The proximal promoter of the human transglutaminase 3 gene

STRATIFIED SQUAMOUS EPITHELIAL-SPECIFIC EXPRESSION IN CULTURED CELLS IS MEDIATED BY BINDING OF Sp1 AND ets TRANSCRIPTION FACTORS TO A PROXIMAL PROMOTER ELEMENT*

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The transglutaminase 3 enzyme is expressed during the late stages of the terminal differentiation of the epidermis and in certain cell types of the hair follicle. The enzyme is thought to be critically involved in the cross-linking of structural proteins and in the formation of the cornified cell envelope, thereby contributing to rigid structures that play vital roles in shape determination and/or barrier functions. To explore the mechanisms regulating the expression of the transglutaminase 3 gene (TGM3), 3.0 kilobase pairs of sequences upstream from the transcription start site were assessed for their ability to control the expression of a chloramphenicol acetyltransferase reporter gene. Deletion analyses in transiently transfected epidermal keratinocytes defined sequences between -126 and -91 as the proximal promoter region of the gene, and which can confer epithelial-specific expression to the TGM3 gene in vitro. Mutation and DNA-protein binding analyses indicated that a complex interaction between adjacent Sp1- and ets-like recognition motifs with their cognate binding factors is required for the function of the TGM3 promoter. As these TGM3 sequences can confer promoter/enhancer activity to reporter genes at a level comparable to the powerful SV40 promoter, they may be useful for gene therapy in keratinocytes.

Transglutaminase (TGase) enzymes are widespread in both plants and animals (1–3). They catalyze the formation of an isopeptide cross-link between the ε-NH₂ side chain of a protein-bound lysine residue and the γ-amide side chain of a protein-bound glutamine residue, thereby forming an insoluble macromolecular aggregate that is used for a variety of cellular functions. To date, there are six known transglutaminase enzymes encoded in the human genome, and interestingly, three of them are active in the epidermis and its appendages. These include: the TGase 1 enzyme (4–16) which can function as membrane-associated (8), soluble full-length, and soluble pro-
currently known, is expressed only during the last stages of terminal differentiation of the epidermis and epidermal appendage cell types such as the inner root sheath and medulla of the hair follicle (22–31). Its expression is initiated well after the transcription of the genes encoding the earlier differentiation markers, such as the keratin proteins K1 and K10, have been repressed (17) and approximately coincident with expression of the profilaggrin (54) and loricin (55) genes. Although TGM3 mRNA represents less than 2% of the TGase transcripts, the activated TGase 3 accounts for up to 75% of the total TGase activity in mammalian epidermis (17). In submammals cultures of undifferentiated normal human epidermal keratinocytes (NHEK) the abundance of TGase 3 mRNA is greatly diminished compared to the foreskin epidermis. In contrast, TGase 1 and TGase 2 mRNA levels are increased. Induction of NHEK cell differentiation with Ca\(^{2+}\) leads to an increase in the TGase 1 and TGase 3 mRNA expression, whereas TGase 2 mRNA is down-regulated (28).

To date, very little is known about the recognition elements and the protein factors that regulate the transcription of these three TGase genes. In a recently published study on TGM2 gene (56), about 1.5 kb of the 5’-upstream region conferred low levels of constitutive activity that could not be modulated by retinoids, so that the sequences which control the high level and the retinoic acid inducibility of TGM2 transcription must be localized elsewhere. In the case of the TGM1 gene, 0.82 kb of upstream sequences were found to induce expression in epithelial cells in the presence of TPA, which could be suppressed by retinoids or protein kinase C (57). Co-transfection experiments indicated that c-jun and c-fos transcription factors were involved, presumably through an AP-1 site in this region, but none of the regulatory sequences have been defined functionally. In the case of the TGM3 gene, no data are available on the factors which control its expression.

The aim of this study has been to explore the mechanisms which regulate the expression of the human TGM3 gene. As an initial step, we have defined sequences in the vicinity of the mRNA start site which provide high promoter activity and which restrict expression to epithelial cells in vitro. Our data indicate that this epithelial-specific activity is provided by cooperative interactions between Sp1 and its transcription factors.

**MATERIALS AND METHODS**

All recombinant DNA technology was done according to standard procedures (58, 59).

**Construction of Recombinant Clones**—Previously, we have isolated a genomic clone of TGM3–6 which extends about 3 kb above the functional promoter region. The 3’-end of the clone was subcloned into the multiclonal cloning vector pBluescript II (Stratagene, La Jolla, CA). In addition, we used a second heterologous promoter derived from the herpes simplex virus thymidine kinase gene. Sequences between +1 and +50 of the thymidine kinase gene from the pBlC2 vector (66) were used to construct tk-promoter vectors. The 5’-sequences of TGM3 with the pECE vector or the 3’-sequences between –126 and –73, derived from synthetic oligonucleotides, were inserted in front of this minimal promoter. The sequence of the resulting construct ptk-TGM3 was verified by sequencing.

**Cell Cultures, Transfections, and Protein Assays**—Cryopreserved NHEK were obtained from Clonetics (San Diego, CA) and grown in calf serum-free (Sigma) coated dishes in serum-free medium (KGM, Clonetics) at 0.05 mM Ca\(^{2+}\), supplemented with 60 µg/ml bovine pituitary extract. Third passage NHEK cells were used for transfection experiments, and preparation of nuclear extracts. A343, Cos-7, HeLa, MCF-7, HepG2, and NIH-3T3 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD) and were grown and maintained following the recommended procedures.

HaCaT cells were a gift from Dr. Norbert E. Fussenegger, and were grown in Dulbecco’s modified Eagle’s medium supplemented with 4.5 g/liter glucose, 10% fetal bovine serum, and non-essential amino acids (Life Technologies, Inc., Bethesda, MD). Neuroblastoma cells (SK-N-AS) were a gift from Dr. Carol Thiele and were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (Life Technologies, Inc., Bethesda, MD). For experiments with transfection cells, culture plates were plated in 6-well culture plates 16–20 h before transfection. Transfections were done when cultures reached 60–70% confluency. Transfection efficiencies were always monitored by use of a thymidine kinase β-galactosidase construct (tk-β-gal) (Clontech, Palo Alto, CA). For Lipofectin transfections, cells culture plates were washed once at 37 °C with phosphate-buffered saline, and then were preincubated for 30 min at 37 °C with either Keratinocyte-SFM (Life Technologies, Inc.) for NHEK cells, or MEM media (Life Technologies, Inc.) for HeLa, A431, HepG2, and NIH-3T3 cells. For each well, 1.5 µg of reporter plasmids and 0.5 µg of tk-β-gal was mixed with 6 µg of Lipofectin, and incubated for 20 min at room temperature. The lipiodNA mixture was then added into each well and incubated for 16–18 h in the case of NHEK and A431 cells, or 3–4 h in the case of HeLa and HepG2 cells. At the end of the transfection period the medium was replaced with the medium in which the cells normally grow, and for NHEK cells the concentration of Ca\(^{2+}\) in the KGM medium was adjusted to 1.2 mM. Cells were harvested 50–60 h post-transfection. For DOTAP transfections, the plasmids (1.5 µg of reporter constructs and 0.5 µg of tk-β-gal) were mixed with 14 µg of DOTAP in HEPES buffer solution (pH 7.3) and incubated for 20 min at room temperature. The transfections were carried out in the medium of each cell type for 16–18 h. The media concentrations were then changed for another 50–60 h. Some NHEK cultures were co-transfected with the TGM3 gene at 250–600 sequences with the pECE vector or this vector containing mouse ets-2 DNA (gift of Dr. Richard Maki, La Jolla Cancer Research Foundation). Similar co-transfection experiments used the human pRSV-Sp1 cDNA or the RSV vector alone (gift of Dr. Robert Tjian, University of California, Berkeley).

Cellular extracts were prepared through at least three freeze-thaw cycles as described (61). Aliquots were used for CAT assays, β-gal assays, and total protein quantitation (62). Cellular extracts of untransfected cells and of cells transfected with the pCAT-Basic vector alone without the TGM3 inserted sequences were used as negative controls, while the pCAT-Basic-Promoter and pCAT-Control vectors served as positive controls. CAT activities were determined (63) using chloramphenicol and [\(^{3}H\)jacyetyl-CoA (DuPont NEN) as substrates. β-Gal activity was assayed by using a commercial enzyme assay system (Promega). The values for CAT were normalized by protein content and β-gal activity. The relative CAT values are the average of at least three independent experiments, each with duplicate samples. All utilized dotblot (64).

**Nuclear Extracts and Mobility Shift Assays**—Nuclear extracts were prepared according to Schreiber et al. (64) with slight modifications. The cell pellets were resuspended and left to swell on ice for 15 min in ice-cold buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EGTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and a mixture of protease inhibitors which included 5 µg/ml benzamidine, 5 µg/ml pepstatin, 0.5 µg/ml isapentin, and 5 µg/ml aprobin. Nonidet P-40 was then added to 0.6% and the suspension was homogenized with 20 strokes in a tightly fitting glass homogenizer. After centrifugation,
the nuclear pellets were resuspended in ice-cold buffer containing 20 mM HEPES (pH 7.9), 0.4M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 20% glycerol, and the mixture of protease inhibitors, and vigorously shaken at 4 °C for 15 min. The nuclear debris was discarded by centrifugation for 15 min at 14,000 × g and the extracts were aliquoted and stored at −70 °C until used.

Mobility shift experiments were performed with 5 μg of nuclear extracts and 2–4 × 10^6 cpm (about 1 ng) of gel-purified 5'- end-labeled double-stranded oligonucleotides containing the desired TGM3 sequences (see figures for sequences). Typically, the binding reactions were carried out in 20 μl containing 10 mM Tris-HCl (pH 7.5), 65 mM NaCl, 5 mM dithiothreitol, 5 mM MgCl₂, 0.05% Nonidet P-40, 10% glycerol, 1 mg/ml bovine serum albumin, and 25 μg/ml poly(dI-dC) as a carrier for 30 min at 4 °C. In competition experiments, a 100-fold molar excess of the cold competitor was preincubated with the extracts for 30 min at 4 °C before the labeled DNA fragment were added. Irrelevant control competitor oligonucleotides for consensus AP1 and AP2 sequences (Santa Cruz Biotechnology) were also used. Recombinant human Sp1 protein was from Promega and used as per the manufacturer’s recommendations. The complexes were resolved on nondenaturating 6% polyacrylamide gels in 0.5× TBE buffer for 1 h at 14 V/cm, and viewed following overnight autoradiography.

RESULTS

The Proximal Promoter of the TGM3 Gene is Highly Active in NHEK Cells—It has been previously shown (15) that NHEK cells grown in 1.2 mM Ca²⁺ express the TGM3 gene, although at a lower level than in the epidermis. Therefore these cells can be used as an in vitro system to explore the transcriptional control of the TGM3 gene. Recently the TGM3 gene was cloned and the primary transcript was characterized (30). In this study we concentrated our interest on the sequences upstream of the transcription initiation site. Several fragments of the 5'-region were subcloned into a promoterless vector containing the CAT gene (pCAT-Basic), of which eight (Fig. 1A) proved to be informative. The constructs were analyzed in transiently transfected NHEK cells grown in media containing 0.05 or 1.2 mM Ca²⁺. The sequences located in the vicinity of the transcription initiation site (construct −126/+10) showed an activity similar to that conferred by the powerful SV40 promoter/enhancer region in pCAT-Control vector (Fig. 1B). There was no significant difference in the CAT activities in cells cultured under conditions promoting differentiation (1.2 mM Ca²⁺) (Fig. 1B) or proliferation (0.05 mM Ca²⁺) (data not shown). The level of expression of the constructs containing the sequences upstream of position −126 was reduced to equal or less than the activity of pCAT-Promoter construct. Thus the positive effect of the proximal promoter region was markedly reduced by the presence of upstream elements. The sequences extending up to position −3050 were unable to overcome this negative effect.

The Activity of TGM3 Proximal Promoter Is Specific to Stratified Squamous Epithelial Keratinocytes—To explore the cell type specificity, the regulatory potential of TGM3 proximal promoter sequences was also analyzed in a variety of other cell types (Table I) including: the spontaneously immortalized keratinocyte cell line HaCaT; two cell lines derived from stratified...
squamous epithelial tissues A431 and HeLa; two cell lines derived from simple epithelial cells MCF-7 and HepG2; the fibroblast cell lines NIH 3T3 and Cos-7; and the SK-N-AS neuroblastoma cell line. The level of expression of the −91/+10 TGM3 construct was low in all cell types tested. However, the −126/+10 TGM3 construct showed high levels of expression in the two epidermal and two stratified squamous epithelial cell lines tested, at levels 10 times that of the −91/+10 construct. In contrast, its expression level in the two cell lines derived from simple epithelial tissues was very low, comparable to that seen in the two other non-epithelial cell types tested. These data suggest the sequences between −126 to −10 confer the cell type specific expression to the TGM3 proximal promoter region.

DNA-Protein Interactions in the Proximal Promoter Region of the TGM3 Gene—To define the regulatory sequences which confer the cell type specific expression to the TGM3 proximal promoter, we explored the DNA-protein interactions at a fragment encompassing the sequences between −186 and +10 of the TGM3 5′-flanking region. In a pilot DNase I footprinting experiment we detected two weak footprints on both DNA strands within the TGM3 promoter (data not shown). The protected region which extended from −73 to −102 contained an Sp1-like binding site. The second protected region was located between positions −111 and −128 and contained two direct copies of the sequence (T/C)TACAGG(C/A)A, which encompass ets-like recognition motifs.

Mobility shift experiments were aimed at characterizing in more detail these DNA-protein interactions. For each labeled probe the specificity of the binding was established in competition experiments with a 100-fold molar excess of either the corresponding unlabeled oligonucleotides (specific competitors), or of poly(dI-dC) or unrelated oligonucleotides as unspecific competitors.

Initially, a 46-base pair probe (−126 to −81, Fig. 2, lane 0) encompassing the putative Sp1 and ets binding sites was used. In the presence of a 100-fold molar excess of poly(dI-dC) (lane 1) or of the two irrelevant oligomers, consensus AP1 (lane 6) or consensus AP2 (lane 10) sequences, five retarded complexes were discerned. Specific competition with the unlabeled probe completely prevented the formation of complexes A, B, and C (lane 2). Competition experiments with wild type oligonucleotides derived from the probe revealed that complexes A and C involved the sequences of the DNase I protected region which encompassed the Sp1 recognition site (lanes 3 and 7), whereas complex B originated from interactions in the ets-like protected region which extended between −111 and −118 (lanes 4 and 8). The contribution of the putative Sp1- and ets-recognition motifs to the formation of these complexes was evaluated in competitive binding with mutant variants of the corresponding sequences. Mutations in the Sp1 binding motif (lanes 4 and 5) prevented competition for complexes A and C. Likewise, mutations in the core ets motif (lanes 7 and 9) strongly interfered with the ability of the mutant oligonucleotide to compete for complex B formation. However, a 100-fold molar excess of the oligonucleotides containing the intact Sp1-binding motif were not able to compete for ets binding (lanes 3 and 7) and, likewise, ets oligomers did not affect the formation of the Sp1 complexes (lanes 4 and 8). Two other bands marked with arrowheads were relatively resistant to self-competition (lane 2) and were formed both with double- and single-stranded oligonucleotides (data not shown), and have not been investigated further.

Binding to the Sp1 Motif—Because the Sp1 complex C was always weaker than complex A, additional experiments were performed to explore whether complexes A and C were due to multicomponent interactions in the NHEK nuclear extract, or due to protein degradation. First, a probe spanning the sequences between −105 and −70 encompassing the Sp1 motif was used (Fig. 3A). Two retarded bands with mobilities corresponding exactly to complexes A and C of Fig. 2 were observed, but in this case of equal intensity (Fig. 4A, lane 1). While an

### Table I

| Cell type        | pCAT Control | TGM3 constructs |
|------------------|--------------|-----------------|
|                  | −91/+10      | −126/+10        | −130/+10        |
| Keratinocyte cells | 3.5 ± 0.4    | 0.2 ± 0.03      | 3.2 ± 0.4       |
|                  | 22 ± 1       | 0.7 ± 0.3       | 7 ± 1           |
| Stratified squamous epithelial cells | 15 ± 3       | 1.3 ± 0.5       | 2.8 ± 0.1       |
| A431             | 3.0 ± 0.4    | 0.1 ± 0.08      | 2.4 ± 0.5       |
| Simple epithelial cells | 5.0 ± 1.3    | 0.2 ± 0.01      | 0.7 ± 0.02      |
| Hela             | 10.2 ± 0.8   | 0.1 ± 0.04      | 0.2 ± 0.02      |
| Non-epithelial cells | 4.4 ± 1      | 0.3 ± 0.1       | 0.4 ± 0.1       |
| Cos-7            | 5.5 ± 1.4    | 0.2 ± 0.01      | 0.4 ± 0.1       |
| NIH 3T3          | 18 ± 2.6     | 0.4 ± 0.1       | 0.8 ± 0.1       |
| SK-N-AS          | 6 ± 1.5      | 0.2 ± 0.01      | 0.4 ± 0.1       |

Fig. 2. Gel mobility shift analysis of the TGM3 proximal promoter region with a probe encompassing the DNase I protected regions. The probe (−126 to −81, lane 0) was incubated with 5 μg of NHEK nuclear extract in the presence of a 100-fold molar excess of: poly(dI-dC) (lane 1); unlabeled probe (lane 2); and irrelevant consensus AP1 (lane 6), or consensus AP2 (lane 10) oligonucleotides. In lanes 3–5 and lanes 7–9, wild type and mutant variants of the probe sequence were used for competition. The letters in bold are the nucleotides of the ets and Sp1 motifs (boxed) that were mutated. In the oligonucleotides listed, dots represent unchanged nucleotides. Mutated nucleotides are as shown. The two Sp1 complexes are designated as A and C; the ets complex is designated B (arrows). Arrowheads mark the complexes of unresolved origin, P, position of the free probe.
oligonucleotide containing the wild type Sp1-like sequence (lane 2) and an Sp1 consensus oligonucleotide (lane 5) both competed for complex A and C formation, a mutation (lane 3) or a deletion (lane 4) of the Sp1 motif abolished the competition. Likewise, incubation of the binding reactions with an Sp1 specific antibody prevented the formation of both complexes (data not shown). Second, we incubated the same probe with recombinant Sp1 protein. In this case, a single retarded band which represented the Sp1-specific complexes A and C. The letters in bold are the nucleotides of the Sp1 motif (boxed) that were mutated; dots represent unchanged nucleotides; P, position of the free probe.

Thus, whereas the Sp1 motif interacted with purified recombinant Sp1 protein to form the faster migrating complex C only, the interaction with the multicomponent NHEK extract resulted in the formation of both complex C and the slower migrating complex A. Moreover, when a probe carrying only the Sp1 motif was used, complexes A and C were of equal intensity (Fig. 3A), but when a longer probe carrying both the Sp1 and ets-like motifs was used, the intensity of complex C was always weaker (Fig. 2). Taken together these data suggest that complex C was not due to protein degradation of complex A, but rather, results from interactions of the Sp1 motif with Sp1 transcription factor alone. Complex A is likely to be due to a multicomponent interaction involving both Sp1, ets and/or other as yet unidentified proteins.

Binding to the ets Motif—To define precisely the nucleotides involved in the ets binding, the wild type and a series of mutant variants of the double stranded sequence between −105 and −70 (lane 0) were used. Of those, complex B was successfully competed by a 100-fold molar excess of the corresponding competitor oligomers (lanes 2–13). The arrow denotes the ets-specific complex B. The letters in bold are the nucleotides in or adjacent to the ets motif (boxed) that were mutated; dots represent unchanged nucleotides; the arrowheads mark the complexes of unknown origin; P, position of the free probe.

Fig. 3. Interactions with NHEK nuclear proteins and recombinant Sp1 protein with oligomers spanning the Sp1 recognition sequences. A, NHEK nuclear extract (lane 1) was combined with a probe spanning the sequences between −105 and −70 (lane 0). Lane 2 represents the competition of the NHEK binding with competitors in a 100-fold molar excess of the unlabeled probe. Lanes 3 and 4 show the competition of NHEK binding with the mutant and deletion variants shown below. Lane 5 is competition with consensus Sp1 oligonucleotide. Fig. 3B shows the competition of the NHEK binding with the unlabeled probe and unlabeled oligomers spanning the Sp1 recognition sequences. The arrows represent the Sp1-specific complexes A and C. The letters in bold are the nucleotides of the Sp1 motif (boxed) that were mutated; dots represent unchanged nucleotides; P, position of the free probe.

Fig. 4. Interactions with NHEK nuclear proteins with oligomers spanning the ets-like recognition sequences. Binding of probe −134 to −102 (lane 0) to the NHEK nuclear extract in the presence of a 100-fold molar excess of poly(dI-dC) (lane 1) or in the presence of a 100-fold molar excess of the corresponding competitor oligomers (lanes 2–13). The arrow denotes the ets-specific complex B. The letters in bold are the nucleotides in or adjacent to the ets motif (boxed) that were mutated; dots represent unchanged nucleotides; the arrowheads mark the complexes of unknown origin; P, position of the free probe.
and mutant variants of the sequences between which were not mutated.

**Legend to Fig. 1.**

**Proximal Promoter of TGM3 Gene**

**Functional Analyses of the TGM3 proximal promoter region by transient CAT assays in NHEK cells.** A, DNA sequence of the human TGM3 promoter region extending from −130 to −91. The ets-like and the Sp1-like recognition motifs are boxed. A-D represent the mutant variants used in the transient CAT assays. The letters in bold denote the mutated nucleotides. The dots represent the sequences which were not mutated. B, transient CAT activities of the wild type and mutant variants of the sequences between −130 and +10. The presentation of the relative CAT activities are as described in the legend to Fig. 1.

**Fig. 5.**

**Synergistic effect of both ets-2 and Sp1 factors on the activity of TGM3 construct −126/+10.** CAT activities were measured in NHEK cells that were co-transfected with the TGM3 construct −126/+10 (1 μg) and 0.5 μg of the pECE vector containing either no insert (designated as 100%) or the mouse ets-2 cDNA; the pRSV-Sp1 vector containing human Sp1 cDNA (1.0 μg); simultaneously with both parental vectors pECE and pRSV (0.5 μg each), containing the respective SV40 and RSV-long terminal repeat regulatory sequences only; simultaneously with the corresponding expression vectors for ets-2 and Sp1 cDNAs (0.5 μg each). The data are the averages of three or more independent experiments.

**Fig. 6.**

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human TGM3 gene, at least between lial cells in culture. We have mapped this region to sequences that contain the information sufficient to support differentiation-specific transcription. The proximal promoter of the TGM3 gene, however, contains the core promoter activity in both epithelial and non-epithelial cell lines, at a level comparable to that of the TGM2 gene (56). In this regard we have examined the upstream sequences of the three transglutaminase genes for regulatory elements involved in the transcription of either of the transglutaminase genes expressed in epithelia. To date in the TGM1 gene, a 0.82-kb fragment could confer epithelial specific expression only in TPA-treated cells (57). In the case of the TGM2 gene, an initial study showed that 1.6 kb of flanking DNA sequences contain low core promoter activity in both epithelial and non-epithelial cell lines, at a level comparable to our construct (−91/+10) (Fig. 1; Table I). Deletion analyses indicated that putative Sp1 binding motifs may be responsible for the TGM2 gene (56). In this regard we have examined the upstream sequences of the three transglutaminase genes for regulatory elements involved. All three contain consensus Sp1 recognition motifs near the transcription start site. Interestingly, the TGM1 gene also possesses an ets-like motif at position −190. Thus it will be interesting to determine whether the ets and Sp1 motifs can also confer high levels of epithelial specific expression to the TGM1 gene. Furthermore, it raises the possibility that members of the ets family of transcription factors may be important in the regulation of late differentiation genes in the epidermis.

Our present data on the expression of the TGM3 gene in keratinocytes represent the first detailed study on the regulatory elements involved in the transcription of either of the three transglutaminase genes expressed in epithelia. To date in the TGM1 gene, a 0.82-kb fragment could confer epithelial specific expression only in TPA-treated cells (57). In the case of the TGM2 gene, an initial study showed that 1.6 kb of flanking DNA sequences contain low core promoter activity in both epithelial and non-epithelial cell lines, at a level comparable to our construct (−91/+10) (Fig. 1; Table I). Deletion analyses indicated that putative Sp1 binding motifs may be responsible for the TGM2 gene (56). In this regard we have examined the upstream sequences of the three transglutaminase genes for regulatory elements involved. All three contain consensus Sp1 recognition motifs near the transcription start site. Interestingly, the TGM1 gene also possesses an ets-like motif at position −190. Thus it will be interesting to determine whether the ets and Sp1 motifs can also confer high levels of epithelial specific expression to the TGM1 gene. Furthermore, it raises the possibility that members of the ets family of transcription factors may be important in the regulation of late differentiation genes in the epidermis.

To date, only two of the several genes involved in the latest stages of epidermal differentiation have been studied, the TGM3 gene, as reported here, and the loricrin gene. About 2.5 kb of upstream sequences are required for epithelial specific expression of the loricrin gene (74), and 10 kb are required for correct temporal expression (74, 75). The proximal promoter region which confers high levels of epithelial specific expression of the TGM3 gene in cultured keratinocytes is located within a narrow window of −126 to −91 base pairs of the transcription start site. This promoter should now be very useful for further studies on the expression of this and other genes in keratinocytes. Furthermore, it may aid in devising strategies to manage lamellar ichthyosis (46–48) and other related recessive genodermatoses involving mutations in the transglutaminase genes.

Our study demonstrates that the correct transcription of the human TGM3 gene, at least in vitro, depends on the simultaneous effect of elements residing in both the proximal promoter and in regions distal from it. The distal elements which are not present within the region extending 3.05 kb upstream of the transcription initiation site are required for late epidermal differentiation-specific transcription. The proximal promoter of the TGM3 gene, however, contains the information sufficient to direct high levels of expression in stratified squamous epithelial cells in culture. We have mapped this region to sequences between −126 and −91, which encompass Sp1 and ets-like binding sites. The overall activity of the proximal promoter region results from the cooperative interactions between these positively acting motifs.

Keratinocytes contain relatively high levels of Sp1 (67). This transcription factor is important for the regulation of a variety of other epidermally-expressed genes (68–70). On the other hand, the TGM3 gene is the first epidermally-expressed gene in which the ets transcription factors have been shown to be important. These transcription factors have been implicated in the regulation of gene expression during a variety of biological processes, including growth control, developmental or transformation programs, and usually function as components of larger transcription complexes (65). Indeed, our transient transfection data (Figs. 5 and 6) demonstrated cooperative interactions between ets and Sp1 transcription factors, as has been previously reported in other gene systems (70–73). However, in the TGM3 gene proximal promoter region, the interactions clearly involved not only Sp1, but also additional proteins in the NHEK nuclear extracts (Fig. 3). Thus the cooperative relationship between the ets and Sp1 factors in the activity of the TGM3 proximal promoter is modulated by interactions with additional so far unidentified nuclear proteins.

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FIG. 7. The proximal promoter element located between −126 and −91 of the TGM3 gene confers high activity to a heterologous promoter in NHEK cells. The TGM3 sequences between −126 and −91 were cloned into a CAT reporter vector (ptk-TGM3) based on the ptk-Promoter (as described under “Materials and Methods”). The data are expressed as percentages of the activity of the ptk-Promoter and are the averages of three independent experiments.

Proximal Promoter of TGM3 Gene

N. G. Markova, unpublished observations.
The Proximal Promoter of the Human Transglutaminase 3 Gene: STRATIFIED SQUAMOUS EPITHELIAL-SPECIFIC EXPRESSION IN CULTURED CELLS IS MEDIATED BY BINDING OF Sp1 AND ets TRANSCRIPTION FACTORS TO A PROXIMAL PROMOTER ELEMENT

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