a*)**, but unlike the known differentiation markers it was also expressed at decreasing levels all the way down to the basal cells.

**Synthesis of gp80 Decreases Under Continued Culture of Normal Keratinocytes and Is Induced in Growth-Arrested HaCaT Cells** In the initial experiments, we compared normal keratinocytes freshly prepared from epidermis with primary cultures and subsequent subcultures. Synthesis of gp80 was evaluated by metabolic labeling and immunoprecipitation using equal amounts of radioactivity. In freshly isolated keratinocytes, very high levels were detected, whereas the amounts of newly synthesized gp80 gradually decreased during continued culture in primary cultures and first and second subcultures (ratio 10:4:2:1) **(Fig 5)**. For comparison, the MH-99 antigen (gp38), a membrane glycoprotein that is expressed by very immature keratinocytes, was examined. In contrast to gp80, gp38 synthesis clearly increased under continued culture, indicating an inverse correlation of the two cell surface glycoproteins **(Fig 5)**.

In other experiments, we cultured keratinocytes with various concentrations of $Ca^{++}$, ranging from 0.09 mM up to 2.00 mM to examine the effect on gp80 synthesis. Significant amounts of gp80 were synthesized by cells cultured in all concentrations of $Ca^{++}$. However, synthesis rates were increased in higher $Ca^{++}$ concen-

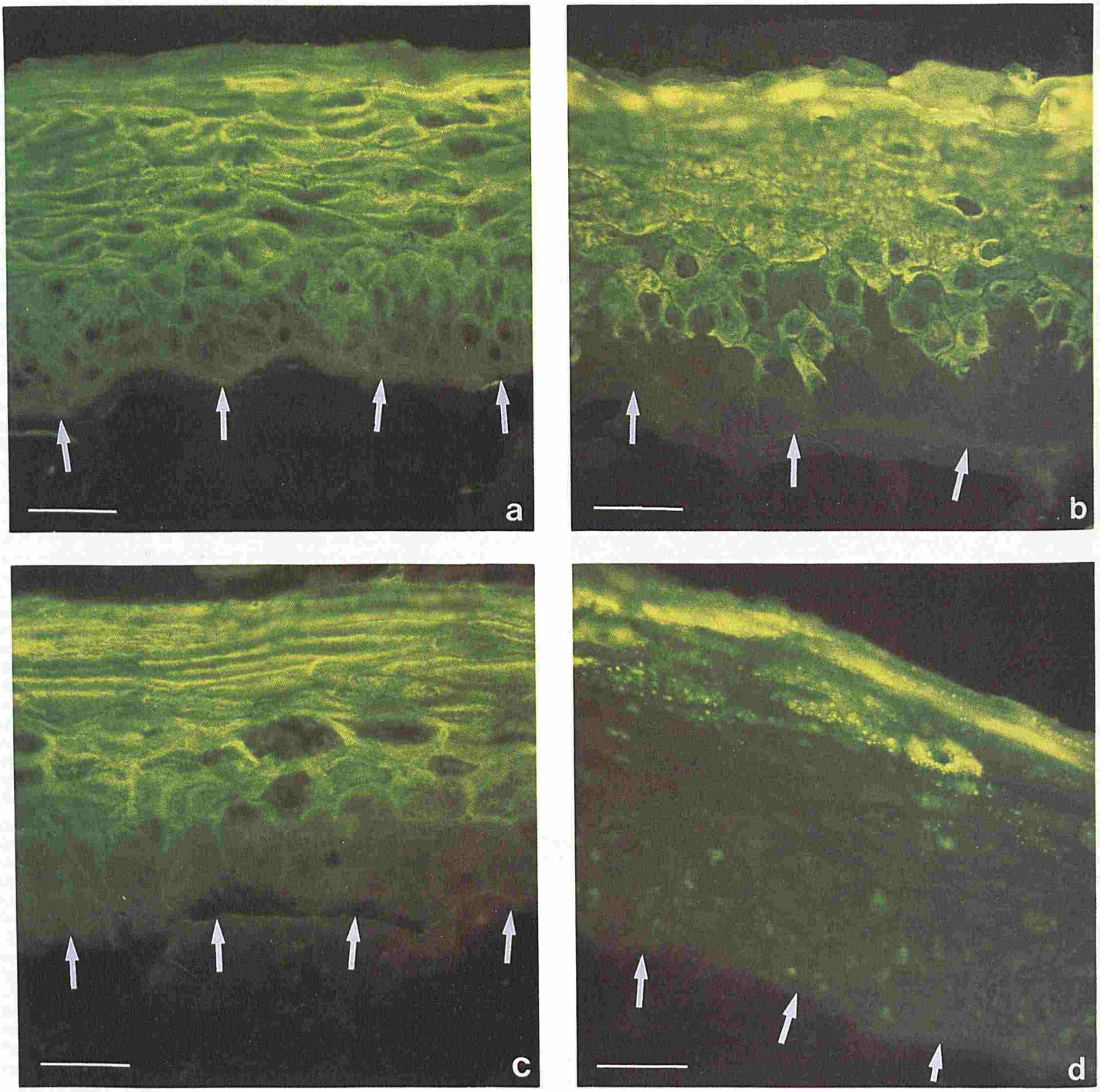


**Figure 4. Expression of gp80 in organotypic keratinocyte cultures is distinct from that of three markers for terminal differentiation.** Normal human keratinocytes were cultured organotypically for 14 d on an artificial dermis equivalent containing human dermal fibroblasts. Cryostat-cut sections of the organoids were stained with MoAb BT 15 (*a*), or with MoAbs against keratin 10 (*b*), involucrin (*c*), and filaggrin (*d*) by indirect immunofluorescence. *Arrows* indicate the basal layer. *Bars*, 100 μm.

trations. As measured by density scanning of the fluorographs, the signal intensity of $^{35}$[S]-immunoprecipitates from cultures incubated with 2.00 mM $Ca^{++}$ were 2–3 times higher than those cultured with 0.09 mM (data not shown). The absence of gp80 in basal cell carcinomas and in most normal basal keratinocytes *in vivo* and in culture led us to investigate its synthesis in the spontaneously immortalized human keratinocyte cell line HaCaT. In these cells, gp80 synthesis was undetectable by radioimmunoprecipitation **(Fig 6)**. Interestingly, in HaCaT cultures treated with 25 or 80 μg/ml mitomycin C, gp80 reappeared at significant levels **(Fig 6)**. Complementary to gp80 again, gp38 synthesis decreased in mitomycin-treated HaCaT cells.

**Post-Translational Processing of gp80** Pulse-chase and glycosylation experiments were designed to elucidate the post-translational processing of gp80. Pulse labeling of primary cultures of normal keratinocytes for 15 min and subsequent immunoprecipitation revealed synthesis of a 55-kD molecule **(Fig 7)**. When the labeling was followed by a 20-min chase in non-radioactive medium, the 55-kD species as well as a clear signal at 80 kD were

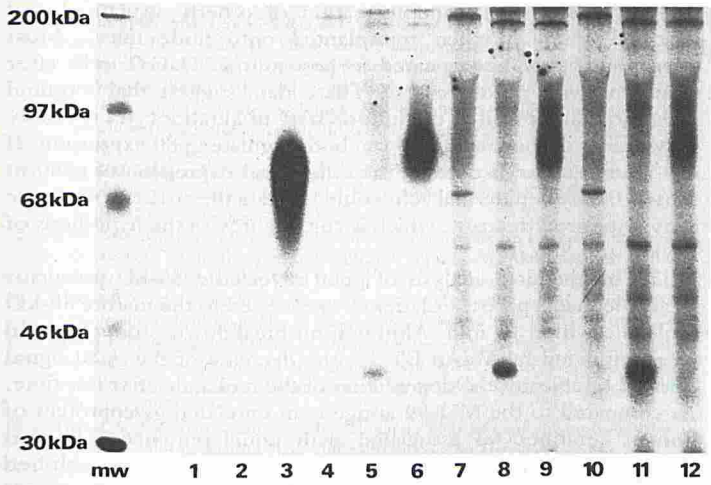


**Figure 5. Synthesis levels of gp80 decrease during serial culture.** Freshly prepared human keratinocytes (*lanes 1–3*), subconfluent primary cultures (*lanes 4–6*), and first (*lanes 7–9*) and second subcultures (*lanes 10–12*) were metabolically labeled with $^{35}$[S]methionine for 8 h. Lysates were immunoprecipitated with normal mouse serum for negative control (*lanes 1, 4, 7, and 10*), with MoAb MH 99 for comparison with an unrelated antigen associated with undifferentiated keratinocytes and for positive control (*lanes 2, 5, 8, and 11*), and with MoAb BT 15 (*lanes 3, 6, 9 and 12*). SDS-PAGE using 9% gels was performed as outlined in *Materials and Methods*.

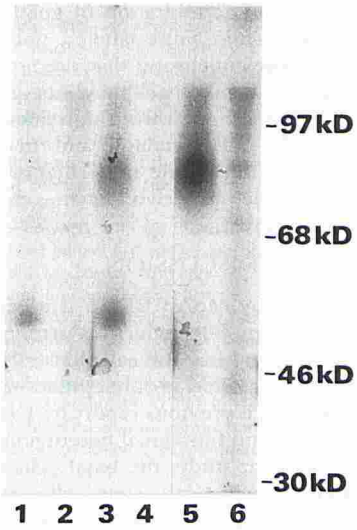


**Figure 7. Post-translational processing of gp80.** Primary cultures of normal human keratinocytes were metabolically labeled for 15 min with $^{35}$[S]methionine and subsequently lysed (*lanes 1 and 2*) or incubated for an additional "chase" period of 20 min (*lanes 3 and 4*) or 90 min (*lanes 5 and 6*) with non-radioactive medium. Immunoprecipitation of the NP40 lysates was performed with MoAb BT 15 (*lanes 1, 3, and 5*) and controls were precipitated with normal mouse serum (*lanes 2, 4, and 6*). SDS-PAGE was performed under non-reducing conditions in a 9% gel.

observed, indicating processing of some of the precursor to the mature protein. After a longer chase period of 90 min, the 55-kD form was no longer present, whereas more of the 80-kD form had accumulated **(Fig 7)**. Maximum levels of mature gp80, labeled during a 15-min pulse, did not accumulate until a chase period of 4–8 h, and the level clearly decreased after 22 h, indicating turnover of the antigen (data not shown).

To clarify whether the 55-kD precursor molecule represents an unglycosylated "protein-backbone" of gp80, we cultured keratinocytes in the presence of 1 to 5 μg/ml tunicamycin to prevent N-(asparagine)–linked glycosylation of newly synthesized proteins **(Fig 8)**. This treatment led to the reduction of the molecular weight of gp80 to 55 kD. In addition, a clear decrease of the signal intensity by about 70% was observed as compared to gp80 from untreated

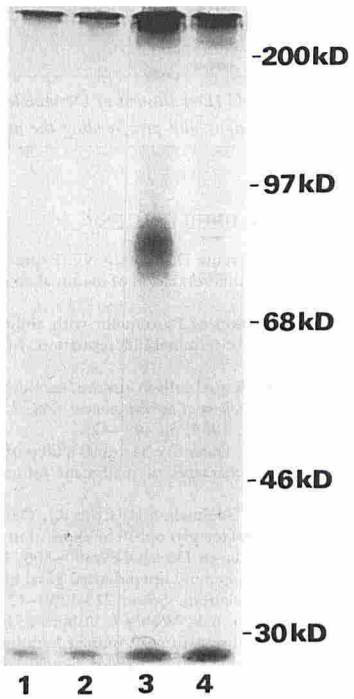


**Figure 6. Lack of gp80 synthesis in untreated HaCaT cells and induction after treatment with mitomycin C.** Lysates from untreated subconfluent HaCaT cells (*lanes 1 and 2*) and from HaCaT cells treated with 8 μg/ml mitomycin C for 8 h (*lanes 3 and 4*) were immunoprecipitated with MoAb BT 15 (*lanes 1 and 3*) and with normal mouse serum (*lanes 2 and 4*). SDS-PAGE was performed under non-reducing conditions in a 9% gel.

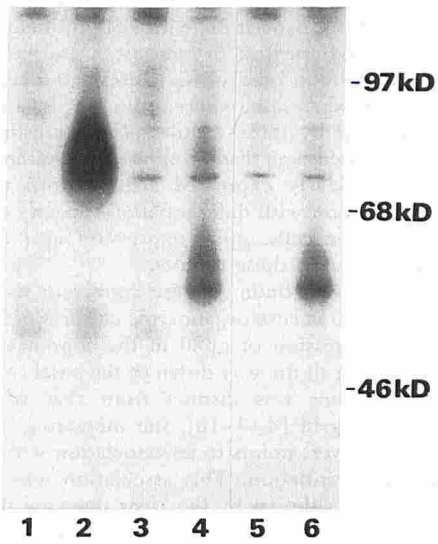


**Figure 8. Tunicamycin inhibits N-linked glycosylation of newly synthesized gp80.** Primary cultures of normal keratinocytes were cultured without (*lanes 1 and 2*), or with 1 μg/ml (*lanes 3 and 4*) and 5 μg/ml (*lanes 5 and 6*) tunicamycin and metabolically labeled with $^{35}$[S]-methionine for 6 h. Immunoprecipitation of the NP40 lysates was performed with MoAb BT 15 (*lanes 2, 4, and 6*) and controls were precipitated with normal mouse serum (*lanes 1, 3, and 5*). Precipitated proteins were run in a 9% gel under non-reducing conditions.

cells. Identical electrophoretic migration of gp80 from tunicamycin-treated and from 15-min pulse-labeled untreated cells was found, consistent with the conclusion that the precursor molecule represents the preglycosylated "backbone" of gp80. In another experiment, immunopurified gp80 from untreated cells was incubated with N-glycanase, O-glycanase, and neuraminidase. N-glycanase led to a reduction of the molecular weight to 55 kD, whereas treatment with the other two enzymes did not reduce the size of gp80 (data not shown).

## DISCUSSION

The antigen recognized by MoAb BT 15 is a cell-surface glycoprotein of $M_r$ 80,000 (gp 80), which is strongly expressed in suprabasal keratinocytes and in basal cells located at the epidermal sulci. The differential expression in subpopulations of basal keratinocytes is consistent with a previous report by Lavker and Sun [6] suggesting morphologic and functional heterogeneity of epidermal basal keratinocytes. In that study, the basal cells at the epidermal ridges were suggested to represent a stem-cell population, whereas those located at the sulci help anchor the epidermis to the dermis. Gp80 expression is absent in the basal cells at the epidermal ridges and the outermost layer of the outer root sheath of the hair follicle and therefore appears to be closely associated with cells undergoing terminal squamous and follicular differentiation. A striking absence of gp80 expression was observed in undifferentiated cells of primary epithelial germs in fetal skin as well as in epithelial germ buds of adult skin arising in early anagen hair follicles, epithelial structures that also represent very immature stages of keratinocyte differentiation. This expression pattern of gp80 is inversely correlated with the MH-99 antigen (gp38), a membrane glycoprotein associated with undifferentiated stages of keratinocyte development [7] and with proliferative activity [17]. Furthermore, the comparison with molecules commonly accepted as markers of terminal differentiation, such as keratins 1 and 10 [1,8–12,25], involucrin [2,14,15], and filaggrin [16] revealed absence of these molecules in basal cells, whereas gp80 was expressed in a subpopulation.

The assumption that gp80 represents a marker for keratinocytes undergoing terminal differentiation was confirmed by investigating several species of epidermal diseases. In benign hyperproliferative disorders, such as keratoacanthoma, psoriasis, common warts, and seborrhoic keratoses, expression of gp80 was again restricted to the suprabasal layers and basal cells at the epidermal sulci, further underscoring the close association with commitment to terminal differentiation of keratinocytes. In contrast, most basal cell carcinomas, which largely consisted of undifferentiated cells [7], completely lacked gp80 expression, whereas the MH-99 antigen (gp38) was highly expressed by these tumors [7]. Interestingly, in two cases of basal cell carcinoma displaying areas of keratotic differentiation, gp80 was clearly expressed around horn pearls, again indicating its association with differentiation capacity of the keratinocyte-derived tumor cells. gp80 expression also may precede terminal differentiation in these tumors.

Moreover, our *in vitro* findings were consistent with the distribution pattern of gp80 *in vivo*: organotypic cultures of keratinocytes showed marked expression of gp80 in the suprabasal layers and decreasing expression all the way down to the basal cells. Although this expression pattern was distinct from that of keratin 10, involucrin, and filaggrin [8,14–16], the increasing expression of gp80 in suprabasal layers points to an association with cells undergoing terminal differentiation. This association was preserved in monolayer cultures as shown by the clear decrease during longer culture as well as by the upregulation of gp80 synthesis by increased concentrations of $Ca^{++}$ ions. Increasing levels of $Ca^{++}$ in a chemically defined medium have been shown to induce stratification, synthesis of involucrin, transglutaminase activity, and formation of cornified envelopes, without influence on the overall synthesis of membrane-bound proteins [3,25,26].

Synthesis of gp80 was not detectable in the immortal human keratinocyte cell line HaCaT [21], although these cells have preserved *in vitro* and *in vivo* differentiation capacity (e.g., expression of keratins 1/10 and formation of orderly structured and stratified epithelia when transplanted onto nude mice). Most interestingly, gp80 reappeared in post-mitotic HaCaT cells after treatment with mitomycin C. These data suggest that terminal differentiation as well as the mitotic state of keratinocytes (possibly as two sides of the same coin) can both regulate gp80 expression. If this interpretation is correct, the differential expression of gp80 in subsets of basal epidermal cells could indicate the absence of mitotic activity or growth arrest, which is consistent with the hypothesis of Lavker and Sun [6].

The biochemical analysis of gp80 revealed a 55-kD precursor molecule that is post-translationally processed to the mature 80-kD molecule within 90 min. Although no breakdown products could be precipitated by MoAb BT 15, the decrease of the gp80 signal after 22 h indicates the degradation of the molecule after this time. As compared to the MH-99 antigen, an unrelated glycoprotein of human keratinocytes associated with undifferentiated cells that already shows a significant decrease after 8–10 h (unpublished data), gp80 is a relatively long-lived molecule on the cell surface. Tunicamycin treatment and glycosidase digestion both revealed a 55-kD core protein, indicating the post-translational increase in molecular mass to be due to asparagine-(N-)–linked glycosylation. In addition, the treatment of living keratinocytes with tunicamycin led to a decrease in the amount of newly synthesized gp80 by approximately 70% as detected by density scanning of the fluorographs. This observation is consistent with previously reported findings; for numerous proteins it has been shown that elimination of glycosylation (e.g., by synthesis in the presence of tunicamycin or by site-directed mutagenesis) leads to markedly decreased production [27,28]. For the great majority of glycoproteins, asparagine-linked glycosylation seems to be crucial for disulfide-bond formation and for proper folding, both of which influence the synthesis levels of these glycoproteins [27].

Our data indicate that gp80 is an N-glycosylated cell surface protein of human keratinocytes whose expression marks a commitment to terminal squamous or follicular differentiation and/or cessation of growth. Its characteristic expression pattern is similar *in vivo* and *in vitro*.

*We thank Dr. James G. Rheinwald (Department of Dermatology, Harvard Medical School, Boston) for helpful discussions and proofreading the manuscript.*

## REFERENCES

1. Bowden PE, Stark H-J, Breitkreutz D, Fusenig NE: Expression and modification of keratins during terminal differentiation of mammalian epidermis. *Curr Topics Dev Biol* 22:35–68, 1987
2. Tseng H, Green H: Association of Basonuclin with ability of keratinocytes to multiply and with absence of terminal differentiation. *J Cell Biol* 126:495–506, 1994
3. Fusenig NE: Mammalian epidermal cells in culture. In: Bereiter-Hahn J, Matoltsy AG, Richards KS (eds.). *Biology of the Integument, Vol. 2, Vertabrates*. Springer-Verlag, Berlin, Heidelberg, 1986, pp 409–422
4. Rheinwald JG, Bechett MA: Defective terminal differentiation in culture as a consistent and selectable character of malignant human keratinocytes. *Cell* 22:629–632, 1980
5. Klein CE, Cordon-Cardo C, Soehnchen R, Cote RJ, Oettgen HF, Eisinger M, Old LJ: Changes in cell surface glycoprotein expression during differentiation of human keratinocytes. *J Invest Dermatol* 89:500–506, 1987
6. Lavker RM, Sun T-T: Heterogeneity in epidermal basal keratinocytes: morphological and functional correlations. *Science* 215:1239–1241, 1982
7. Klein CE, Hartmann B, Schön MP, Weber L, Alberti S: Expression of a 38-kD cell-surface glycoprotein in transformed human keratinocyte cell lines, basal cell carcinomas, and epithelial germs. *J Invest Dermatol* 95:74–82, 1990
8. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R: The catalog of human cytokeratin polypeptides: patterns of expression of specific cytokeratins in normal epithelia, tumors and cultured cells. *Cell* 31:11–24, 1982
9. Moll R, Moll I, Wiest W: Changes in the pattern of cytokeratin polypeptides in epidermis and hair follicles during skin development in human fetuses. *Differentiation* 23:170–178, 1982
10. Fuchs E, Green H: Changes in keratin gene expression during terminal differentiation of the keratinocyte. *Cell* 19:1033–1042, 1980
11. VanMujen GNP, Warnaar SO, Ponec M: Differentiation-related changes of

cytokeratin expression in cultured keratinocytes and in fetal, newborn, and adult epidermis. *Exp Cell Res* 171:331–345, 1987

12. Stark H-J, Breitkreutz D, Limat A, Bowden P, Fusenig NE: Keratins of the human hair follicle: "hyperproliferative" keratins consistently expressed in outer root sheath cells in vivo and in vitro. *Differentiation* 35:236–248, 1987
13. Rice RH, Qin Q, Pilato A, Rubin AL: Keratinocyte differentiation markers: involucrin, transglutaminase, and toxicity. *Monogr Natl Cancer Inst* 13:87–91, 1992
14. Watt FM, Boukamp P, Hornung J, Fusenig NE: Effect of growth environment on spatial expression of involucrin by human epidermal keratinocytes. *Arch Dermatol Res* 279:335–340, 1987
15. Yaffe MB, Murthy S, Eckert RL: Evidence that involucrin is a covalently linked constituent of highly purified cultured keratinocyte cornified envelopes. *J Invest Dermatol* 100:3–9, 1993
16. Manabe M, O'Guin WM: Keratohyalin, trichohyalin and keratohyalin-trichohyalin hybrid granules: an overview. *J Dermatol* 19:749–755, 1992
17. Schön MP, Schön M, Klein CE, Blume U, Bisson S, Orfanos CE: Carcinoma-associated 38-kD membrane glycoprotein MH 99/KS 1/4 is related to proliferation and age of transformed epithelial cell lines. *J Invest Dermatol* 102:987–991, 1994
18. Klein CE, Hainzl A, Kaufmann R, Weber L: Cell surface glycoproteins define distinct states of eccrine gland differentiation. *Arch Dermatol Res* 283:357–361, 1991
19. Eisinger M, Lee JS, Hefton M, Darzynkiewicz Z, Chiao JW, deHarven E: Human epidermal cell cultures: growth and differentiation in the absence of dermal components or medium supplements. *Proc Natl Acad Sci USA* 76:5340–5344, 1979
20. Limat A, Hunziker T, Boillat C, Bayreuther K, Noser F: Post-mitotic human dermal fibroblasts efficiently support the growth of human follicular keratinocytes. *J Invest Dermatol* 92:758–762, 1989
21. Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE: Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol* 106:761–771, 1988
22. Limat A, Breitkreutz D, Hunziker T, Boillat C, Wiesmann U, Klein CE, Noser F, Fusenig NE: Restoration of the epidermal phenotype by follicle outer root sheath cells in recombinant culture with dermal fibroblasts. *Exp Cell Res* 194:218–227, 1991
23. Mattes MJ, Cairncross JG, Old LJ, Lloyd KO: Monoclonal antibodies to three widely distributed human cell surface antigens. *Hybridoma* 2:253–263, 1983
24. Schön MP, Schön M, Mattes MJ, Stein R, Weber L, Alberti S, Klein CE: Biochemical and immunological characterization of the human carcinoma-associated antigen MH 99/KS 1/4. *Int J Cancer* 55:988–995, 1993
25. Fusenig NE, Breitkreutz D, Dzarlieva RT, Boukamp P, Herzmann E, Bohnert A, Pöhlmann J, Rausch C, Schütz S, Hornung J: Epidermal cell differentiation and malignant transformation in culture. *Cancer Forum* 6:209–240, 1982
26. Pillai S, Bikle DD, Mancianti ML, Cline P, Hincenbergs M: Calcium regulation of growth and differentiation of normal human keratinocytes: modulation of differentiation competence by stages of growth and extracellular calcium. *J Cell Physiol* 143:294–302, 1990
27. Blithe DL: Biological functions of oligosaccharides on glycoproteins. *Trends Glycosci Glycotech* 5:81–98, 1993
28. Varki A: Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* 3:97–130, 1993