Defining a Region of the Human Keratin 6a Gene That Confers Inducible Expression in Stratified Epithelia of Transgenic Mice*

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Injury to the epidermis and other stratified epithelia triggers a repair response involving the rapid induction of several genes, including keratin 6 (K6). The signaling pathways and mechanisms presiding over this induction in keratinocytes at the wound edge remain to be defined. We reported previously that of the multiple genes encoding K6 isoforms in human, K6a is dominant in skin epithelia (Takahashi, K., Paladini, R., Coulombe, P. A. (1995) J. Biol. Chem. 270, 18581–18592). Using bacterial LacZ as a reporter gene in transgenic mice, we show that the proximal 5.2 kilobases of 5'-upstream sequence from the K6a gene fails to direct sustained expression in any adult tissue, including those where K6 is constitutively expressed (e.g. hair follicle, nail, oral mucosa, tongue, esophagus, forestomach). In contrast, the proximal 960 base pairs of 5'-upstream sequence suffice to mediate an induction of β-galactosidase expression in a near-correct spatial and temporal fashion after injury to epidermis and other stratified epithelia. Transgene expression also occurs following topical application of phorbol esters, all-trans-retinoic acid, or 2-4-dinitrofluorobenzene, all known to induce K6 expression in skin. Our data show that critical regulatory sequences for this inducibility are located between −960 and −550 bp in the 5'-upstream sequence of K6a and that their activity is influenced by enhancer element(s) located between −2500 and −5200 base pairs. These findings have important implications for the control of gene expression after injury to stratified epithelia.

The regulation of the corresponding genes offers an opportunity to decipher the molecular mechanisms underlying the onset of the wound repair response in stratified epithelia. Indeed, even though the levels of several potent growth factors are greatly elevated in the wound site early after injury to the skin (reviewed in Ref. 3), those playing a critical role in this vital homeostatic response remain to be identified.

We recently cloned several human genes and cDNAs predicted to encode highly related keratin 6 (K6) isoforms (4). K6 (56 kDa) is a type II keratin that belongs to the superfamily of intermediate filament proteins and is usually co-expressed with one or two type I keratins, K16 and K17 (5, 6). The K6 isoforms show a complex pattern of expression in epithelia, with constitutive and inducible components (7). They are normally found in the outer root sheath (ORS)1 of hair follicles, in glandular tissues, in tongue, gingiva and oral mucosa, esophagus, forestomach, and certain reproductive tract epithelia (e.g. Ref. 8). With the exception of palm and sole, K6 is not expressed in normal interfollicular epidermis (5, 7, 8). The K6 isoforms are better known for their much enhanced expression during hyperproliferation and abnormal differentiation in stratified epithelia (4, 9, 10). Thus, K6 and K16 are induced in wound edge keratinocytes as early as 4–6 h after injury to human skin and disappear after closure (11, 12). K6 expression is induced as well in a variety of diseases affecting complex epithelia, such as infections, squamous metaplasia, carcinoma, and chronic hyperproliferative disorders, including psoriasis (9, 10). In these conditions, K6 expression may be very abundant, but is usually restricted to the suprabasal compartment of the epithelium (10). In mouse skin, K6 expression is induced but is usually restricted to the suprabasal compartment of the epidermis (10). In mouse skin, K6 expression is induced after topical application of a variety of chemicals (e.g. phorbol esters, retinoic acid; see Ref. 13). K6 induction also occurs in primary cultures of mitotically active keratinocytes from epidermis, esophagus, trachea, and cornea (7, 14). Understanding the regulation of K6 gene expression is thus of great interest at various levels, one being the control of gene expression in contexts such as wound repair, psoriasis, and carcinoma. Using a transgenic mouse approach, we report here on the identification of a segment of 5'-upstream sequence in the human K6a gene that is both necessary and sufficient for the inducible expression of an heterologous reporter gene in adult mouse epithelia.

**EXPERIMENTAL PROCEDURES**

**DNA Constructs and Production of Transgenic Mice—**Our starting template was the human K6a gene, the dominant K6 isoform in hair follicle outer root sheath, foot sole epidermis, and skin squamous carcinoma samples (4). Segments containing 5.2 kb (Smal-NcoI), 2.56 kb (HindIII-NcoI), 0.96 kb (EcoRI-NcoI), and 0.55 kb (SacI-NcoI) of 5'...
upstream sequence from the translation initiation codon were isolated from the human K6a gene (GenBank accession numbers L42575–L42583; see Ref. 4) by restriction digestion and subcloned into a LacZ vector. Key: a, very strong expression; b, strong expression; c, very sporadic expression; d, moderately strong expression; e, weak expression; f, no expression.

TABLE I

| Promoter region | TG line | TG copy number | Intact trunk skin* | Wounded tissue* | Other tissues |
|-----------------|---------|----------------|-------------------|----------------|--------------|
| 0.5 kb          | KT4–1p  | 2              | −                 | −              | −            |
|                 | KT4–2p  | 4              | −                 | +              | −            |
|                 | KT4–3p  | 4              | −                 | +              | −            |
|                 | KT4–1m  | 4              | −                 | +              | −            |
|                 | KT4–2m  | 1              | −                 | −              | −            |
|                 | KT4–3m  | 2              | −                 | +              | −            |
| 1.0 kb          | KT3–2p  | 3              | −                 | +              | −            |
|                 | KT3–1m  | 1              | −                 | −              | −            |
|                 | KT3–2m  | 5              | −                 | +              | −            |
|                 | KT3–3m  | 1              | −                 | +              | −            |
|                 | KT3–4m  | 4              | −                 | +              | −            |
| 2.5 kb          | KT2–1p  | 2              | −                 | +              | −            |
|                 | KT2–2m  | 4              | −                 | +              | −            |
| 5.2 kb          | KT1–1p  | 1              | −                 | +              | −            |
|                 | KT1–2p  | 2              | −                 | +              | −            |
|                 | KT1–2m  | 2              | −                 | +              | −            |
|                 | KT1–3m  | 4              | −                 | +              | −            |
|                 | KT1–4m  | 1              | −                 | +              | −            |
|                 | KT1–5m  | 3              | −                 | +              | −            |
| K6 expression in mouse skin | −       | +              | +              | +              | −            |
| K6 expression in human skin | −       | +              | +              | +              | −            |

*Expression was assessed using adult mouse tissues incubated with X-gal staining solution, paraffin-embedded, and sectioned for light microscopy. Key: −, no expression; +, very sporadic expression; +, modest but consistent expression; ++, moderately strong expression; ++++, very strong expression.

Wounded tissues (skin and oral mucosa) were examined at 24 h following full-thickness injury (see “Experimental Procedures”).

Expression is very sporadic and restricted to a very small number of outer root sheath keratinocytes in occasional hair follicles (fewer than 1:100).

dExpression in these other tissues is sporadic as well, with only a small subset of cells positive for β-galactosidase activity. Abbreviations: esoph, esophagus; kid, kidney; trach, trachea; pericard, pericardium; sm, small intestine.

upstream sequence from the translation initiation codon were isolated from the human K6a gene (GenBank accession numbers L42575–L42583; see Ref. 4) by restriction digestion and subcloned into a LacZ vector. Key: a, very strong expression; b, strong expression; c, very sporadic expression; d, moderately strong expression; e, weak expression; f, no expression.

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|                 | KT4–2p  | 4              | −                 | +              | −            |
|                 | KT4–3p  | 4              | −                 | +              | −            |
|                 | KT4–1m  | 4              | −                 | +              | −            |
|                 | KT4–2m  | 1              | −                 | −              | −            |
|                 | KT4–3m  | 2              | −                 | +              | −            |
| 1.0 kb          | KT3–2p  | 3              | −                 | +              | −            |
|                 | KT3–1m  | 1              | −                 | −              | −            |
|                 | KT3–2m  | 5              | −                 | +              | −            |
|                 | KT3–3m  | 1              | −                 | +              | −            |
|                 | KT3–4m  | 4              | −                 | +              | −            |
| 2.5 kb          | KT2–1p  | 2              | −                 | +              | −            |
|                 | KT2–2m  | 4              | −                 | +              | −            |
| 5.2 kb          | KT1–1p  | 1              | −                 | +              | −            |
|                 | KT1–2p  | 2              | −                 | +              | −            |
|                 | KT1–2m  | 2              | −                 | +              | −            |
|                 | KT1–3m  | 4              | −                 | +              | −            |
|                 | KT1–4m  | 1              | −                 | +              | −            |
|                 | KT1–5m  | 3              | −                 | +              | −            |
| K6 expression in mouse skin | −       | +              | +              | +              | −            |
| K6 expression in human skin | −       | +              | +              | +              | −            |

*Expression was assessed using adult mouse tissues incubated with X-gal staining solution, paraffin-embedded, and sectioned for light microscopy. Key: −, no expression; +, very sporadic expression; +, modest but consistent expression; ++, moderately strong expression; ++++, very strong expression.

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ting (data not shown), as well as by enzymatic assays performed in soluble extracts prepared from intact skin of adult transgenic mice (Table II). A notable exception occurs in the vibrissae follicles of whisker pads in several KT1 transgenic lines, which show weak LacZ activity in a greater number of ORS keratinocytes (Fig. 1, compare D and D-inset). Expression remains patchy, however, and is not seen in transgenic lines made with shorter K6a promoter-based constructs (KT3, KT4; not shown). A survey of other stratified epithelia known to express K6, such as nail, cornea (limbus), tongue, oral mucosa, esophagus, and forestomach fails to reveal β-galactosidase activity in the majority of transgenic lines produced (Fig. 1, E–H), albeit with a few exceptions (Table I). Among such exceptions are lines KT3-2m and KT3-4m, which show β-galactosidase activity in a very small subset of epithelial cells in tongue, esophagus, and/or cornea (typically, only 1–3 cells/entire histological section; Table I). The three other lines made with the KT3 construct do not show expression in these epithelia (Table II).

**TABLE II**

**Quantitation of β-galactosidase activity in transgenic mouse skin extracts**

| Promoter | TG line | Intact trunk skin | Wounded skin | TPA-treated skin | RA-treated skin |
|----------|---------|-------------------|--------------|-----------------|----------------|
| 1.0 kb   | KT3–2p  | 0.22              | 0.60         | ND              | ND             |
|          | KT3–1m  | 0.03              | 0.24, 0.41   | ND              | ND             |
|          | KT3–2m  | 0.13              | 0.55         | ND              | ND             |
|          | KT3–3m  | 0.13              | 0.67         | 1.48            | 1.81           |
|          | KT3–4m  | 0.04              | 0.52, 0.74   | 0.70            | 1.17           |
| 2.5 kb   | KT2–1p  | 0.11              | 0.43, 0.58   | 0.46            | 0.89           |
|          | KT2–2m  | 0.08              | 1.14, 0.68, 1.69 | 0.69       | 0.91           |
| 5.2 kb   | KT1–1p  | 0.09, 0.10        | 3.30         | 1.77            | 3.74           |
|          | KT1–3m  | 0.08              | 2.50, 2.64, 2.50 | 2.94       | 3.68           |
|          | KT1–4m  | 0.02              | 1.16, 1.85   | ND              | ND             |
|          | KT1–5m  | 0.06              | 1.91, 1.79   | 2.62            | 2.72           |
| Wild-type mice | 0.05, 0.06, 0.07 | 0.03, 0.14 | 0.03 | 0.05 |

* All transgenic animals used were heterozygous at the transgene locus. See Table I for transgene copy number per mouse genome.
* Expression was estimated in a 4-mm punch biopsy (full thickness) of skin tissue.
* A 2-mm-wide band of skin tissue was dissected at the edge of a full-thickness wound made 48 h earlier.
* ND, nondetermined.
* Each value represents the average of three different samples obtained from a single mouse. Multiple values correspond to different mice in a given transgenic line.

Fig. 1. **Expression of [hK6a 5'-]LacZ transgenes in intact mouse tissues.** The sections shown were prepared from adult mouse tissues incubated with X-gal, embedded, sectioned, and stained with eosin (frames A, B, D–I) or alternatively processed for histochemistry with anti-mouse K6 followed by a peroxidase conjugate (frames C, D–I). A, KT2-2m trunk skin; B, KT1-3m trunk skin; the arrow points to a single β-galactosidase positive keratinocyte in the outer root sheath of a hair follicle (F); C, KT2-2m tail skin, K6 immunostaining; the outer root sheath of hair follicles (F) is strongly positive, while the epidermis (EPI) shows only background staining (* marks sebaceous glands); D, KT3-1m whisker pad skin; the arrow depicts many β-galactosidase-positive keratinocytes in a vibrissae follicle (V); D inset, KT1-1p whisker pad skin, showing a β-galactosidase-negative vibrissae follicle (V); E and E', KT2-2m nail tissue; the K6-positive epidermis in the nail fold (arrowheads) shows no β-galactosidase activity; M, nail matrix; F and F', KT3-3m eye tissue; the K6-positive conjunctival (C) epithelium and limbus area of the cornea (L) are both negative for β-galactosidase; G and G', KT1-4m tongue and H and H' KT1–1p esophagus, in both these cases the suprabasal layers of the epithelia are strongly positive for K6 and yet shown no β-galactosidase activity; P, filiform papillae; I and I' KT1–3p retinal epithelium; the arrow depicts a transgene-positive ganglion cell in this K6-negative tissue. In A, B, C, E, F, G, and H the arrowheads highlight the interface between the stratified epithelium and underlying connective tissue. Bars = 1 μm.
I). Expression of the LacZ transgene is occasionally detected in other types of tissues as well (Table I). Thus, for each K6α promoter construct tested, one or two lines show sporadic expression in the retina, where K6 is not detectable by immunohistochemistry (Fig. 1, I and I’). Since this “ectopic” expression does not appear to correlate with transgene copy number (Table I), it appears likely that the transcriptional activity of the LacZ transgenes is somewhat sensitive to their site of integration in the mouse genome.

**Induction of Transgene Expression by Injury in Stratified Epithelia of Transgenic Mice—K6 expression is induced following injury to the skin and other stratified epithelia (Fig. 2A), and this prompted us to examine transgene expression under such conditions. Full-thickness injury to the skin of adult mice induces LacZ expression in epidermis and hair follicles at the wound edge in most KT1, KT2, and KT3 transgenic lines, but in none of the KT4 lines (Table I). In the responsive lines, β-galactosidase activity occurs in keratinocytes proximal to the wound edge as early as 2.5 h after injury (Fig. 2, B and C) and extends further away from the wound site at later time points, in a pattern analogous to mouse endogenous K6 (Fig. 2D). Immunostaining for the β-galactosidase protein indicates that it is restricted to suprabasal keratinocytes in wounded skin tissue (Fig. 2E). In contrast, mouse endogenous K6 typically extends down to the basal layer in epidermal tissue at the proximal edge of the wound (Fig. 2A), underscoring a potential difference in the regulation of mouse endogenous K6 and our human K6α promoter-based transgenes (see Ref. 8 for similar observations when using the bovine K6β promoter in transgenic mice). Induction of LacZ expression also occurs after injury to other stratified epithelia, including oral mucosa (Fig. 2F, Table I), cornea (Fig. 2G), tongue (not shown), and foot pad epidermis (Fig. 2H), with all but the shortest transgene (KT4). Thus, these observations establish that the critical information required to mediate rapid induction following injury is contained within the proximal 5′-upstream sequence of the human K6α gene. They further suggest that the signaling pathways involved in K6 activation after injury are likely to be related, if not the same, in these four different stratified epithelia. The histochemistry findings are supported by β-galactosidase enzymatic assays performed on soluble extracts prepared from wounded skin tissue, which also reveals differences in the extent of transgene induction depending upon the amount of K6α 5′-upstream sequence involved. At 48 h after skin injury, KT1 transgenic mice show a greater induction of β-galactosidase activity compared to KT2 and KT3 mice (Table II). We selected transgenic lines showing strong expression (KT1-3m, KT2-2m, KT3-3m) to compare the extent of β-galactosidase...
induction as a function of time after skin injury. The data obtained (Fig. 3) confirmed the rapid induction of the three types of transgene in wound edge tissue. However, peak enzymatic activities were reached at a later time and were three to five times as large in KT1 mice compared with KT2 and KT3 mice (Fig. 3). These data suggest the presence of enhancer elements sensitive to injury and located upstream from $-2500$ bp in the $5'$-upstream sequence of K6a.

Induction of Transgene Expression by Other Acute Stimuli in the Skin of Adult Transgenic Mice—Topical application of the phorbol ester PMA and of all-trans-retinoic acid (RA), both known to induce K6 expression in mouse epidermis (e.g. Refs. 8 and 13), also result in LacZ induction in [hK6a 5'-]LacZ transgenic mice (Fig. 2, K and L'). As following injury, the extent of $\beta$-galactosidase enzymatic activity in extracts prepared from skin treated with PMA or RA is clearly greater in KT1 transgenic lines than in KT2 and KT3 lines (Table II). Under the treatment regimens tested, RA appears stronger than PMA in its ability to induce LacZ expression (note that unlike PMA, treatment with RA significantly alters terminal differentiation of skin keratinocytes; see Ref. 13). We also tested whether DNFB, a potent contact allergen that triggers a delayed-type hypersensitivity reaction (20), could induce transgene expression. We found that challenging presensitized skin with a second application of DNFB to a distinct body site causes a modest LacZ induction in many KT1 transgenic lines (Fig. 2, K and L'). Collectively, our data demonstrate that the 5'-upstream region of the human K6a gene contains sufficient regulatory information for its chemical induction in adult transgenic mouse skin using agents that produce enhanced proliferation (via PMA), altered differentiation (via RA), or a contact dermatitis-like reaction (via DNFB).

We next examined the activity of the [hK6a 5'-]LacZ transgenes in contexts featuring chronic hyperproliferation and altered differentiation in adult mouse skin. First, we applied the two-step 7,12-dimethylbenz[a]anthracene-12-O-tetradecanoyl-phorbol-13-acetate skin carcinogenesis protocol (21) to produce skin papillomas in the various lines of transgenic mice. As expected (22), abundant expression of K6 occurs in premalignant papilloma lesions produced in our various lines of transgenic mice (data not shown). Somewhat surprisingly, a relatively small number of keratinocytes express the transgene in fully developed papillomas isolated from KT1 and KT2 transgenic mice, and the LacZ-positive keratinocytes tend to be located in the uppermost portion of the much thickened epidermis (Fig. 2, L and L'). Second, we took advantage of K16-overexpressing transgenic mice available in our laboratory to produce double-transgenic animals via matings with KT2-2m transgenic mice (Table I). A particular line of transgenic mice containing 8–10 copies of the full-length human K16 gene (5-7-K16) develops striking lesions in hair follicle ORS and epidermis in the first week after birth, coinciding with the emergence of fur (17). As expected, double-transgenic mice developed similar skin lesions affecting the hair follicle ORS and adjacent epidermis in the first week after birth. However, only patchy LacZ transgene expression could be evidenced in the skin of various body sites in these mice, even though mouse endogenous K6 was present at high levels (Fig. 2, M and M'). We verified that the transgene retained its ability to respond to acute skin injury in the hK16-LacZ double transgenic mice (data not shown). Together with the data gathered on chemically induced skin papillomas, these findings suggest up to 5.2 kb of proximal 5'-upstream sequence from the human K6a gene may not contain sufficient information for its sustained expression in contexts akin to chronic hyperproliferative diseases.

DISCUSSION

The Organization of Regulatory Elements in the K6a Gene Appears Unique among Skin Keratin Genes—Several of the keratin genes are expressed in a stable and predictable fashion in well defined epithelial contexts (5, 7). In normal interfollicular epidermis and a few other cornifying epithelia, for instance, the K5-K14 and K1-K10 genes are expressed in a pairwise and constitutive fashion in the progenitor and differentiating layers, respectively (23–26). In striking contrast, the K6 isoform genes show a complex regulation with constitutive and inducible components in various stratified epithelia, such that there is no obvious relationship between K6 expression and a defined program of terminal differentiation (see Refs. 7 and 14). Yet, the predicted genomic structure and amino acid sequence of the human K6 isoform genes are very related to K5 (a type II keratin as well), and accordingly these have been postulated to originate from a common ancestral gene (4, 27). Since the relevant gene duplication event, however, the regulation of these genes has diverged significantly more than their coding sequences (28). Byrne and Fuchs (18) showed that 6 kb of 5'-upstream region from the human K5 gene can direct the expression of a LacZ reporter in a tissue-specific fashion in transgenic mice. Similar results were obtained with the human type I K14 and K10 genes (29, 30), but not with the type II K1 gene (31), whose faithful regulation seems to necessitate sequences located outside of the proximal 5'-upstream sequence (32). Here, we show that the proximal 5.2 kb of 5'-upstream sequence from the dominant K6 isoform gene in human skin, K6a (4), does not support consistent expression of a heterologous reporter sequence at a detectable level in any tissue of adult transgenic mice (with the potential exception of vibrissae; see below). In separate studies, we found that the presence of the 3'-untranslated region of the human K6a gene in the context of the KT1 and KT2 transgene constructs did not alter the expression pattern of a distinct coding sequence (a mutant K6a cDNA) in transgenic mice (33). We therefore conclude that when assessed in transgenic mice, the constitutive aspect of human K6a expression necessitates sequences that are located: (i) upstream from the proximal 5.2 kb of 5'-upstream sequence; (ii) distal to the 3'-noncoding region; and/or (iii) in introns, as is the case for the simple epithelial K18 gene (34). The organization of regulatory sequence elements in the human K6a gene

![Fig. 3. Kinetics of $\beta$-galactosidase induction after injury in various transgenic mouse lines.](image-url)
thus appears distinct from that documented for the evolution-
ary related K5 gene as well as other major keratin genes that
are constitutively expressed in skin epithelia.

More consistent expression of the KT1 transgene occurs in
vibrissae follicles, although it still represents a small fraction of
the K6-positive tissue. This may imply that the regulatory
sequences involved in directing constitutive expression of K6
are somewhat distinct between hair follicles and vibrissae.
Alternatively, however, it could be that the KT1 transgene
activity is “constantly induced” at a low level by the mild
frictional trauma incurred due to the frequent rubbing of this
area associated with grooming. Of all the transgene constructs
tested in our studies, indeed, the KT1 was the most responsive
to trauma.

The results reported here contrast with those reported for
the bovine K6b gene, which encodes a keratin protein most
related to human K6 in its predicted amino acid sequence and
expression pattern (the BK6b gene was originally designated
BKIV; see Ref. 8). Two groups observed a near-tissue-specific
expression of heterologous coding sequences in transgenic mice
when using either 5.2 or 8.8 kb of 5′-upstream sequence from
the BK6b gene (8, 35). The occurrence of such significant dif-
ferences is surprising, given the extensive homology in the
proximal 5′-upstream sequences of the human K6a, K6b, and
bovine K6b genes (data not shown; Ref. 38). Multiple K6 iso-
form genes have been identified in both the human and bovine
genomes (4, 36, 37), and we do not know whether the bovine
K6b gene is the actual ortholog of human K6a. This notion
could explain the differences observed in the activity of the
5′-upstream sequence of these genes in transgenic mice. An
in-depth comparison of the promoter sequences of these genes
should provide significant insights into the unique aspects of
the regulation of the human K6a gene.

The keratin 6 gene(s) are co-expressed with the K16 and/or
K17 genes as type I keratin partners in stratified epithelia
under basal or challenged conditions (see Introduction). We
previously reported that a full-length genomic clone (11 kb)
containing the entire human K16 gene yielded cell type-specific
expression in the trunk skin of transgenic mice under both
basal and injury conditions (17), but not in specialized skin
epithelia such as foot pad epidermis and nail matrix. As these
studies did not address the contribution of the various seg-
ments of the human K16 gene to the pattern of expression
observed in transgenic mice, the organization of regulatory
sequences in the human K6a and K16 genes can not be com-
pared at the present time.

Role of the Proximal 5′-Upstream Sequence in K6a Gene
Expression after Various Acute Stimuli—We demonstrated
here that the proximal 960 bp of 5′-upstream sequence in the
human K6a gene successfully mediates the rapid induction of a
heterologous reporter gene in adult transgenic mouse skin
after acute injury or treatment with appropriate chemical in-
ducers, while the proximal 550-bp segment can not. At least
when studied in transgenic mice, therefore, we conclude that
cis-acting sequences located between −550 and −960 bp in
the human K6a gene are necessary for its induction when sub-
jecting stratified epithelia to a variety of acute stimuli (injury,
12-O-tetradecanoylphorbol-13-acetate, RA, DNFB). Moreover,
these regulatory sequences are at least partly distinct from
those underlying its constitutive expression in the relevant
epithelia. Whether these inducible elements activate transcrip-
tion by acting directly on core promoter elements or alterna-
tively by negating a repressor element located within the pro-
imal 550-bp segment remains to be defined. Moreover, given
our observation that the product of the KT3 transgene is spa-
tially restricted to the suprabasal layers after its induction
(Fig. 2), as is the case for the K6 isoforms after injury to human
skin (11), we also conclude that the critical elements control-
ing the cell type specificity of human K6a expression are likely to
be present within the proximal 960 bp of its 5′-upstream se-
quence. These data extend the findings of Ramirez et al. (8),
who observed an induction of a LacZ reporter transgene fea-
turing 8.8 kb of 5′-upstream sequence from the bovine K6b
gene after treatment with 12-O-tetradecanoylphorbol-13-acet-
te and RA and after injury to the skin. On the other hand, our
conclusions differ from those reached by Jiang et al. (39, 40),
who found that the proximal 390 bp of 5′-upstream sequence
from the human K6b gene conferred positive and cell type-
specific expression of a CAT reporter in human keratinocytes
in culture, a context that allegedly mimicks hyperproliferation
(14). The “promoter region” of many keratin genes has been
found to behave differently when transfected in cultured cell
lines compared with when stably integrated within the mouse
genome (e.g. Refs. 18, 34, and 41), a notion that may be at play
here. Other explanations for this discrepancy include the ex-
istence of distinct regulatory mechanisms for human K6a and
K6b (see Ref. 4) or alternatively, the potential presence of
strong silencer element(s) located between −390 bp and −550
bp in both these genes.

We observed a much stronger induction of transgene expres-
sion following acute chemical induction or injury to adult trans-
genic mice bearing a construct featuring 5.2 kb of human K6a
5′-upstream sequence compared with those having shorter 5′
sequences (Table II; Fig. 3). For each acute challenge tested
(PMA, RA, injury), indeed, the extent of LacZ induction in
mouse skin showed a similar dependence upon the amount of
5′-upstream sequence in the transgene. This notion suggests
that the relevant regulatory elements in the proximal core
promoter are subject to positive regulation by powerful en-
hancer element(s) located between −2500 and −5200 bp in
the human K6a gene. Our data also suggest that the molecular
mechanisms that trigger K6 induction after acute stimuli in
skin may differ to some extent from those underlying its sus-
tained expression in chronic lesions such as those typical of
psoriasis and benign and malignant neoplasia. Further char-
acterization of the human K6a gene in transgenic mice should
enable us to define the identity and mode of action of the
various functional elements involved in controlling the complex
regulation of this gene.

It should be emphasized that the results reported here
apply to post-natal mouse skin and that our interpretation of
the expression pattern is based on the comparison of the dis-
tribution of the transgene product with that of mouse K6 pro-
tein(s). Transient expression of K6 has been detected in epider-
mis at a late stage of human fetal development (week 36; see
Ref. 7). Studies are in progress to examine whether the [5′
K6a]-LacZ transgene is expressed pre-natally in developing
hair follicles or epidermis in our lines of transgenic mice. At
another level, a close examination of the pattern of [5′ hK6a]-
LacZ transgene expression in adult transgenic mouse skin sug-
gests that after induction not all suprabasal keratinocytes
show a β-galactosidase-positive nucleus, whereas the majority
of them stain positive for mouse K6 protein(s) under the same
conditions (e.g. Fig. 2). As apparent from previous transgenic
mouse studies (18), the β-galactosidase protein may be rela-
tively short-lived in skin keratinocytes, even when targeted to
the nucleus (this study). Given that keratin proteins are very
stable in epiblast cells (7), a survey of the mouse K6 mRNA(s)
distribution would provide a more suitable reference against
which to compare the distribution of the transgene protein. In
a parallel set of transgenic mouse studies involving the same
promoter sequences, we found that after induction by PMA
application (33) or injury (data not shown), a Myc epitope-tagged transgenic keratin protein shows more consistent expression in suprabasal epidermis. A characterization of the mouse K6 isoform family has yet to be completed and should enable the design of suitable probes for the specific detection of K6 mRNA(s). These issues are of significant importance for our understanding of the control of de novo keratin gene transcription at a spatial and temporal levels in stratified epithelia subjected to various types of challenges. This information is also needed to better exploit the 5′-upstream region of the human K6α gene for inducible expression or inducible gene rearrangements in stratified epithelia of transgenic mice and Dr. K. McGowan for his advice and assistance. We also thank Dr. D. Paulin for providing the nls-LacZ reporter sequence, Dr. D. Roop for providing an antisera to mouse K6, and Drs. E. Colucci-Guyon and C. Byrne for their advice.

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