Regulation of the Transglutaminase I Gene

IDENTIFICATION OF DNA ELEMENTS INVOLVED IN ITS TRANSCRIPTIONAL CONTROL IN TRACHEOBRONCHIAL EPITHELIAL CELLS*

Alexander Medvedev, Nicholas A. Saunders‡, Hironori Matsuura, Anna Chistokhina, and Anton M. Jetten§

From the Cell Biology Section, Laboratory of Pulmonary Pathobiology, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709

The transglutaminase I (TGase I) gene encodes an enzyme that catalyzes the cross-linking of structural proteins involved in the formation of the cornified envelope during squamous cell differentiation. To identify DNA elements important for the transcriptional control of the TGase I gene, we analyzed the ability of a 2.9-kilobase pair (kb) upstream regulatory region to control the expression of a reporter gene *in vivo* and *in vitro*. Transgenic mice bearing the pTG(-2.9kb)CAT construct exhibited the same pattern of tissue-specific expression of CAT as reported for TGase I. Deletion analysis in transiently transfected rabbit tracheal epithelial cells indicated that two sequences from bp -490 to -470 and from -54 to -37 are involved in the activation of TGase I transcription. Point mutation analysis and mobility shift assays showed that the sequence located between -54 and -37 is a functional Sp1-like transcription element. Sp1 and Sp3, but not Sp2, are part of nuclear protein complexes from differentiated RbTE cells binding to this site. The element TGATGTCA between bp -490 and -470 is contained in a larger 22-bp palindrome and resembles the consensus cAMP response element-binding protein (CREB)/AP-1 element recognized by dimeric complexes of members of the CREB, ATF, Fos, and Jun families. Mutations in this sequence greatly reduced promoter activity. Supershift analysis identified CREB1, JunB, c-Fos, Fra-1, and c-Jun in protein complexes isolated from differentiated rabbit tracheal epithelial cells binding to this site. Our study shows that the Sp1- and CREB/AP-1-like sites act in concert to stimulate transcription of the TGase I gene. The 2.9-kb promoter region could guide expression of specific genes in the granular layer of the epidermis and could be useful in gene therapy.

Transglutaminases (TGases)[1] (EC 2.3.2.13) constitute a family of Ca^{2+}-dependent enzymes that covalently cross-link proteins by catalyzing the formation of isopeptide bonds between the γ-amide group of glutamine and the ε-amino group of lysine (1–5). TGases have functions in a variety of biological processes, such as differentiation, apoptosis, and blood clotting (4–9), and are implicated in a number of diseases (4, 10–12). Factor XIII catalyzes the cross-linking of a number of proteins in plasma and plays an important role in fibrinogenesis (1, 2, 4, 5). Tissue TGase (TGase II) has been implicated in the activation of certain cytokines (13, 14) and cross-linking of specific components of the extracellular matrix (4). This TGase has also been reported to have GTPase activity and as such may have a role in signal transduction (15). In addition, evidence has been provided indicating that TGase II may catalyze cross-linking of intracellular proteins during apoptosis (8, 16). TGase I and III are involved in the formation of the cross-linked envelope during squamous cell differentiation (5–7,17–19).

Squamous differentiation is a terminal pathway of differentiation that occurs as a multistep process (21–23). In epidermal keratinocytes this differentiation represents a normal process, whereas in tracheobronchial epithelial cells, it is an aberrant pathway of differentiation that occurs under conditions of severe vitamin A deficiency or after injury (20–22). Early during differentiation, cells become committed to irreversible growth arrest that is accompanied by alterations in the expression of several growth-regulatory genes (6, 23–25). In subsequent stages in the differentiation process, cells begin to express a variety of squamous-specific genes (20–22). In tracheobronchial epithelial cells, squamous differentiation is associated with induction of keratin 13 (26, 27), relaxin (28), cholesterol sulfotransferase (29), epithelial membrane protein 1 (30), and several proteins involved in the formation of the cross-linked envelope (31–38).

The formation of the cross-linked envelope is a characteristic event in the final stages of squamous differentiation. The envelope consists of a layer of cross-linked protein deposited just beneath the plasma membrane (5–7, 18). It contributes to the cohesiveness and rigidity of the cornified layer and provides a vital barrier function. The envelope is formed by the cross-linking of many precursor proteins, including involucrin (38), cornifins/small proline-rich proteins (SPRRs) (32, 35, 36), and loricrin (37) catalyzed by TGase I and III (5–7, 17–19). TGase I is synthesized as a soluble enzyme in the cytosol and becomes associated with the cell membrane after acylation by palmitic and myristic acid at a cluster of five cysteines at its amino terminus (39, 40). The TGase I gene has been cloned from various species (33, 41, 42). The human TGase I gene, which spans 14.3 kb and contains 15 exons, has been mapped to chromosome 14q11 (TGM1 locus) (43–45). The importance of this enzyme in the morphogenesis and function of the skin was recently corroborated by studies implicating mutations in the TGase I gene that result in a lack of TGase I activity in the

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‡ Present address: Dept. of Medicine, University of Queensland, Princess Alexandra Hospital, Brisbane 4102, Australia.

§ To whom correspondence should be addressed. Tel.: 919-541-2768; Fax: 919-541-4133; E-mail: jetten@niehs.nih.gov.

[1] The abbreviations used are: TGase, transglutaminase; CAT, chloramphenicol acetyltransferase; RbTE, rabbit tracheobronchial epithelial; NHEK, normal human epidermal keratinocyte(s); LUC, luciferase; CREB, cAMP response element-binding protein; SPRR, small proline-rich protein; EMSA, electrophoretic mobility shift assay; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase pair(s).
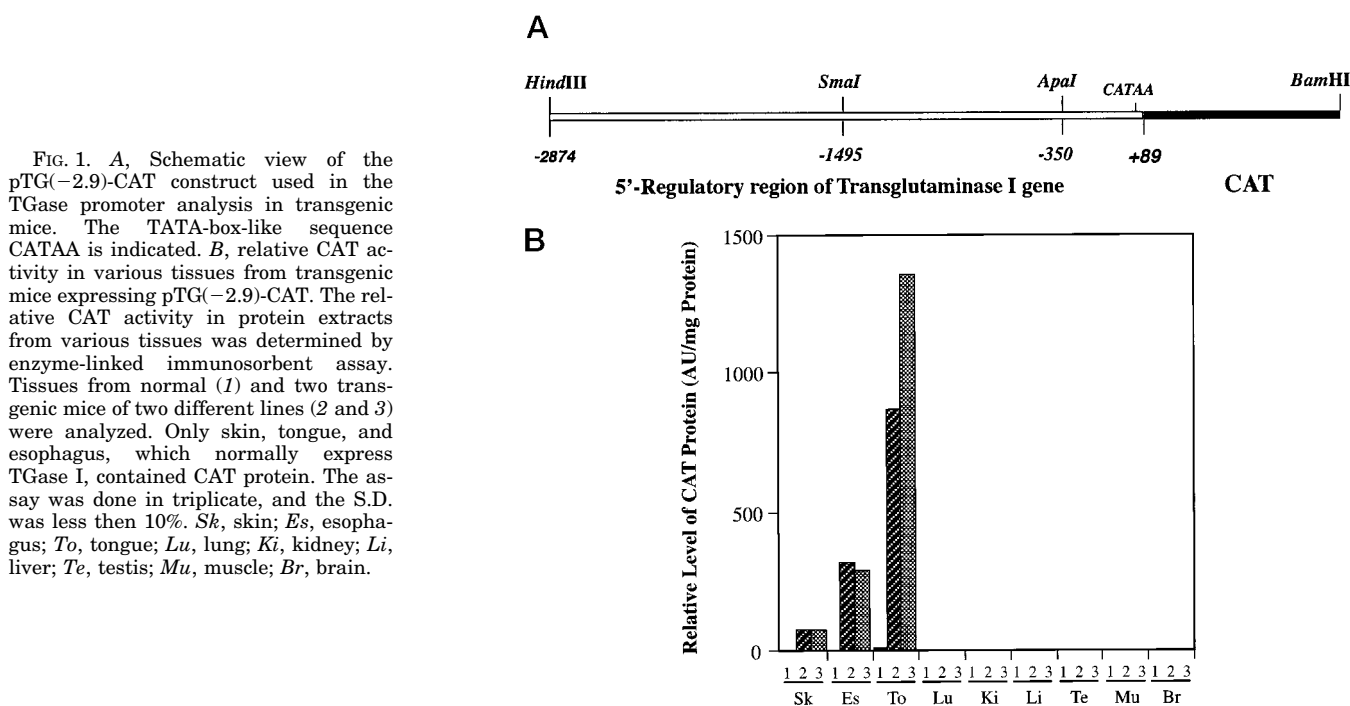
A

B

FIG. 1. *A*, Schematic view of the pTG(−2.9)-CAT construct used in the TGase promoter analysis in transgenic mice. The TATA-box-like sequence CATAA is indicated. *B*, relative CAT activity in various tissues from transgenic mice expressing pTG(−2.9)-CAT. The relative CAT activity in protein extracts from various tissues was determined by enzyme-linked immunosorbent assay. Tissues from normal (*1*) and two transgenic mice of two different lines (*2* and *3*) were analyzed. Only skin, tongue, and esophagus, which normally express TGase I, contained CAT protein. The assay was done in triplicate, and the S.D. was less then 10%. *Sk*, skin; *Es*, esophagus; *To*, tongue; *Lu*, lung; *Ki*, kidney; *Li*, liver; *Te*, testis; *Mu*, muscle; *Br*, brain.

autosomal recessive skin disorder lamellar ichthyosis (11, 12). Recent studies demonstrated that TGase I-null mice exhibit severe deficiencies in several skin functions, including impairments in cell envelope assembly and barrier function (46).

Analysis of the TGase I promoter may provide insight not only into the molecular mechanisms controlling this gene in squamous epithelia and squamous metaplasia but also into the molecular pathogenesis of lamellar ichthyosis and lead to new methods of treatment, including drug and gene therapy. In this study, we analyzed the ability of a 2.9-kb promoter regulatory region of the TGase I gene to control the expression of a chloramphenicol acetyltransferase (CAT) reporter gene *in vivo* and *in vitro*. We show that this region directs expression of the transgene to the suprabasal layers, comprising the late spinous and granular layers, of several squamous tissues. This pattern of expression correlates well with that reported for TGase I (47, 48). Deletion analysis identified two DNA elements in the 2.9-kb regulatory region containing an Sp1- and a CREB/AP-1-like site that are involved in the transcriptional regulation of TGase I.

EXPERIMENTAL PROCEDURES

Cell Culture—Rabbit tracheobronchial epithelial (RbTE) cells were cultured in Ham's F-12 medium supplemented with 10 ng/ml epidermal growth factor, 5 µg/ml transferrin, and insulin as described previously (49). Normal human epidermal keratinocytes (NHEK) were purchased from Clonetics (San Diego, CA) and cultured in KGM medium (Clonetics). Rabbit tracheal fibroblasts were grown in Ham's F-12/RPMI 1640 (1:1) supplemented with 10% fetal bovine serum.

Plasmids and Reporter Constructs—The plasmid pTG(−2.9kb)-LUC containing the luciferase reporter gene under the control of the −2.9 kb promoter flanking region of the TGase I gene has been previously described (34). The deletion mutants pTG(−1.5kb)-LUC and pTG(−350)-LUC were constructed using the *Sma*I and *Apa*I restriction sites (Fig. 1*A*). The minimal promoter construct pTG(−37)-LUC and pTG(−54)-LUC were made by cloning the respective PCR products into the promoterless luciferase vector pGL2-Basic (Promega). Deletion mutants in region −750 to −350 bp were constructed by PCR-directed cloning into the construct pTG(−37)-LUC (Fig. 2*A*). All PCR reactions were conducted with proofreading Vent DNA polymerase (New England Biolabs), and the original 2.9-kb construct was used as template. All primers contained a six-base 5′-leader sequence followed by a six-base restriction site (*Kpn*I, *Mlu*I, or *Xho*I) and a 20-base promoter sequence. Point mutation constructs were made by direct cloning of chemically synthesized oligonucleotides upstream of pTG(−37)-LUC. The sequences of the PCR products were checked for accuracy using the dideoxynucleotide chain termination method and the Sequenase Quick-denature plasmid sequencing kit (Amersham Pharmacia Biotech).

Search for Transcription Factor Binding Sites—Promoter flanking regions were analyzed for the presence of potential transcription factor binding sites using the TFSEARCH Program.[2]

Transfections and Reporter Gene Assays—Plasmid DNA used in transfections were isolated and purified with a Wizard Miniprep kit (Promega). RbTE cells were grown to confluence in six-well dishes, and transfections were carried out in triplicate using lipofectamine (Life Technologies, Inc.) according to the manufacturer's protocol. Cells were incubated in 1.0 ml of Ham's F-12 medium without growth factors and antibiotics in the presence of 1.5 µg of total DNA and 5 µl of lipofectamine. Cotransfection with β-actin-CAT reporter plasmid was carried out to correct for differences in transfection efficiency. After a 5-h incubation, medium was replaced with complete F-12 or KGM medium. Cells were collected 48 h after transfection and assayed for reporter activity. Whole cell extracts were prepared with the lysis buffer included in the CAT ELISA kit (Boehringer Mannheim) according to the manufacturer's protocol and used in CAT and luciferase assays. Luciferase assays were performed with the Luciferase Assay Kit from Promega. The relative luciferase activity was normalized for CAT reporter activity.

Electrophoretic Mobility Shift Assays (EMSAs)—Nuclei were isolated from differentiated rabbit tracheobronchial epithelial cells, and nuclear extracts were prepared by the method of Dignam *et al.* (50) with slight modifications. Briefly, cells were washed twice in ice-cold PBS, collected into microtubes, and centrifuged for 10 s in a microcentrifuge. The cell pellet was then resuspended in 1 ml of ice-cold, low salt buffer (10 mM HEPES, pH 7.9, 1 mM EDTA, 0.5 mM EGTA, 10 mM KCl, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 5 µg/ml leupeptin and aprotinin, 0.1 mM sodium vanadate). After incubation on ice for 20 min, Nonidet P-40 was added to a final concentration of 0.5%. The cell suspension was vigorously vortexed for 20 s followed by centrifugation for 30 s in a microcentrifuge to collect the nuclei. The nuclei were resuspended in 10 volumes of high salt buffer (10 mM HEPES, pH 7.9, 1 mM EDTA, 0.42 M NaCl, 10% glycerol, and protease inhibitors) and incubated on ice for 20 min followed by a 10-min centrifugation in a microcentrifuge at 4 °C. Nuclear extracts were collected and stored in aliquots at −70 °C.

The Sp1 consensus oligonucleotide (CTCCG**CCCCGCCC**GATC-GAAT), the AP-1 consensus (CGCTTGA**TGAGTCA**GCCGGAA), the

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[2] This program is available on the World Wide Web at http://pdap1.trc.rwcp.or.jp/research/db/TFSEARCH.html.

CREB consensus (GTACTGGCTGC**TGACGTCA**CCAGCCA), and other oligonucleotides were synthesized using a model 392 DNA synthesizer (Applied Biosystems). Oligonucleotides for EMSA were end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase (Promega) and purified with NAP-5 columns (Amersham Pharmacia Biotech). Approximately 0.1 ng (50,000 cpm) of the oligonucleotide probe and 0.5 μg of nuclear extract were used in the binding reaction in buffer containing 20 mM HEPES, pH 7.9, 50 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10% glycerol. To prevent nonspecific binding, 1 μg of poly(dI-dC) and 0.2 μg of salmon sperm DNA were included in the reaction. The binding reactions were carried out at room temperature for 25 min in the presence or absence of a 10- or 100-fold molar excess of oligonucleotide competitors. For supershift analysis, various antibodies were included in the incubation mixture. DNA-protein complexes were separated on 5% nondenaturing polyacrylamide gel electrophoresis at room temperature in 0.5× TBE. Antibodies against Sp1, Sp2, Sp3, c-Jun, Ser^{63}-phosphorylated c-Jun, JunB, JunD, Fra-1, Fra-2, c-Fos, CREB1, CREB2, ATF-2, and ATF-4 were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Footprint Analysis—A TGase I promoter fragment from −750 to −350 bp was generated by PCR with proofreading Vent DNA polymerase (NE Biolabs). One of the primers used for PCR was labeled with T4 polynucleotide kinase (Promega). The binding mixture contained 20 mM HEPES (pH 8.0), 10% glycerol, 50 mM NaCl, 10 mM MgCl$_2$, 10–20 μg of nuclear extract, 1 ng of labeled DNA, and 1 μg of poly(dI-dC). The binding reaction was carried out for 25 min at room temperature and then treated with different concentrations of DNase I. After 1 min of incubation with DNase I, 1 volume of stop buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA, and 1% SDS) was added. DNA was purified by phenol extraction and concentrated with Microcon-30 (Amicon). DNA fragments were separated on a 6% sequencing gel.

Generation and Identification of Transgenic Mice—The construction of the pTG-(−2.9)CAT reporter was carried out by cloning the 2.9-kb promoter fragment (*Kpn*I/*Nhe*I restriction fragment) from pTG(−2.9)LUC into the *Xba*I site of the promoterless pCAT reporter plasmid (Promega). Compatible end ligation of the *Nhe*I site into the *Xba*I site was followed by incubation with T4 DNA polymerase (Amersham Pharmacia Biotech). The resulting blunt ended *Xba*I and *Kpn*I sites were then autoligated. Orientation of the clones was confirmed by sequencing. This plasmid was linearized, and a 4.5-kb *Hin*dIII/*Bam*HI DNA fragment containing the −2873 to +89 bp regulatory region of TGase I gene and the CAT reporter excised. The fragment was purified and microinjected into the pronuclei of one-cell mouse embryos obtained from C57BL/6J females mated with SJL F2 males. The embryos were then transferred to the oviduct of pseudopregnant females, and normal gestation was allowed. Positive founder transgenic mice carrying the construct were identified by PCR analysis of genomic DNA isolated from tail biopsies. A rabbit TGase I promoter-specific primer, 5′-TCG-GCCCGCCCTCCCCA, and a CAT-specific primer, 5′-AACGGTGG-TATATCCAGTGA, were used for the analysis. Tissues for CAT assay, RNA isolation, and *in situ* hybridization were taken from 3- or 12-month-old mice.

In Situ Hybridization—Transgenic mice were sacrificed, and tissues isolated and fixed for 3 h with 4% paraformaldehyde in phosphate-buffered saline, pH 7.4. After rinsing in phosphate-buffered saline, tissues were dehydrated in a graded ethanol series, permeated with xylene, and embedded in paraplast. *In situ* hybridization was performed as described (51) with the following minor modification. Serial sections (5 μm) were mounted on Silane-prep™ slides (Sigma), deparaffinized, acetylated, and pretreated with 2× sodium citrate (SCC), 50% formamide, 10 mM dithiothreitol at 50 °C. Radiolabeled sense and antisense CAT probes were generated with either T3 or T7 RNA polymerase and ^{35}S-labeled UTP and CTP (Amersham Pharmacia Biotech; 1000 Ci/mmol), as described (51). Probes were subjected to limited alkaline hydrolysis to reduce the size of the transcripts to about 150 bases. After hybridization at 50 °C for 16 h, the sections were washed and treated with RNase. The slides were dipped in NTB-2 emulsion (Eastman Kodak Co.) diluted 1:1.5 and exposed for 14 days at 4 °C. Slides were developed and counterstained with hematoxylin.

RESULTS

A 2.9-kb TGase I Promoter Region Is Sufficient for Tissue- and Differentiation-specific Expression—The cloning and sequence of the 2.9-kb 5′-flanking region of the rabbit TGase I gene has been reported previously (26). We also mapped the transcription start site and showed that this promoter region was able to induce transactivation of the luciferase reporter
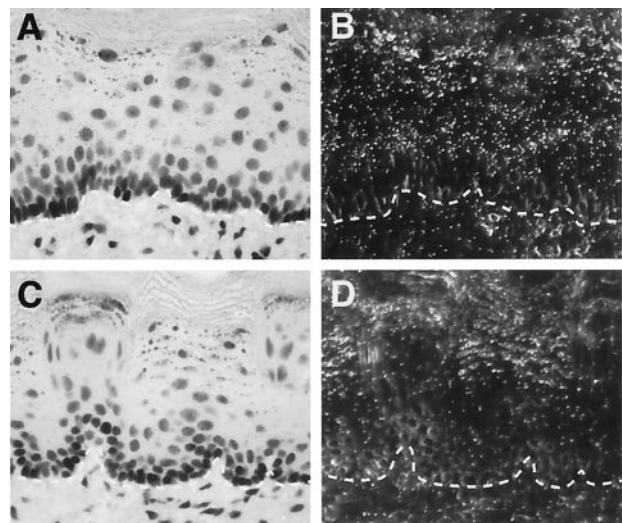

FIG. 2. **Localization of CAT reporter mRNA in the stratified, squamous-differentiated epithelium of the tongue from transgenic mice containing pTG(−2.9)-CAT.** Sections prepared from the tongue of transgenic (*A* and *B*) and control mice (*C* and *D*) were analyzed by *in situ* hybridization using radiolabeled antisense CAT probe to detect expression of CAT mRNA. *A* and *C*, bright field; *B* and *D*, dark field exposures. The *dotted line* indicates the position of the basement membrane.

gene during differentiation of cultured RbTE cells (26). To determine whether this region is able to control the expression of TGase I *in vivo* in a cell type- and differentiation-specific manner, we constructed an expression vector in which the CAT reporter is under the control of the 2.9-kb 5′-flanking region of the rabbit TGase I gene and introduced this plasmid into the mouse germ line. The presence of this transgene was corroborated by PCR. Two independent founders, which contained and expressed the integrated transgene, were selected for subsequent analysis. Protein extracts prepared from various tissues of control and transgenic mice were analyzed for CAT protein concentration. As expected, no CAT protein was detectable in any of the tissues isolated from control mice (Fig. 1*B*). In the transgenic mice, CAT protein was only detected in crude protein extracts from tissues that normally express TGase I, including skin, tongue, and esophagus. The highest CAT levels were found in extracts from tongue and esophagus; lower levels were found in skin. This pattern of CAT expression is consistent with the known expression levels of TGase I in these tissues (34). These results demonstrate that this 2.9-kb 5′-flanking region of the TGase I gene contains regulatory elements that are sufficient to control the transcription of this gene in a tissue-specific manner.

During squamous differentiation, cells transit from the basal into the suprabasal layers of the epithelium and begin to express a variety of differentiation-specific genes. Initiation of TGase I expression occurs within the late spinous and granular cell layers of the squamous epithelium. To localize the expression of the transgene in squamous tissues, we performed *in situ* hybridization using sense and antisense riboprobes to the CAT mRNA. A specific hybridization signal was observed in the suprabasal layers, in particular in the late spinous and granular layers of the squamous epithelium of the tongue from transgenic mice, as shown in Fig. 2, *A* and *B*. The hybridization signal in the basal layer was at background levels. A low background hybridization signal was observed in squamous epithelia of control mice with no significant differences between the different layers (Fig. 2, *C* and *D*). These results indicate that the 2.9-kb region contains DNA elements that are able to regulate transcription of CAT at a specific stage during
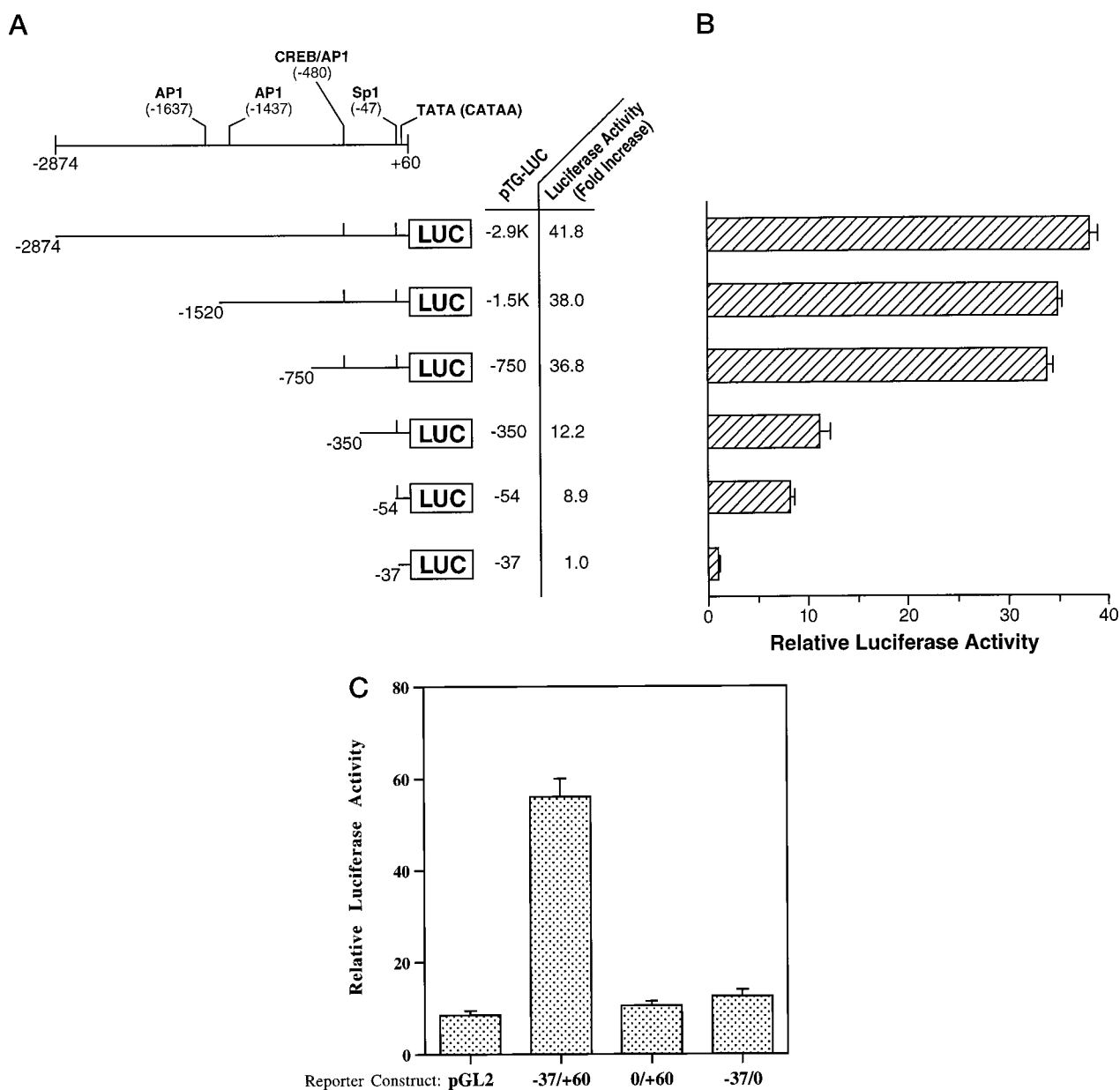
Transcriptional Regulation of Transglutaminase I

FIG. 3. **Analysis of the promoter activity of the 5'-regulatory region of the rabbit TGase I gene.** A series of 5' deletion mutants were made to the pTG(-2.9)-LUC reporter construct and analyzed by transient transfection into differentiating RbTE cells. The constructs are named by the size of the regulatory region. *A*, the relative luciferase activity for each construct was calculated and plotted. *B*, -fold increase in relative luciferase activity is shown on the *right* in *A*. *C*, effect of deletions within the minimal promoter region ($-37/+60$) on promoter activity. The $0/+60$ construct contains the region downstream from the TATA-box-like sequence CATTAA, while the $-37/0$ construct contains the upstream region including the TATA box.

differentiation that is similar to the pattern of endogenous TGase I expression (47).

Deletion Analysis of the TGase I Promoter—In an effort to locate regulatory sequences important in the transcriptional control of the TGase I gene, a series of deletion constructs were made using several unique restriction sites within the 5'-flanking region or fragments generated by PCR (Fig. 3*A*). Transcriptional activity of these pGL2-LUC constructs was analyzed by transient transfection into primary rabbit tracheobronchial cells cultured under differentiation-inducing conditions. Compared with the activity induced by the minimal promoter (bp -37 to $+60$), the full-length 2.9-kb region was able to increase luciferase activity by about 30–50-fold, depending on the experiment. Deletions up to bp -750 had only a small effect (10% reduction) on luciferase activity, indicating that the region between bp -2874 and -750 does not contribute significantly

to differentiation-specific activation when transfected into squamous-differentiated RbTE cells (Fig. 3). In contrast, deletion of the region spanning bp -750 to -348 resulted in a 70% decrease in luciferase activity. Further deletions of the region up to bp -54 had only minor effects; however, deletion of the region between bp -54 and -37 caused an additional 90% reduction in promoter activity (Fig. 3*B*). As demonstrated in Fig. 3*C*, the minimal TGase I promoter (pTG($-37/+60$)LUC) containing the TATA box-like sequence CATAA still exhibited transactivating activity compared with the promoterless pGL2-LUC. Deletion of this sequence (pTG($0/+60$bp)LUC) or the region downstream of CATAA (pTG($-37/0$)LUC) completely abolished the ability to activate transcription (Fig. 3*C*).

Analysis of the promoter activity of TGase I deletion constructs in differentiated NHEK showed that the relative transcriptional activation of the reporter gene by the various pro-
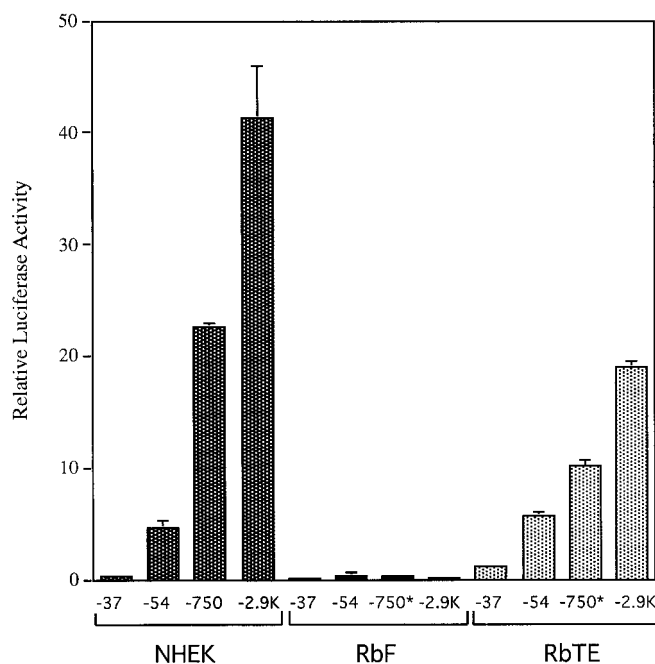
FIG. 4. **Comparison of TGase I promoter activity in NHEK, RbTE, and rabbit tracheobronchial fibroblasts (*RbF*).** Cells were transfected with β-actin-CAT and either pTG(−54)-LUC, pTG(−37)-LUC, pTG(−750)-LUC, pTG(750*)-LUC, or pTG(−2.9)-LUC reporter plasmid DNA as described under "Experimental Procedures" and 48 h later assayed for luciferase activity and CAT protein levels. The relative luciferase activity for each construct was calculated and plotted. pTG(750*)-LUC is identical to pTG(750)-LUC except that it does not contain the region between bp −37 and −350.

moter regions was very similar to that observed in RbTE cells (Fig. 4). These results suggest that regulation of TGase I expression during squamous differentiation of tracheal bronchial epithelial cells and epidermal keratinocytes appears to occur largely through the same upstream-regulatory regions. However, deletion of the −2.9 kb to −750 bp region caused a consistently greater reduction (45%) in promoter activity in NHEK than RbTE cells, indicating species- or cell type-specific differences in the promoter activity of DNA elements in this region. In contrast to RbTE and NHEK, rabbit tracheal fibroblasts transfected with several deletion constructs showed very low levels of luciferase activity, and no significant difference in promoter activity was observed between the various regions (Fig. 4). This finding supports the conclusion that this upstream-regulatory region of the TGase I promoter is important for squamous-specific regulation of TGase I in keratinocytes and is in agreement with previous findings (34, 52–54).

The Proximal TGase I Promoter Contains a Functional Sp1 Element—The proximal region was analyzed with TFSearch for the presence of potential transcriptional factor binding sites. This computer search indicated that the −54/−37 region responsible for 5–10-fold transactivation of the basal TGase I promoter contains an Sp1 consensus sequence at −47 to −40 (Fig. 5A). A two-base mutation in this Sp1 element reduced transcriptional activity of the −54/+60 fragment to that of the minimal promoter (−37/+60), indicating that the increase in transcriptional activation is related to this sequence (Fig. 5B). Gel shift assays, using a ^{32}P-labeled oligonucleotide TG-Sp1 corresponding to the −54/−37 region and nuclear protein extracts from squamous differentiated RbTE cells, revealed the formation of several specific DNA-protein complexes. Unlabeled TG-Sp1 was able to compete for these complexes, while the mutated oligonucleotide TG-mtSp1 competed much less efficiently (Fig. 5C). A commercially available Sp1 consensus

oligonucleotide (*Sp1cons.*) also competed well for binding. An antibody against Sp1 protein caused a supershift of the upper band, indicating that Sp1 is a major part of this protein complex (Fig. 5D). The second and third faster migrating bands were supershifted by antibodies against Sp3. Antibodies against Sp2 or Egr-1 (not shown), a transcription factor that binds the consensus sequence GCGGGGGCG, failed to interact with any of the protein complexes binding TG-Sp1.

Deletion Analysis of −750/−350 Region of the TGase I Promoter—To identify elements in the −750/−350 region that are involved in the regulation of the TGase I gene, a series of overlapping deletion mutants was constructed by inserting various segments of this regulatory region into pTG(-37)-LUC (Fig. 6A). Transient transfection analysis showed that the region most important for transactivation was located between bp −490 and −470 (Fig. 6B). Deletion of this sequence within the bp −750 to −350 fragment decreased transactivation by more than 80%. When ligated to the minimal TGase I promoter (D10; Fig. 6B) this fragment increased the level of reporter gene activity by more than 10-fold. The −490 to −470 sequence consists of the almost perfect palindromic structure CTGGCT-GC**TGATGTCA**CCAGCCAG (referred to as TG$_{pal}$) containing a CREB/AP-1-like sequence (TGATGTCA) in the middle (Fig. 6C). It is interesting to note that this palindromic region including the CREB/AP-1-like element is highly conserved in the corresponding 5′-flanking region of the human TGase I gene (Fig. 6C).

Fig. 7 shows that the CREB/AP-1-like element (TG$_{pal}$) and the Sp1 site are acting in concert to optimally increase transcription. Each element used separately with the minimal promoter increased transactivation of the reporter, while together (TG$_{pal}$/−54) they synergistically enhanced transactivation to a level slightly less than that of the full 2.9-kb 5′-flanking region.

To directly map protein binding sites in the −750/−350 region of the TGase I promoter, nuclear extracts from squamous differentiated RbTE cells and a 400-bp DNA fragment (−750/−350) were used for DNase I footprint analysis. Although several DNA elements were found to be protected, the region between bp −490 and −470 containing the TG$_{pal}$ was one of the most strongly protected sequences (Fig. 8).

TGase I Promoter Contains a Functional CREB/AP-1-like Element—To determine which bases in the TG$_{pal}$ element are optimal for its transactivation function, mutated oligonucleotides M1–M8 (Fig. 9A) were inserted upstream of the −37/+60 basic promoter region of pTG(−37)-LUC. The transcriptional activity of these constructs was tested in differentiating RbTE cells by transient transfection assays. As demonstrated in Fig. 9B, transactivation of the LUC reporter under the control of TG$_{pal}$ was increased about 10-fold compared with the activity of the basic promoter. The mutations (M2–M7) that disrupt transcriptional activation most are within the CREB/AP-1-like sequence (TGATGTCA).

To map the exact position of the enhancer element within the −490/−470 region of the TGase I promoter, we performed EMSA with a ^{32}P-labeled TG$_{pal}$ oligonucleotide and nuclear extracts isolated from differentiated RbTE. Several oligonucleotides (M1–M8; Fig. 9A), containing different point mutations in this region, were tested for their ability to compete with ^{32}P-labeled TG$_{pal}$ for protein binding. The oligonucleotide M1 competed as effectively as the unlabeled TG$_{pal}$ itself (Fig. 10A). The oligonucleotides M2–M4 and M8 were less effective competitors than unlabeled TG$_{pal}$, while M6, M7, and to a lesser degree M5 did not compete well for binding. These results indicate that TCA within the CREB-like element is optimal for the formation of DNA-protein complexes. Since TG$_{pal}$ contains an element that shows similarity to CREB and AP-1 sites, we
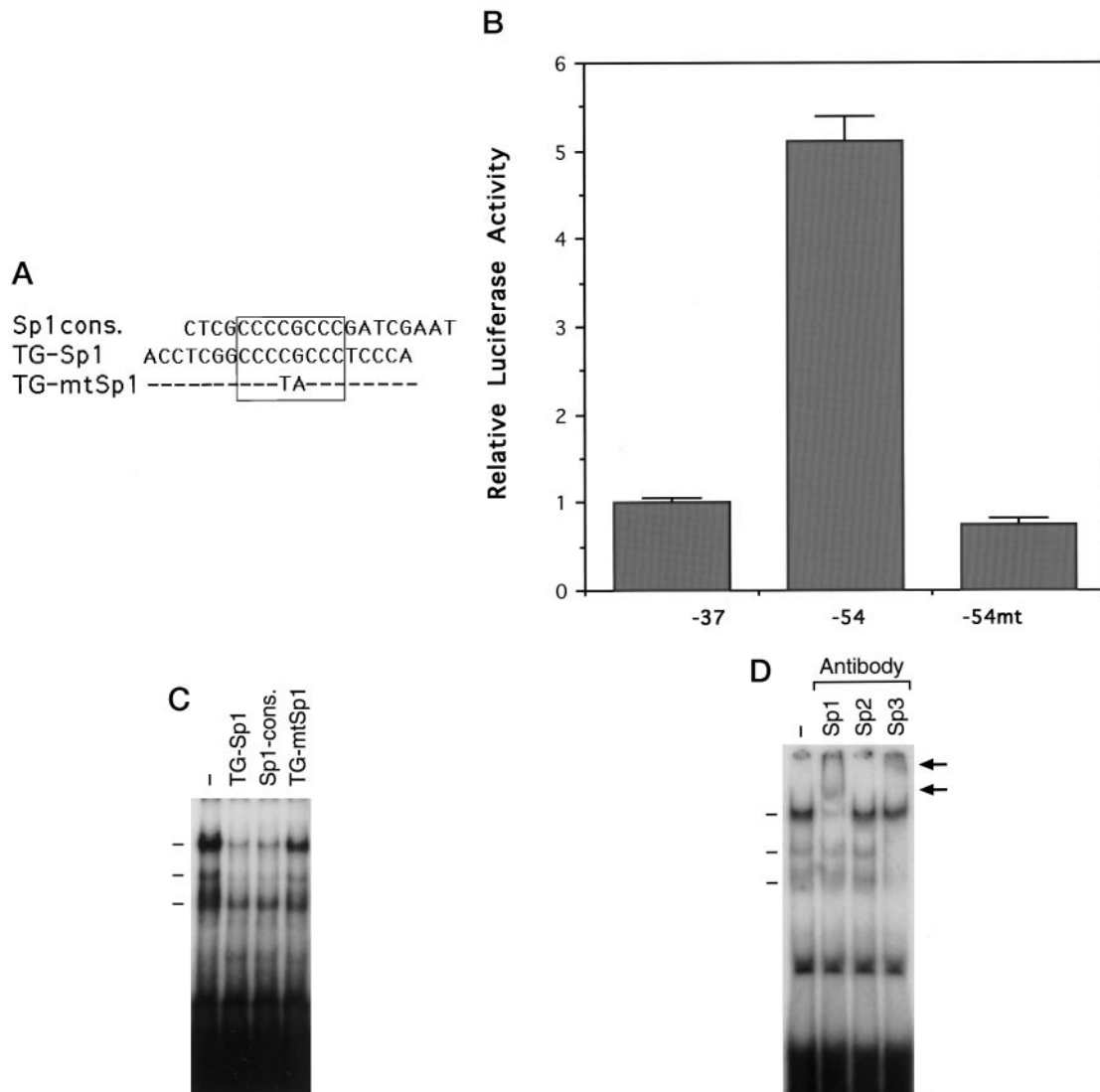
FIG. 5. **The proximal TGase I promoter contains a functional Sp1 element.** *A*, sequence of the oligonucleotide TG-Sp1 encoding the region from −54 to −35 bp. The location of the two mutations in the oligonucleotide TG-mtSp1 or reporter construct pTG-(−mt54)-LUC are indicated. The sequence of the commercial Sp1 oligonucleotide (*Sp1cons.*) is also shown. *B*, RbTE cells were transfected with pTG(−37)-LUC (−37), pTG(−54)-LUC (−54) or pTG(−mt54)-LUC (−54mt) and 48 h later collected and assayed for luciferase activity as described under "Experimental Procedures." The data shown are the mean of triplicate dishes. *C*, EMSA was carried out with ^{32}P-labeled TG-Sp1 and nuclear extracts from squamous-differentiated RbTE cells in the presence or absence of a 100-fold excess of unlabeled TG-Sp1, TG-mtSp1, or the commercial Sp1 oligonucleotide. *D*, supershift (*arrow*) of protein complexes was carried out by including specific antibodies against Sp1, -2, or -3 in the incubation mixture.

assessed the ability of consensus AP-1 (TGAGTCA) and CREB (TGACGTCA) oligonucleotides to compete with ^{32}P-labeled TG_{pal} for binding to nuclear proteins from squamous differentiated RbTE cells. As shown in Fig. 10*B*, both the consensus AP-1 and CREB oligonucleotides were able to compete with TG_{pal}; however, they were less efficient than TG_{pal} itself.

To determine the identity of some of the proteins in the complexes binding to TG_{pal}, supershift analysis was carried out with nuclear extracts from squamous differentiated RbTE cells and antibodies against various members of the Jun, Fos, CREB, and ATF families of transcription factors. These analyses identified CREB1, c-Jun Fra-1, and c-Fos as part of protein complexes bound to TG_{pal} (Fig. 11*A*). These proteins were also found in complexes bound to a consensus CREB oligonucleotide and, except for CREB1, also in complexes bound to a consensus AP-1 oligonucleotide (Fig. 11, *B* and *C*). The anti-c-Fos antibody caused a supershift of almost all of the protein-AP-1 complexes, while this shift is much less dramatic with TG_{pal} or CREB oligonucleotides. The supershift with anti-Fra-1 antibody was most pronounced with AP-1 oligonucleotide and

very weak with TG_{pal}. These differences in the degree of the supershift are likely to be an indication of the differences in the affinity of the transcriptional factor for the particular DNA element and the amount of the protein present in the bound complexes. Antibodies specific for Ser[63]-phosphorylated (active) c-Jun or JunB were also able to cause a supershift of TG_{pal}-protein complexes (not shown), whereas antibodies against JunD, ATF2, ATF4, Fra-2, and CREB2 did not cause supershifts (not shown).

DISCUSSION

In this study, we analyzed the promoter activity of the 2.9-kb 5′-flanking region of the rabbit TGase I promoter *in vivo* and *in vitro*. Analysis of the TG(−2.9)-CAT transgenic mice revealed that CAT mRNA was only expressed in squamous tissues and was restricted to the suprabasal layers comprising the late spinous and granular cell layers. This pattern of transgene expression correlates well with that reported for endogenous TGase I mRNA (47). These results demonstrate that this 2.9-kb region contains elements that are sufficient to direct the tissue-
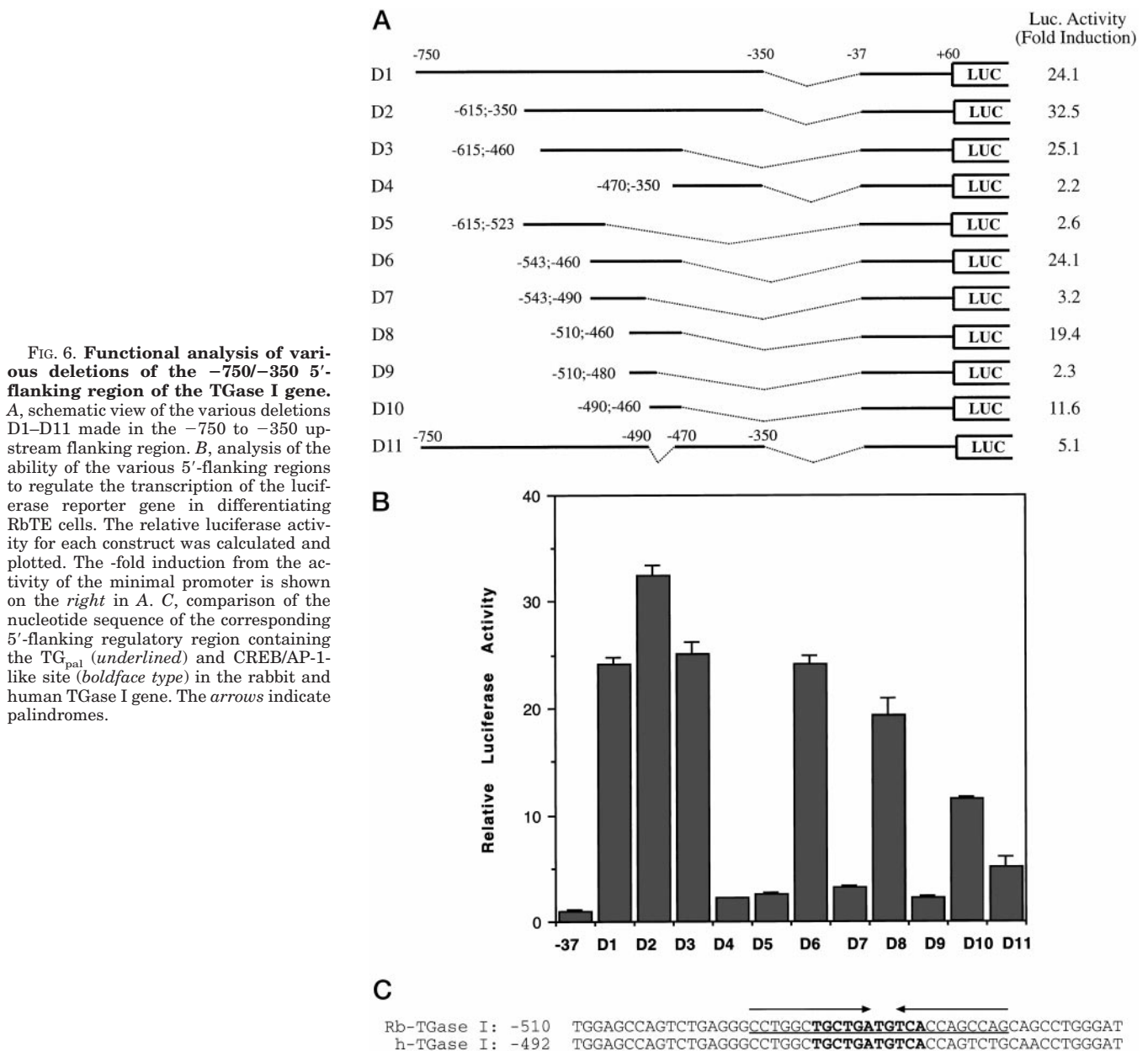
FIG. 6. **Functional analysis of various deletions of the −750/−350 5′-flanking region of the TGase I gene.** *A*, schematic view of the various deletions D1–D11 made in the −750 to −350 upstream flanking region. *B*, analysis of the ability of the various 5′-flanking regions to regulate the transcription of the luciferase reporter gene in differentiating RbTE cells. The relative luciferase activity for each construct was calculated and plotted. The -fold induction from the activity of the minimal promoter is shown on the *right* in *A*. *C*, comparison of the nucleotide sequence of the corresponding 5′-flanking regulatory region containing the TG$_{pal}$ (*underlined*) and CREB/AP-1-like site (*boldface type*) in the rabbit and human TGase I gene. The *arrows* indicate palindromes.

and differentiation-specific expression of TGase I *in vivo* and are in agreement with a recent report on the promoter activity of the human TGase I in transgenic mice (54). This conclusion is supported by observations in cultured cells showing that the 2.9-kb region of the TGase I promoter is able to control the transcription of a reporter gene in a cell type- and differentiation-specific manner (Fig. 4; Refs. 34 and 52).

In order to locate the regulatory elements required to drive TGase I expression during squamous differentiation, a series of deletions were made to the 2.9-kb 5′-flanking sequence. This analysis mapped the minimal promoter to −37/+60 and identified two major sites that are important in the transcriptional control of TGase I: a palindromic sequence from −490 to −470 bp (TG$_{pal}$) containing a CREB/AP-1-like site in the middle and the region from bp −53 to −37 consisting of a functional Sp1 site. We showed that these two elements work in concert to regulate the basal promoter activity of the TGase I gene. Previous studies implicated several AP-2-like sites in the transcriptional regulation of human TGase I in NHEK cells (51); these sites may cooperate with the Sp1 and TG$_{pal}$ elements.

The GC-rich region containing the Sp1 consensus element is located just upstream from the TATA-like box. Point mutations in this element reduce promoter activity to that of the minimal TGase I promoter (Fig. 5). Although another consensus Sp1 sequence is present at bp −194, no evidence was obtained indicating that this site is important in the regulation of the TGase I gene. Sp1 elements have been reported to be able to bind a number of transcriptional factors including members of the Sp1 family (56). Sp1 contains a DNA-binding domain consisting of three zinc fingers and binds the consensus sequence 5′-GGGCGG. In addition to Sp1, three related genes (Sp2, -3, and -4) have been identified (56, 57). These transcription factors can act alone or in cooperation with other transcription factors and can enhance or repress transcription depending on promoter context and cell type. Sp1 elements have been linked to the regulation of several genes during squamous differentiation. The Sp1 site is needed for optimal transcriptional activation of the involucrin gene, and protein complexes bound to this site were found to contain Sp1 but not Sp2, -3, or -4 (57). Sp1 sites have also been implicated in the regulation of SPRR-2A (59) and TGase III (60). Our mobility shift analysis demonstrated that in differentiated RbTE cells, Sp1 and to a
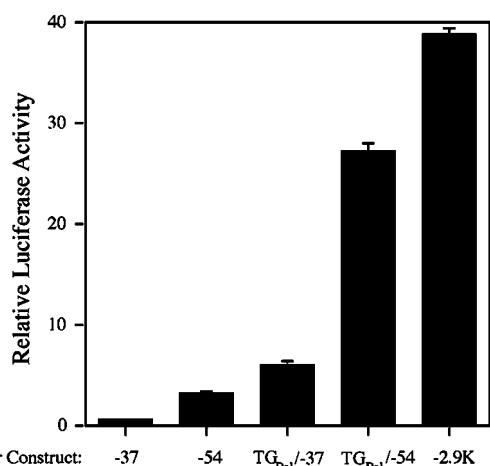
FIG. 7. **The Sp1 and CREB-like site act in concert to enhance transcription of the TGase I gene.** Reporter plasmids containing the LUC reporter gene under the control of the minimum TGase I promoter (−37), the −54 to +60 promoter region including the Sp1 site (−54), the minimal promoter plus TG$_{pal}$ (*TG$_{pal}$/−37*), the −54 to +60 promoter region plus TG$_{pal}$ (*TG$_{pal}$/−54*), or the −2.9 kb regulatory region (−2.9K) were transfected into differentiated RbTE cells. After 48 h, cells were assayed for reporter gene activities, and the relative LUC activity was calculated.
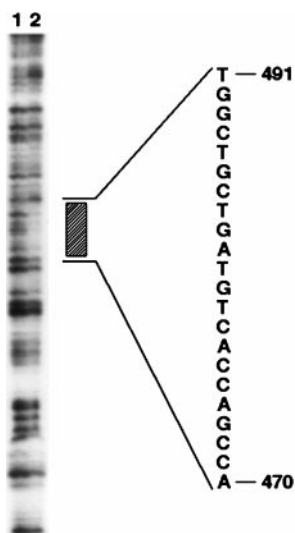

FIG. 8. **DNase I footprint analysis of the −350/−450 promoter flanking region of the TGase I gene.** Footprint analysis was carried out as described under "Experimental Procedures." *Lane 1*, no nuclear extract; *lane 2*, nuclear extract from squamous differentiated RbTE cells. The protected region coincides with that of TG$_{pal}$ as indicated by the sequence on the *right*.

FIG. 9. **Effect of various mutations on the ability of TG$_{pal}$ to increase transcription of the luciferase reporter.** *A*, sequence of TG$_{pal}$ (*wt*) and mutated oligonucleotides M1–M8. *B*, the different oligonucleotides were inserted into pTG(−37)-LUC just upstream from the minimal promoter, and their ability to regulate the transcription of the luciferase reporter gene was analyzed.

minor extent Sp3 are part of protein complexes that interact with the TG-Sp1 element. A recent study has demonstrated that the ratio of Sp1 to Sp3 is low in undifferentiated and high in squamous-differentiated NHEK cells and that Sp1 is involved in the differentiation-specific regulation of HPV-16 genes (61). This differential expression may hint at a possible role for Sp1-Sp3 antagonism during squamous differentiation. Antagonism of Sp1-mediated transcriptional activation by Sp3 has been demonstrated in several other cell systems (56). In contrast to these observations, the differentiation-dependent, transient induction of the cyclin-dependent kinase inhibitor p21$^{WAF1/Cip1}$ during early stages of squamous differentiation in NHEK cells has been reported to depend on the interaction of Sp3 rather than Sp1 with a GC-box in the proximal promoter region of p21$^{WAF1/Cip1}$ (62). The reported increase in the Sp1/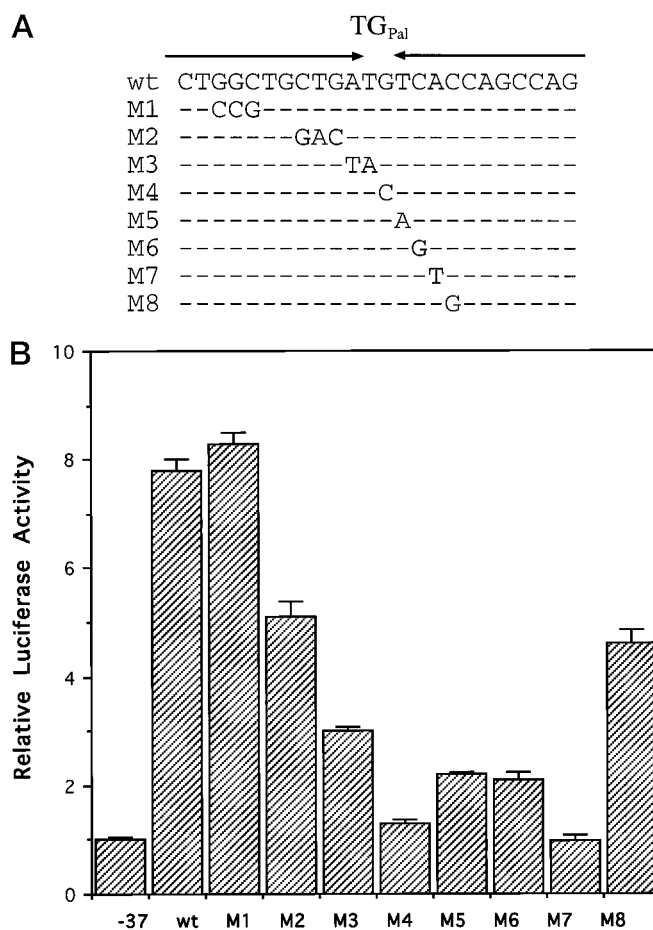Sp3 ratio during differentiation may be a later event and be part of the down-regulation of p21$^{WAF1/Cip1}$. Moreover, the activation or repressor function of Sp3 may be determined by the sequence or context of the GC-box.

The functionally important CREB/AP-1-like element TGAT-GTCA is contained in a 22-bp palindrome that is protected in DNase I footprinting assays. Point mutations in the CREB/AP-1-like site reduced promoter activity of this site, supporting the importance of the CREB/AP-1 motif. The CREB/AP-1-like element has been shown to bind a variety of transcriptional factors, including members of the CREB, ATF, and Fos/Jun families (63). Mobility shift assays using an array of antibodies against different CREB/AP-1-binding proteins demonstrated that TG$_{pal}$ interacted with protein complexes containing CREB1, c-Jun, JunB, Fra-1, and c-Fos in nuclear extracts from differentiated RbTE cells. CREB/AP-1-like elements have been implicated in the transcriptional regulation of several other squamous marker genes, including involucrin, profilaggrin, and SPRR-1 genes (64–67). It is likely that the regulation of squamous-specific genes, which are induced at different stages during differentiation, involves control by different CREB/AP-1 protein complexes. The differential expression of members of the Jun/Fos family during different stages of squamous differentiation is in agreement with this concept (68). A role for CREB/AP-1-like elements is further supported by studies showing that PKC activation, which regulates the expression of Jun/Fos family members, is an important component of the signaling pathways controlling squamous differentiation and
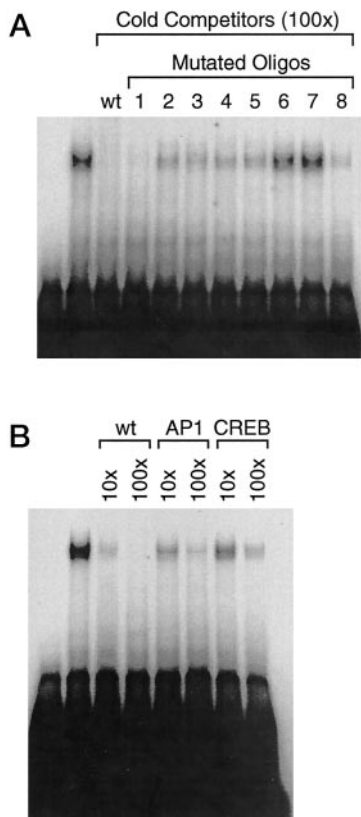
FIG. 10. **Interaction of nuclear proteins isolated from squamous-differentiated RbTE cells with oligomers spanning the TG$_{pal}$ regulatory element.** EMSA was carried out using ^{32}P-labeled TG$_{pal}$ and nuclear extracts from squamous differentiated RbTE cells. Incubations were carried out in the presence or absence of a 100-fold excess of unlabeled TG$_{pal}$, oligonucleotides M1–M8 (*A*) (see Fig. 9*A*) or the consensus AP-1 site or consensus CREB-site (*B*).

FIG. 11. **EMSA characterization of protein interactions with TG$_{pal}$ (*A*), consensus AP-1 (*B*), and consensus CREB (*C*) oligonucleotides.** Nuclear extracts from differentiated RbTE cells were preincubated with 2 μg of each antibody as indicated, followed by incubation with ^{32}P-labeled TG$_{pal}$, AP-1, or CREB. The protein-DNA complexes were separated by polyacrylamide gel electrophoresis.

TGase I (34, 68–71). Cholesterol sulfate, which is highly induced during squamous differentiation (71), can function as an endogenous second messenger and through the activation of different PKC isoforms enhance the expression of several squamous-specific genes, including TGase I (52, 70).

The role of specific CREB/AP-1 complexes in squamous differentiation is not yet well understood. Recent studies have demonstrated that expression of c-Fos appears not to be essential for squamous differentiation in the epidermis, since epidermal differentiation is normal in c-Fos null mice (68). Others have suggested that JunB may be involved in the induction of early markers, including involucrin and TGase I, while JunD may play a role in the control of later markers, such as profilaggrin (65). A role for JunB in TGase I regulation is supported by immunohistochemical studies showing that JunB is predominantly present in the granular layer where TGase I is also expressed (47, 68) and by EMSA demonstrating the presence of JunB in protein complexes binding TG$_{pal}$. It is clear that multiple protein complexes in squamous differentiated cells can compete for binding to TG$_{pal}$. Some of these complexes may act as repressors, while others may function as stimulators of gene transcription. Therefore, increased transcription of squamous-specific genes may depend on the level and activity of these complexes during the differentiation process. This may involve changes in heterodimerization partners that could alter the affinity and activity of the complex. This concept is supported by recent findings showing that ectopic expression of a dominant-active c-Jun has a repressive effect on the expression of several squamous-specific genes, including involucrin and SPRR-1 (73). In addition, it was demonstrated that c-Jun is
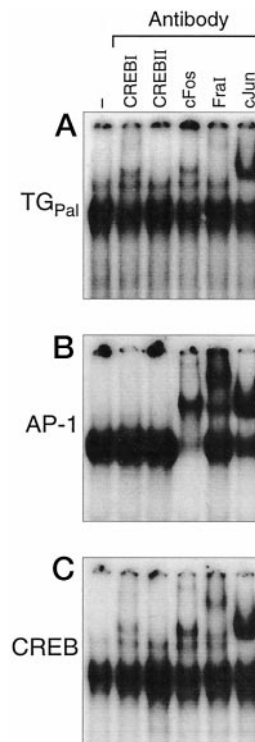
expressed predominantly in basal and early spinous layer and that the level of c-Jun and phosphorylated c-Jun is dramatically down-regulated during squamous differentiation and is probably related to the observed reduction in JNK activity (68).[3] These findings support a negative regulatory role of c-Jun in the control of squamous differentiation. The latter is in agreement with observations showing that squamous cell markers are not induced in squamous cell carcinoma cell lines overexpressing c-Jun (73).

In this study, we show that the 2.9-kb 5′-flanking region of the TGase I gene can regulate this gene in a tissue- and differentiation-specific manner and identify two DNA elements important in its transcriptional control. It is known that certain forms of lamellar ichthyosis are linked to defects in the expression of the TGase I gene (11, 12). In some cases, this genetically heterogeneous disease might be caused by mutations in transcriptionally important promoter regions of TGase I. Therefore, the study of this promoter region could help in understanding the nature of this disease and provide a tool for gene therapy of lamellar ichthyosis and other skin disorders.

REFERENCES

1. Folk, J. E. (1983) *Adv. Enzymol. Relat. Areas Mol. Biol.* **54,** 1–56
2. Lorand, L., and Conrad, S. M. (1984) *Mol. Cell. Biochem.* **58,** 9–35
3. Parameswaran, K. N., Cheng, X. F., Chen, E. C., Velasco, P. T., Wilson, J. H., and Lorand, L. (1997) *J. Biol. Chem.* **272,** 10311–10317
4. Aeschlimann, D., and Paulsson, M. (1994) *Thrombosis Haemostasis* **71,** 402–415
5. Greenberg, C. S., Birckbichler, P. J., and Rice, R. H. (1991) *FASEB J.* **5,** 3071–3077
6. Jetten, A. M. (1989) *Environ. Health Perspect.* **80,** 149–160
7. Rice, R. H., and Green, H. (1978) *J. Cell Biol.* **76,** 705–711

[3] H. Adachi and A. M. Jetten, unpublished observations.

8. Fesus, L., Davies, P. J. A., and Piacentini, M. (1991) *Eur. J. Biochem.* **56,** 170–177
9. Davies, P. J. A., Murtaugh, M. P., Moore, W. T., Jr., Johnson, G. S., and Lucas, D. (1985) *J. Biol. Chem.* **260,** 5166–5174
10. Lorand, L. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 14310–14313
11. Russell, L. R., DiGiovanna, J. J., Hashem, N., Compton, J. G., and Bale, S. J. (1994) *Am. J. Hum. Genet.* **55,** 1146–1152
12. Huber, M., Rettler, I., Bernasconi, K., Frenk, E., Lavrijsen, S. P., Ponec, M., Bon, A., Lautenschlager, S., Schorderet, D. F., and Hohl, D. (1995) *Science* **276,** 525–527
13. Eitan, S., and Schwartz, M. (1993) *Science* **261,** 106–108
14. Kojima, S., Nara, K., and Rifkin, D. B. (1993) *J. Cell Biol.* **121,** 439–448
15. Nakaoka, H., Perez, D. M., Baek, K. J., Das, T., Husain, A., Misono, K., Im, M-J., and Graham, R. M. (1994) *Nature* **264,** 1593–1696
16. Tarcsa, E., Kedei, N., Thomazy, V., and Fesus, L. (1992) *J. Biol. Chem.* **267,** 25648–25651
17. Thacher, S. M., and Rice, R. H. (1985) *Cell* **40,** 685–695
18. Reichert, U., Michel, S., and Schmidt, R. (1993) in *Molecular Biology of the Skin: The Keratinocyte* (Darmon, M. and Blumenberg, M., eds) pp 107–150, Academic Press, Inc., New York
19. Kim, I-G., Gorman, J. J., Park, S-C., Chung, S-I., and Steinert, P. M. (1993) *J. Biol. Chem.* **268,** 12682–12690
20. Fuchs, E., and Byrne, C. (1994) *Curr. Opin. Genet. Dev.* **4,** 725–736
21. Jetten, A. M., Nervi, C., and Vollberg, T. M. (1992) *J. Natl. Cancer Inst. Monogr.* **13,** 93–100
22. Jetten, A. M., and Harvat, B. L. (1997) *J. Dermatol.* **24,** 711–725
23. Saunders, N. A., and Jetten, A. M. (1994) *J. Biol. Chem.* **269,** 2016–2022
24. Harvat, B. L., Seth, P., and Jetten, A. M. (1997) *Oncogene* **14,** 2111–2122
25. Missero, C., Calautti, E., Eckner, R., Chin, J., Tsai, L. H., Livingston, D. M., and Dotto, G. P. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 5451–5455
26. Jetten, A. M., George, M. A., Smits, H. L., and Vollberg, T. M. (1989) *Exp. Cell Res.* **182,** 622–634
27. Huang, F. L., Roop, D. R., and De Luca L. M. (1986) *In Vitro Cell Dev. Biol.* **22,** 230–237
28. Jetten, A. M., Bernacki, S. H., Floyd, E. E., Saunders, N. A., Pieniazack, J., and Lotan, R. (1992) *Cell Growth Differ.* **3,** 549–556
29. Rearick, J. I., Albro, P. W., and Jetten, A. M. (1987) *J. Biol. Chem.* **262,** 13069–13074
30. Marvin, K. W., Fujimoto, W., and Jetten, A. M. (1995) *J. Biol. Chem.* **270,** 28910–28916
31. Smits, H. L., Floyd, E. E. R., and Jetten, A. M. (1987) *Mol. Cell. Biol.* **7,** 4017–4023
32. Marvin, K. W., George, M. D., Fujimoto, W., Saunders, N. A., Bernacki, S. H., and Jetten, A. M. (1992) *Proc. Natl. Acad. Sci U. S. A.* **89,** 11026–11030
33. Floyd, E. E. R., and Jetten, A. M. (1989) *Mol. Cell. Biol.* **9,** 4846–4851
34. Saunders, N. A., Bernacki, S. H., Vollberg, T. M., and Jetten, A. M. (1993) *Mol. Endocrinol.* **7,** 387–398
35. Austin, S. J., Fujimoto, W., Marvin, K. W., Vollberg, T. M., Lorand, L., and Jetten, A. M. (1996) *J. Biol. Chem.* **271,** 3737–3742
36. Gibbs, S., Lohman, F., Teubel, W., van der Putte, P., and Backendorf, C. (1990) *Nucleic Acids Res.* **18,** 4401–4407
37. Hohl, D., Mehrel, T., Lichti, U., Turner, M. L., Roop, D. R., and Steinert, P. M. (1991) *J. Biol. Chem.* **266,** 6626–6636
38. Eckert, R. L., and Green, H. (1986) *Cell* **46,** 583–589
39. Chakravarty, R., and Rice, R. H. (1989) *J. Biol. Chem.* **264,** 625–629
40. Steinert, P. M., Kim, S. Y., Chung, S. I., and Marekov, L. N. (1996) *J. Biol. Chem.* **271,** 26242–26250
41. Phillips, M. A., Stewart, B. E., Qin, Q., Chakravarty, R., Floyd, E. E. R., Jetten, A. M., and Rice, R. H. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 9333–9337
42. Kim, H. C., Idler, W. W., Kim, I. G., Han, J. H., Chung, S. I., and Steinert, P. M.

43. Phillips, M. A., Stewart, B. E., and Rice, R. H. (1992) *J. Biol. Chem.* **267,** 2282–2286
44. Yamanishi, K., Inazawa, J., Liew, F-M., Nonomura, K., Ariyama, T., Yasuno, H., Abe, T., Doi, H., Hirano, J., and Fukushima, S (1992) *J. Biol. Chem.* **267,** 17858–17863
45. Polakowska, R. R., Eickbush, T., Falciano, V., Razvi, F., and Goldsmith, L. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 4476–4480
46. Matsuki, M., Yamashita, F., Ishida-Yamamoto, A., Yamada, K., Kinoshita, C., Fushiki, S., Ueda, E., Morishima, Y., Tabata, K., Yasuno, H., Hashida, M., Iizuka, H., Ikawa, M., Okabe, M., Kondoh, G., Kinoshita, T., Takeda, J., and Yamanishi, K. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 1044–1049
47. Michel, S., Bernard, F., Jetten, A. M. Floyd, E. E. R., Shroot, B., and Reichert, U. (1992) *J. Invest. Dermatol.* **98,** 364–368
48. Schroeder, W. T., Thacher, S. M., Stewart-Galetka, S., Annarella, M., Chema, D., Siciliano, M. J., Davies, P. J. A., Tang, H., Sowa, B. A., and Duvic, M. (1992) *J. Invest. Dermatol.* **99,** 27–34
49. Jetten, A. M., and Shirley, J. E. (1986) *J. Biol. Chem.* **261,** 15097–15101
50. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489
51. Matsuura, H., Myokai, F., Arata, J., Noji, S., and Taniguchi, S. (1994) *J. Dermatol. Sci.* **8,** 25–32
52. Mariniello, L., Qin, Q., Jessen, B. A., and Rice, R. H. (1995) *J. Biol. Chem.* **270,** 31358–31363
53. Ueda, E., Ohno, S., Kuroki, T., Livneh, E., Yamada, K., Yamanishi, K., and Yasuno, H. (1996) *J. Biol. Chem.* **271,** 9790–9794
54. Yamada, K., Matsuki, M., Morishima, Y., Ueda, E., Tabata, K., Yasuno, H., Suzuki, M., and Yamanishi, K. (1997) *Hum. Mol. Genet.* **13,** 2223–2231
55. Choate, K. A., and Khavari, P. A. (1997) *Hum. Gene Ther.* **8,** 1659–1665
56. Hagen, G., Muller, S., Beato, M., and Suske, G. (1994) *EMBO J.* **13,** 3843–3851
57. Kingsley, C., and Winoto A. (1992) *Mol. Cell. Biol.* **12,** 4251–4261
58. Banks, E. B., Crish, J. F., Welter, J. F., and Eckert, R. L. (1998) *Biochem. J.* **331,** 61–68
59. Fisher, D. F., Gibbs, S., van de Putte, P., and Backendorf, C. (1996) *Mol. Cell. Biol.* **16,** 5365–5374
60. Lee, J-H., Jang, S-I., Yang, J-M., Markova, N. G., and Steinert, P. M. (1996) *J. Biol. Chem.* **271,** 4561–4568
61. Apt, D., Watts, R. M., Suske, G., and Bernard, H. U. (1996) *Virology* **224,** 281–291
62. Prowse, D. M., Bolgan, L., Molnár, A., and Dotto, G. P. (1997) *J. Biol. Chem.* **272,** 1308–1314
63. Meyer, T. E., and Habener, J. F. (1993) *Endocr. Rev.* **14,** 269–290
64. Welter, J. F., Crish, J. F., Agarwal, C., and Eckert, R. L. (1995) *J. Biol. Chem.* **270,** 12614–12622
65. Jang, S-I., Steinert, P. M., and Markova, N. G. (1996) *J. Biol. Chem.* **271,** 24105–24114
66. Lu, B., Rothnagel, J. A., Longley, M. A., Tsai, S. Y., and Roop, D. R. (1994) *J. Biol. Chem.* **269,** 7443–7449
67. Reddy, S. P. M., Chuu, Y-J., Lao, P. N., Donn, J., Ann, D. K., and Wu, R. (1995) *J. Biol. Chem.* **270,** 26451–26459
68. Rutberg, S. E., Saez, E., Glick, A., Dlugosz, A. A., Spiegelman, B. M., and Yuspa, S. H. (1996) *Oncogene* **13,** 167–176
69. Lee, Y-S., Dlugosz, A. A., McKay, R., Dean, N. M., and Yuspa, S. H. (1997) *Mol. Carcinog.* **18,** 44–53
70. Denning, M. F., Kazanietz, M. G., Blumberg, P. M., and Yuspa, S. H. (1995*) Cell Growth Differ.* **6,** 1619–1626
71. Dlugosz, A. A., and Yuspa, S. H. (1994) *J. Invest. Dermatol.* **102,** 409–414
72. Rearick, J. I., and Jetten, A. M. (1986) *J. Biol. Chem.* **261,** 13898–13904
73. Lohman, F. P., Gibbs, S., Fischer, D. F., Borgstein, A. B., van de Putte, P., and Backendorf, C. (1996) *Oncogene* **14,** 1623–1627

Regulation of the Transglutaminase I Gene: IDENTIFICATION OF DNA ELEMENTS INVOLVED IN ITS TRANSCRIPTIONAL CONTROL IN TRACHEOBRONCHIAL EPITHELIAL CELLS

Alexander Medvedev, Nicholas A. Saunders, Hironori Matsuura, Anna Chistokhina and
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