Skin-specific Expression of a Truncated *Ela* Oncoprotein Binding to p105-Rb Leads to Abnormal Hair Follicle Maturation Without Increased Epidermal Proliferation

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Abstract. In cultured cells, mutants of the Adenovirus *Ela* oncoprotein which bind to a reduced set of cellular proteins, including p105-Rb, p107, and p60-cyclin A, are transformation defective but can still interfere with exogenous growth inhibitory and differentiating signals, such as those triggered by TGF-β. We have tested the ability of one such mutant, NTdl646, to interfere with keratinocyte growth and differentiation in vivo, in the skin of transgenic mice. Keratinocyte-specific expression of the transgene was achieved by using a keratin 5 promoter. Two independent lines of transgenic mice were obtained which expressed *Ela* specifically in their skin and exhibited an aberrant hair coat phenotype with striking regional variations. Affected hair shafts were short and crooked and hair follicles exhibited a dystrophic or absent inner root sheath. Interfollicular epidermis was normal, but its hyperplastic response to acute treatment with TPA (12-*O*-tetradecanoylphorbol-13-acetate) was significantly reduced. Primary keratinocytes derived from these animals were partially resistant to the effects of TPA and TGF-β. The rate of spontaneous or chemically induced skin tumors in the transgenic mice was not increased.

Thus, expression of a transgene which interferes with known negative growth regulatory proteins causes profound disturbances of keratinocyte maturation into a highly organized structure such as the hair follicle but does not lead to increased and/or neoplastic proliferation.

SELF-renewing epithelia are characterized by a fine balance between growth and differentiation. An important problem is how these processes are controlled. In the epidermis, proliferation is normally confined to keratinocytes of the basal layer while cells of the upper layers terminally differentiate (Fuchs, 1990). A more complex program of keratinocyte growth and differentiation is involved in formation of skin appendages such as hair follicles (Stenn et al., 1991). The mature hair follicle consists of several concentric epithelial layers, of which the innermost ones make up the hair shaft. Each follicle undergoes repeated cycles of growth (anagen), regression (catagen), and quiescence (telogen). In the growth phase, the relatively undifferentiated and actively dividing cells of the lower portion, or "bulb," move upwards and differentiate into the several concentric epithelial layers of the upper region. In turn, the proliferating bulbar cells appear to arise from stem cells situated in the "bulge," in the upper region of the hair follicle (Cotsarelis et al., 1990). The importance of this region for keratinocyte self-renewal is indicated by both hair follicle (Oliver, 1966; Inaba et al., 1979) and epidermal (Lenoir et al., 1988; Limat et al., 1991) regeneration experiments. This upper portion of the follicle, rather than the epidermis, is also thought to contain target cells for cutaneous carcinogenesis (Berenblum et al., 1959; Argyris, 1980. Keratinocytes in both epidermis and hair follicles are dependent on intimate interactions with the underlying mesenchymal tissues (for example, see Billingham and Silvers, 1967; Saiag et al., 1985; Oliver and Jahoda, 1988; Coulomb et al., 1989; Hirai et al., 1992).

Previous studies have shown that the Adenovirus *Ela* oncogene can be used to probe into complex regulatory functions, involved in keratinocyte growth and differentiation (Barrandon et al., 1989; Pietenpol et al., 1990; Missero et al., 1991*a*,*b*). The immortalizing and transforming activity of this oncogene depends on its binding to a specific set of cellular proteins, including p105-Rb, p107, and p60-cyclin A (for review see Nevins, 1992). It has been shown that *Ela* can interfere with the function of these latter proteins by causing their dissociation from specific transcriptional complexes (Bagchi et al., 1990; Chellapan et al., 1991; Mudryj et al., 1991; Cao et al., 1992; Ewen et al., 1992). Besides transformation, binding of *Ela* to these proteins interferes with transmission of exogenous growth inhibitory and differentiating signals of the kind triggered by TGF-β (Pietenpol et al.,

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1990; Missero et al., 1991*a*). Transformation-deficient *Ela* mutants which bind to p105, p107, and p60-cyclin A can still interfere with the normal TGF-β response, even if to a lesser extent than wild-type *Ela*. Like with TGF-β, the sensitivity of keratinocytes to phorbol esters (12-*O*-tetradecanoylphorbol 13-acetate[TPA]), elevated cAMP levels or corticosteroids can be at least partially suppressed by these *Ela* mutants (Missero et al., 1991*a*; Florin-Christensen et al., 1992, 1993)

In parallel with these activities, *Ela* can block growth-inhibitory and differentiating signals involved in control of skin tumor development (Missero et al., 1991*b*). Tumorigenicity of keratinocytes transformed by a single *ras* oncogene can be suppressed by dermal fibroblasts, through the production of a diffusible TGF-β factor. Resistance to dermal fibroblast-tumor inhibition was observed in keratinocytes concomitantly transformed by *ras* and *Ela* or a truncated *Ela* mutant which binds to p105, p107, and p60.

Complex growth inhibitory and/or differentiating signals may be operative in the intact skin, which cannot be taken into account in culture and/or in simple tumorigenicity assays. Our previous studies suggest that heterotypic cell–cell interactions, such as those mediated by TGF-β production, can play an important role in skin tumor control and, more generally, in normal skin homeostasis (Missero et al., 1991*b*). To further test this model, we have generated transgenic mice which specifically express in their epidermis a transformation defective *Ela* mutant (NTdl646) which retain the ability to bind to the p105, p107, and p60 proteins (Whyte et al., 1989). Transgenic mice expressing the entire Adenovirus *E1* region from the MMTV promoter have been previously described (Koike et al., 1989). The complex phenotype that was found in that case, multifocal tumors at the squamocolumnar junction of the stomach, is likely to be a result of the cooperative activity of the *Ela* and *Elb* oncogenes. For our studies, the *Ela* NTdl646 mutant was chosen to disrupt negative regulatory loops involved in maintenance of a normal epidermal tissue without eliciting direct growth stimulatory and/or transforming effects. Transgenic mice with skin-targeted expression of positive growth-regulatory molecules such as activated *ras* (Bailleul et al., 1990) and BNLF-1 (Wilson et al., 1990) oncogenes, and TGF-β (Vassar and Fuchs, 1991) have been previously reported. In the latter two cases, an hyperplastic epidermis was observed. With *ras*, an hyperkeratotic epidermis was formed which proceeded to yield benign papillomas. In contrast, the epidermis of transgenics carrying the truncated *Ela* oncogene was normal and its rate of tumor conversion was not increased. Rather than in the epidermis, there were widespread abnormalities in the hair follicles, with aberrant hair shafts and absent or markedly dystrophic inner root sheaths.

Materials and Methods

Plasmid Construction and Characterization

A 900-bp portion of the keratin 5 promoter was obtained from a mouse genomic DNA library by PCR amplification, from position −807 to position +90 of the published sequence (Lersch et al., 1989) and cloned into the ClaI site of Bluescript KS$^+$ (Stratagene, La Jolla, CA). The SV-40 polyadenylation site (from position +2560 to +3007 of the SV-40 sequence) was inserted behind the K5 promoter at the PstI–BamHI sites. Finally, a G418-resistance gene driven from the SV-40 early promoter (from nucleotide +3393 to +5734 of pSV2-neo) (Southern and Berg, 1982) was inserted at the NotI site.

The coding region of the *Ela* NTdl646 gene was excised from the plasmid vector (Whyte et al., 1989) by digestion with NcoI (which cuts just upstream of the *Ela* initiation codon) and PstI (at the 3′ end of the gene) and inserted at the unique EcoRV site of the plasmid vector (K5T), behind the K5 promoter and in front of the SV-40 polyA site.

Transgenic Mice Generation

A 2.6-kb DNA fragment containing the K5 promoter, the *Ela* NTdl646 gene and the SV-40 polyadenylation signal (BamHI cassette) was purified and injected into the male pronucleus of fertilized eggs from (C57BL/6 × SJL) F_1 mice as described (Hogan et al., 1985). 18 offsprings were obtained which were screened for foreign DNA after reaching 3 wk of age. Dot blot analysis was performed with 10 μg of purified genomic DNA using an *Ela*-specific probe (nucleotides +646 to +1007 of the *Ela* sequence). DNA from the *Ela*-positive mice was cleaved with EcoRI and BglII and analyzed by 1% agarose gel electrophoresis and Southern blotting with the *Ela* probe to test whether the integrated DNA was intact.

For subsequent analysis of newborn litters, tail DNA was subjected to PCR analysis with *Ela*-specific primers corresponding to nucleotides +877 to +897 and +1073 to +1093 of the *Ela* gene, followed by agarose gel electrophoresis. PCR reactions with primers for the endogenous mouse actin gene were run as controls.

Histology and Immunohistochemistry

Formalin-fixed tissues were sectioned and stained with haematoxylin and eosin. For EM, specimens were fixed in glutaraldehyde as previously described (Filvaroff et al., 1990). For immunohistochemistry, tissues were fixed in 3% paraformaldehyde for 1 h at 4°C. Paraffin sections were subsequently incubated with anti-*Ela* M73 mAbs (1:10 dil., Oncogene Science, Inc., Manhasset, NY) overnight at 4°C, with biotinylated goat anti-mouse antibodies for 1 h at room temperature, and with Streptavidin peroxidase (1:20 dilution; Zymed Labs, Inc., San Francisco, CA) for 30′. Slides were reacted with 0.1% (wt/vol) 3-3′diaminobenzidine (Sigma Immunochemicals, St. Louis, MO). Sections were counterstained with 5% Light Green SF Yellowish (Fisher Scientific, Pittsburgh, PA).

Ribonuclease Protection Assays

Tissues were pulverized after quick freezing in liquid nitrogen. Total RNA was extracted by the method of Chomszynsky and Sacchi (1987). Ribonuclease protection assays were performed as previously described (Missero et al., 1991*b*) using 50 μg of tissue RNA and an RNA probe corresponding to nucleotides +646 to +1007 of the *Ela* gene. An RNA probe for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Tso et al., 1985) was also used to control for mRNA levels in the various samples. Probes were prepared after plasmid linearization, by in vitro transcription with T7 polymerase (Stratagene) in the presence of α^{32}P-UTP (10 mCi/ml, 400 Ci/mmol, Amersham Corp., Arlington Heights, IL).

Immunoblotting

Cells or pulverized tissues were dissolved in sample buffer (Laemmli, 1970), boiled for 5′ and analyzed by SDS-PAGE and immunoblotting with anti-*Ela* M73 mAbs (1:20 dilution; Oncogene Science, Inc.). Antibody binding was detected by incubation with ^{125}I-protein A (100 μCi/ml; ICN Biochemicals, Irvine, CA). The various samples were normalized for protein content by gel electrophoresis and staining with Coomassie blue.

Cell Culture and Assays

Pam212 cells (Yuspa et al., 1980) were transfected with the K5T and K5T/*Ela*646 plasmids, as previously described (Missero et al., 1991*a*). G418-resistant colonies were pooled and amplified before testing. Primary keratinocytes from newborn mice were prepared and cultured as previously described (Hennings et al., 1980; Dotto et al., 1988).

Mitogenicity assays were performed as previously described (Missero et al., 1991*a*). TPA was purchased from Chemsyn Science Laboratories

1. *Abbreviations used in this paper*: DMBA, 7,12-dimethylbenz[A]anthracene; IRS, inner root sheath; ORS, outer root sheath; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

(Lenexa, KA) and TGF-β 1 from R&D Systems (Minneapolis, MN). Cells were labeled by a 1-h pulse with [Methyl-^{3}H]thymidine (3.3 μCi/ml; 40–60 Ci/mmol; ICN Biomedicals, Costa Mesa, CA). Epidermal transglutaminase activity was measured by [^{3}H]putrescine incorporation as previously described (Filvaroff et al., 1990).

Tumor Induction Experiments

Ela-positive and -negative mice in the resting phase of the hair cycle (6–7 wk old) were shaved and treated with DMBA (20 μg in 200 μl acetone) 48 h after shaving. Mice were subsequently treated twice a week with TPA (10^{-4} M in acetone) for 7 mo. These experiments were performed with transgenics in a Sencar background (F_4 and F_5 generations) and the Ela-negative mice used as controls were derived from the same litters as the transgenics. DMBA was dissolved in acetone just before use. TPA solution was stored at −70°C. Papillomas were excised for histological examination at different stages of their growth.

For experiments involving DMBA, Ela-positive and -negative mice from the same litters were exposed to a single dose of this agent 1–3 d after birth via intraperitoneal injection. Relative to topical treatment of adult mice, this way of administration is equally effective for skin tumor induction (Hennings et al., 1981) and has the potential advantage of hitting target keratinocyte populations in mice of younger age, at a time when the effects of *Ela* expression appear to be more evident. For these experiments DMBA was first dissolved in DMSO, diluted in SSV buffer (Hennings et al., 1981) and injected at 20 μg per mouse in a 25 μl volume. Mice were kept for examination until 8 mo of age.

Results

Construction of a Keratinocyte-specific Ela Expression Vector and Generation of Transgenic Mice

A previous study has indicated that the first 900 nucleotides in front of the human keratin 5 gene are sufficient to drive specific expression of this gene in cultured keratinocytes (Lersch et al., 1989). For the present work, this 900-bp promoter sequence was used to drive specific expression of the *Ela* NTdl646 gene in keratinocytes of transgenic mice. This gene lacks the sequence for the first 29 amino acids of *Ela*. This deletion prevents binding of *Ela* to a p300 cellular protein without affecting association with p105, p107, and p60. The NTdl646 gene lacks the immortalizing and transforming activity of the full *Ela* gene (Whyte et al., 1989), but retains the ability to interfere with the growth inhibitory and differentiating effects of TGF-β, TPA, cAMP, and corticosteroids (Missero et al., 1991*a*; Florin-Christensen et al., 1992; our own unpublished observations).

A construct carrying the *Ela* NTdl646 gene behind the K5 promoter (K5T/*Ela*646; Fig. 1 *A*) was transfected into established Pam212 keratinocytes (Yuspa et al., 1980). As previously described for the *Ela* NTdl646 gene driven by its own adenoviral promoter (Missero et al., 1991*a*), K5T/*Ela*646 induced ~40% resistance to TGF-β growth inhibition (data not shown).

K5T/*Ela*646 was used to generate transgenic mice, by injection of purified insert DNA (BamHI cassette, Fig. 1 *A*) into fertilized eggs from (C57BL/6 × SJL) F_1 mice. 5 out of 18 newborn mice were found to be positive for the transgene, and each carried ~4 copies of unrearranged DNA, as judged by Southern blotting and dot blotting of chromosomal DNA alongside serial dilutions of vector DNA standards. Transgenic lines for further analysis were generated by breeding all positive founders with Sencar mice. This strain of mice was chosen because of its intrinsic sensitivity to skin carcinogenesis (Hennings et al., 1981), which might enhance whatever growth-related effect the truncated *Ela* transgene might have. One of the *Ela*-positive founders failed to transmit the gene to the progeny. Of the other four, two, 901T and 906T, showed similar hair coat abnormalities (see below), which were transmitted to the offspring. The 901T founder was albino and gave rise only to albino mice. 906T was agouti and, when crossed with the Sencar albinos, gave rise to *Ela*-positive offsprings with all possible coat color combinations, indicating that the inserted transgene was unlinked to either the agouti or albino loci.

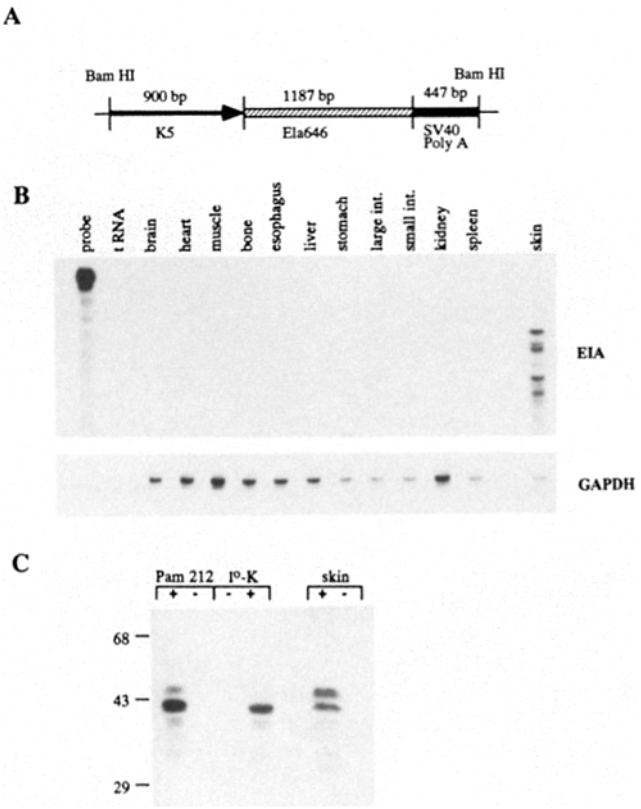

Figure 1. Map of the K5T/Ela646 construct and expression of the *Ela* transgene. (*A*) The BamHI cassette used for transgenic mice construction, containing the K5 promoter, the *Ela* NTdl646 gene and the SV-40 polyadenylation site. (*B*) Ribonuclease protection assay with an *Ela*-specific probe to determine tissue specificity of expression in an F_2 906T mouse. Ribonuclease protection with a GAPDH RNA probe was used as an independent measure of mRNA amounts present in the individual tissue preparations. A weak positive signal, detectable in the stomach and intestine samples, was lost after photographic reproduction. A similar pattern of *Ela* expression was observed in two other 906T and one 901T mice. (*C*) Immunoblotting with anti-*Ela* mAbs to determine expression of the *Ela* NTdl646 protein in Pam212 keratinocytes transfected with the K5T/*Ela*646 construct (+) or the K5T vector alone (−), primary keratinocytes derived from *Ela*-positive mice (+) and *Ela*-negative littermates (−), and whole skin of a 906T *Ela*-positive mouse (+) and an *Ela*-negative littermate (−). Molecular weight markers are indicated in kD.

Macroscopic Abnormalities in the Ela NTdl646 Transgenics

Positive mice were clearly distinguishable a few days after birth because of a striped hair coat, resulting from areas of shorter and wrinkled hair shafts alternating with bands of

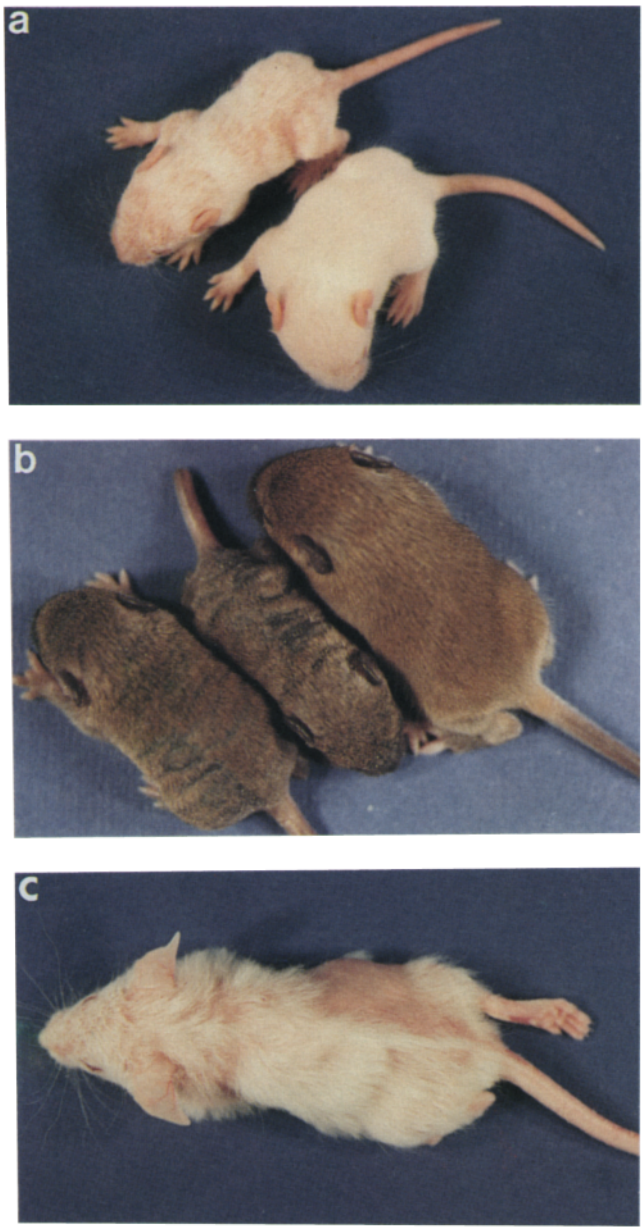

Figure 2. Aberrant hair coat phenotype in the *Ela* NTdl646 transgenics. (*a*) An *Ela*-positive 901T mouse at 7 d of age along with an *Ela*-negative littermate; (*b*) two *Ela*-positive 906T mice along with an *Ela*-negative sibling at 10 d of age; and (*c*) an *Ela*-positive 906T mouse with particularly strong phenotype at 1 mo of age. The well demarcated bald area occurred spontaneously.

normal looking hair (Fig. 2, *a* and *b*). The stripes of affected hair rarely crossed the midline and their location remained constant throughout the lifetime of the animal.

This striped pattern occurred independently of hair color, sex, and sex of affected parent. Some 906T mice, especially in the F_2 and F_3 generations, exhibited a stronger phenotype, which included larger and irregular areas of very poor hair growth and/or baldness (Fig. 2 *c*). As with the stripes, these areas of baldness did not cross the midline but could sharply terminate there (Fig. 2 *c*). In these same areas, thin pigmented lines could be detected. With time (as early as 3–4 wk of age) some of the bald areas evolved into ulcers (data not shown). Since such destructive lesions could be found at the base of the neck, and in mice that were caged individually, it is unlikely that they were the result of trauma, such as biting or scratching. In the most severely affected animals, the hair abnormality was present universally on all skin surfaces with some hair gradually growing later in life. The weak and strong phenotypes were equally observed in inbred litters of *Ela*-positive transgenics and outbred litters of *Ela*-positive males with normal Sencar females.

Besides hair coat abnormalities, the only other macroscopical phenotypic alteration present in some newborn animals was a persistent crust in the eye, without effects on the opening of the lid. The crust was eventually eliminated by 2–3 wk of age. *Ela*-positive mice were often smaller than their *Ela*-negative littermates at birth, but this difference tended to disappear by 3–4 wk of age. Autopsy revealed no macroscopic or histologic abnormalities of the various internal organs.

Histological Abnormalities

Use of a dissecting microscope revealed that, relative to neighboring regions, hair shafts in the affected stripes were shorter and crooked (Fig. 3, *a–c*). These hair abnormalities were particularly evident in agouti mice, where the alternating transversal bands of yellow and black pigment—intrinsic to the agouti phenotype—produced an impression of abnormal pigmentation. Very short and irregular shafts were present also in the bald areas. Overhairs—including vibrissae—and underhairs were similarly affected. The shafts appeared thinner and wavy but the tips were tapered as in normal hairs and fractured forms were not seen (Fig. 3, *c–e*). Hair shafts from individual mice collected and analysed for amino acid content. Variations in individual aminoacid were <1% except for citrulline and ornithine—an enzymatic conversion product of trichohyalin (Rogers et al., 1991): a 30% decrease of these particular aminoacids was consistently observed in each of five *Ela*-positive mice versus five *Ela*-negative siblings.

Hair follicles were directly visualized after removal of dermal collagen by acid extraction (Schweizer and Marks, 1977). This technique revealed that hair follicle density was only slightly reduced in the *Ela*-positive mice, even in their striped and bald areas, but follicles were generally smaller and erratic in shape compared with the controls.

Severely affected follicles were often located next to relatively normal ones. By high power light microscopy, no abnormalities were notable in the dermal papilla, or the lower bulb. Cells in the upper area of the bulb were slightly reduced in number and size and had an abnormal shape and organization. The most obvious changes were apparent in the inner root sheath (IRS) with disruption of the Huxley's and Henle's layers and dilated cells with a reduced content of trichohyalin granules (Fig. 4, *c* and *d*). The hair shaft was often prematurely separated from the IRS and showed substantial irregularities in width and a reduction of trichohyalin granules in the medulla. In contrast to IRS and hair shaft, the outer root sheath was usually normal. Focal disruption of the outer root sheath (ORS) could however be found at sites of ulceration (see below). These findings were confirmed by EM, with cells of the IRS lacking the typical differentiation features of both Huxley's and Henle's layers (Fig. 5).

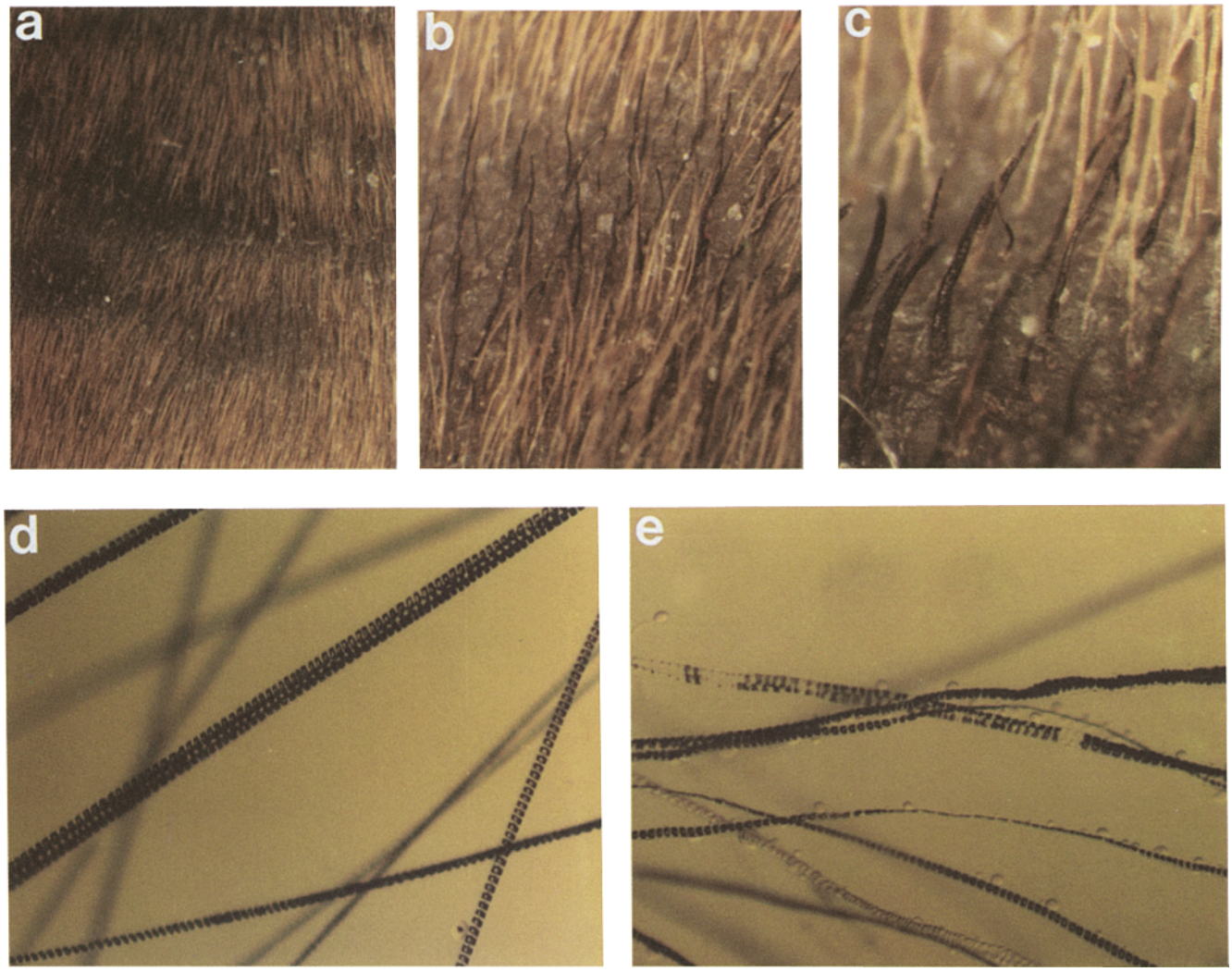

Figure 3. Hair coat abnormalities as observed microscopically. (*a–c*) striped hair coat of a 906T mouse as seen under a dissecting microscope at increasing magnification. (*d* and *e*) Hair shafts from an *Ela*-negative and -positive littermate, respectively, as seen by light microscopy. Bars: (*a*) 300 μm; (*b*) 150 μm; (*c*) 60 μm; and (*d* and *e*) 75 μm.

The epidermis of the *Ela*-positive mice was normal (Fig. 4, *a* and *b*), except for mild thickness and scaling in some markedly affected newborns. The healing process after full thickness wounding was normal. Areas of spontaneous ulceration were characterized by a marked epidermal hyperplasia surrounding the ulcer and an inflammatory and reparative response of the dermis which contained very few residual hair follicles. No signs of infections were detectable at these locations. To determine the histogenesis of these lesions, we examined a set of eight mice at a rather young age (3 wk), with a very strong phenotype and with already small ulcers. Limited foci of epidermal hyperplasia were found in the bald but intact areas. Much more pronounced hyperplasia was found along the margin of the small ulcers. The wall of the hair follicles in these areas was irregularly thinned and showed focal infiltration of acute inflammatory cells. At least in some cases, it was possible to demonstrate that the ORS was focally disrupted. This histological picture is similar to human perforating folliculitis and is consistent with a deep folliculitis being responsible for the observed ulceration and scarring.

No signs of spontaneous tumorigenic conversion was ever detected over a period of 12 mo either at these locations or anywhere else in the skin of the affected animals.

Correlation of Phenotype with Preferential K5T/Ela646 Expression in the Skin

Total RNA was prepared from the skins of F_1 mice and analyzed by ribonuclease protection assays with an *Ela*-specific probe. *Ela* expression was found in the two families with positive phenotype, 901T and 906T, while the other two families showed no detectable expression (data not shown). *Ela* expression in 901T mice was found to be consistently lower (two- to-threefold in three independent measurements) than in the 906T. For most of the biochemical and functional assays reported hereafter we have focused on the 906T mice.

Tissue specific expression of the transgene was assessed by ribonuclease protection assays with total RNA derived from various tissues. As shown in Fig. 1 *B*, *Ela* was preferentially expressed in the skin, with much weaker levels of expression detectable in the stomach and intestine. In all other organs

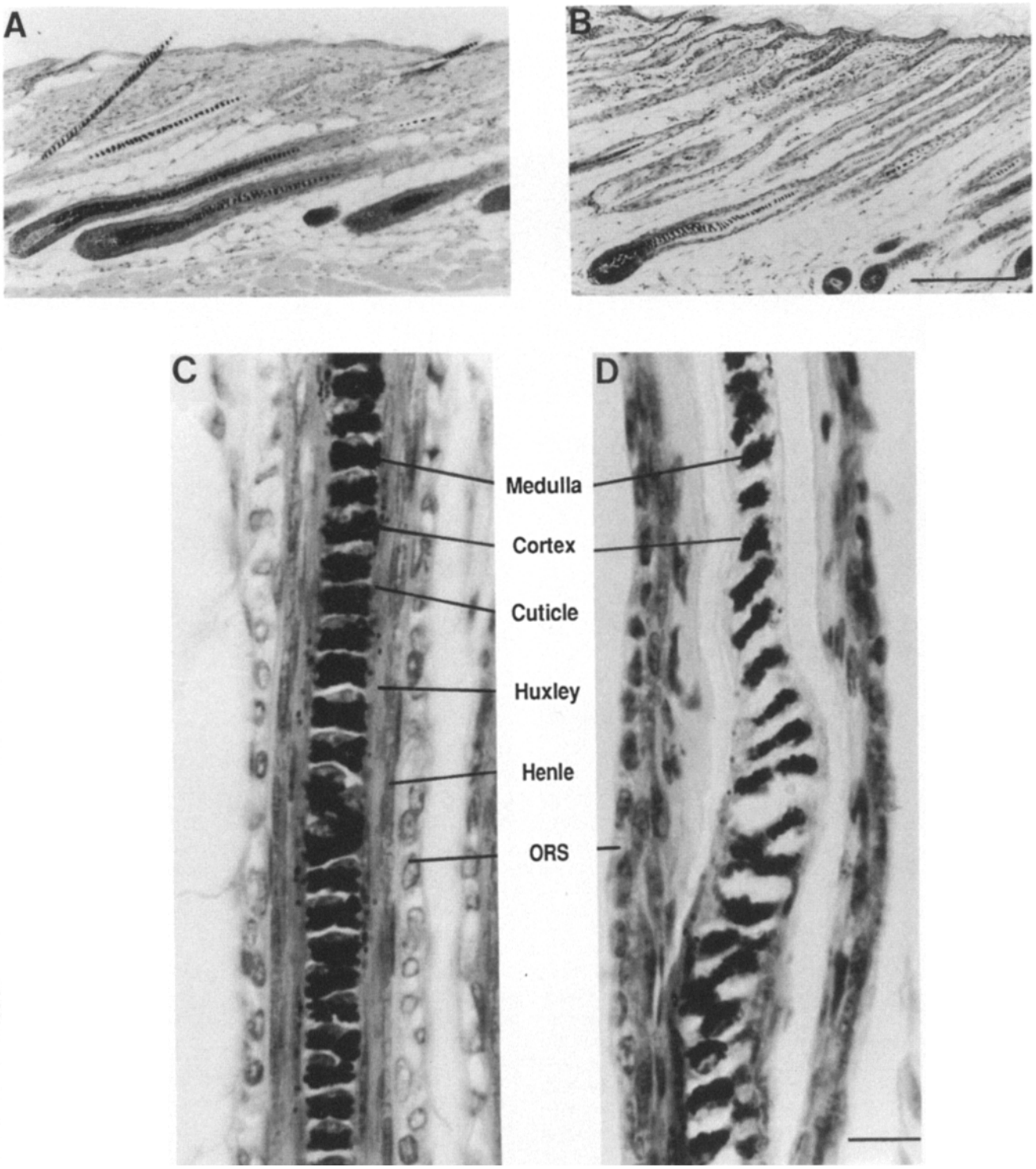

Figure 4. Histology of the skin of *Ela* NTdl646 transgenics. (*A* and *B*) Low power magnification of the skin from an *Ela*-negative (*A*) and *Ela*-positive (*B*) littermates. Note the abnormal and crooked hair follicles with a normal, nonhyperplastic epidermis in the affected animal. Early and irregular separation of the hair shaft from the sheath could be typically observed. (*C* and *D*) High power magnification of a hair follicle from the *Ela*-negative and *Ela*-positive mice. Note the normal layering of the control follicle (*C*) with clearly distinguishable ORS, Henle's and Huxley's layers, cuticle, cortex, and medulla. Notice that the medulla has a regular width and the medullary spaces are filled with melanin and trichohyalin granules. The affected follicle (*D*) contains an irregularly shaped shaft with open medullary spaces, with normal melanin but substantially reduced trichohyalin granules. The wall lacks the typical layering of the normal follicle. In particular, the region corresponding to the putative IRS shows a markedly dystrophic structure and lacks the typical features of both Huxley's and Henle's layers. Haematoxylin and eosin. Bars: (*A* and *B*) 200 μm; (*C* and *D*) 15 μm.

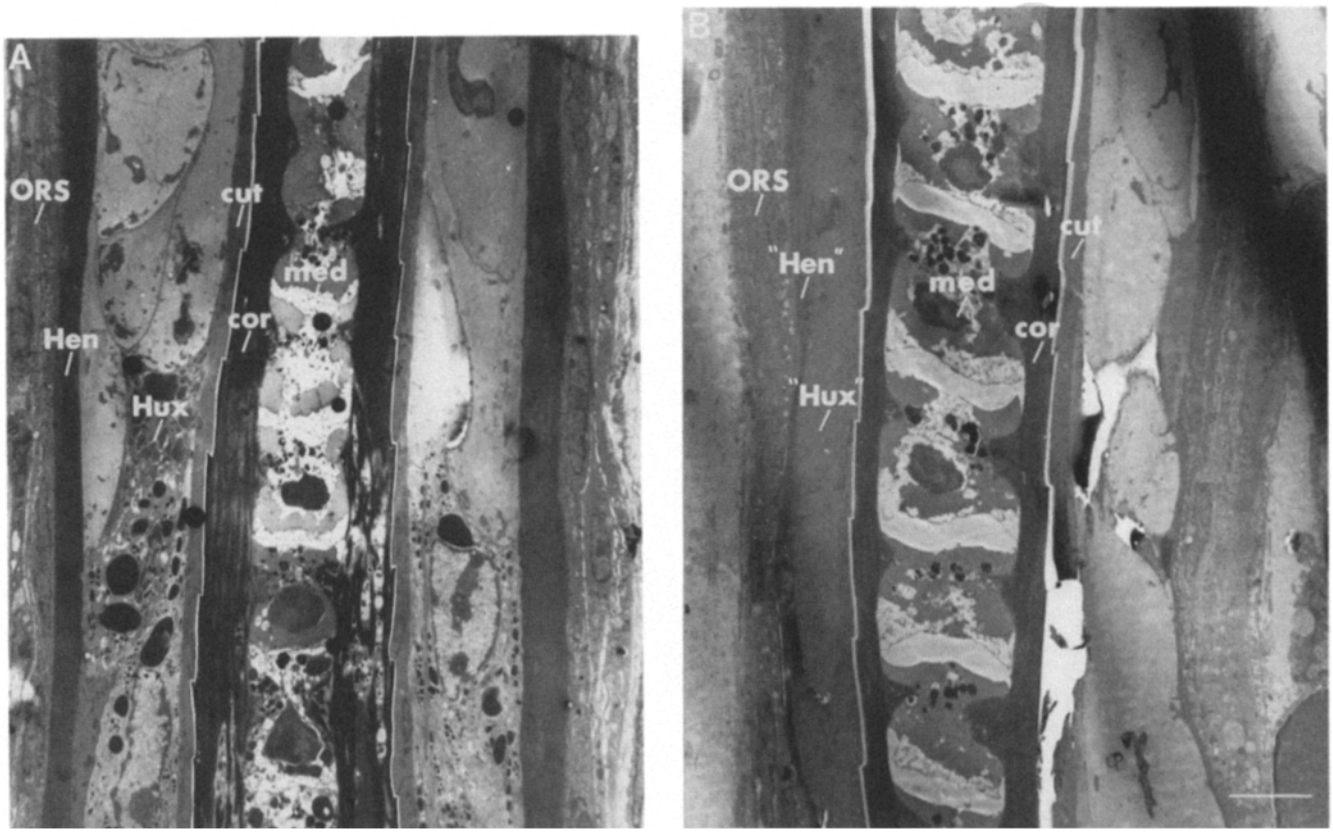

Figure 5. EM of hair follicles from control (*A*) and transgenic (*B*) littermates. Note the normal structure of the control follicle with the ORS, Henle's (*Hen*), and Huxley's layers (*Hux*), cuticle (*cut*), cortex (*cor*), and medulla (*med*). The wall of the affected follicle is distinctly disorganized with swollen cells without trichohyalin granules in the area of the Huxley's layer (*Hux*) and an irregular to absent Henle's layer (*Hen*). The layering of the shaft is relatively normal, but the medullary spaces are wider and have a reduced number of granules. The differences in thickness of the cortex and keratinization levels between *A* and *B* are not significant as they are due to the slightly different level of the follicle from which these two sections were taken. Bar, 5 μm.

Ela expression remained undetectable, including the esophagus. This organ contains a stratified epithelium where the K5 keratin is usually expressed (Fuchs and Green, 1980; Moll et al., 1982). The lack of expression of the *Ela* transgene in this location may be due to the relatively small K5 promoter region used for these studies.

The *Ela* gene products migrate as a set of bands as a result of differential splicing and phosphorylation (Boulanger and Blair, 1991). Expression of *Ela* NTdl646 proteins was verified by immunoblot analysis of whole skin extracts of the transgenic mice with anti-*Ela* M73 mAbs (Fig. 1 *C*). Primary keratinocytes derived from the *Ela* transgenics were also found to express these proteins (Fig. 1 *C*), while dermal fibroblasts did not (data not shown). This analysis was complemented by an immunohistochemical study with the same antibodies to determine expression of the *Ela* protein in the skin in situ. Specific nuclear labeling was found in keratinocytes of both hair follicles (primarily in the ORS) and, to a lesser extent, epidermis (Fig. 6). Interestingly, staining was not homogeneous, but in patches, suggesting local variations in levels of *Ela* expression between neighboring hair follicles and epidermal keratinocytes.

Response of Transgenic Keratinocytes to TGF-β Growth Inhibition

Primary keratinocytes derived from the *Ela* transgenics were tested for TGF-β sensitivity. Newborn litters obtained from crosses of F_2 906T males with Sencar females were separated into *Ela*-positive and -negative groups by PCR analysis of tail DNA. Primary keratinocytes were prepared from the two groups of mice and *Ela* expression was confirmed by immunoblotting with *Ela*-specific antibodies (Fig. 1 *C*). Mitogenic response of these cells was determined after 20 h incubation with TGF-β at various concentrations (Fig. 7 *A*). In five independent experiments, DNA synthesis of the *Ela*-positive cells was found to be consistently less inhibited than that of their *Ela*-negative counterparts. However, this difference was never greater than 20% and was not observed at high TGF-β concentrations (>1 ng/ml).

Thus, expression of a truncated *Ela* NTdl646 gene in the transgenic keratinocytes interferes only partially with TGF-β growth inhibition.

Resistance of Transgenic Keratinocytes to TPA Effects both In Vitro and In Vivo

Phorbol esters such as TPA (12-*O*-tetradecanoylphorbol-13-acetate) act as potent modulators of keratinocyte growth and differentiation, under both in vitro and in vivo conditions (Steinert and Yuspa, 1978; Balmain, 1978; Bhisey et al., 1982; Yuspa et al., 1982; Reiners and Slaga, 1983). Mitogenicity assays similar to the ones described above were used as a first test of the sensitivity of *Ela*-positive and -negative

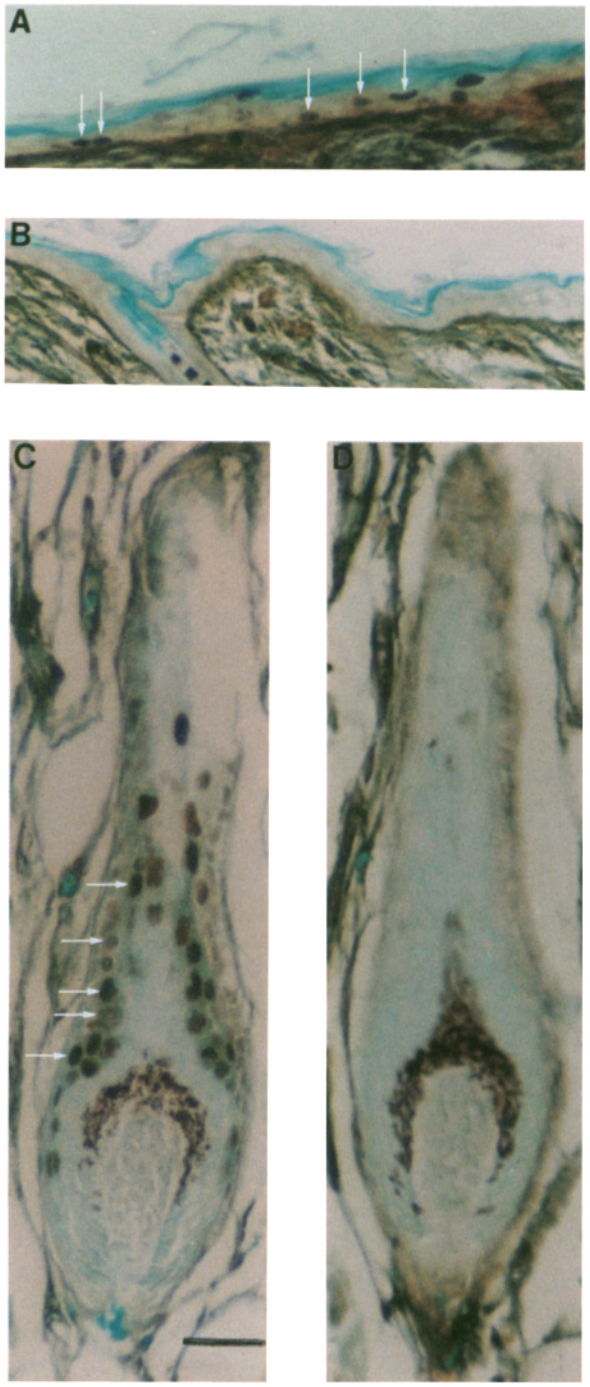

Figure 6. Expression of the *Ela*NTdl646 gene in the skin of transgenic mice as determined by immunohistochemistry. (*A* and *B*) Epidermis from a 906T *Ela*-positive mouse (2 wk old) stained with anti-*Ela* mAbs (*A*) or nonimmune antibodies (*B*); (*C* and *D*) a hair follicle of the same mouse stained with anti-*Ela* mAbs (*C*) or nonimmune antibodies (*D*). White arrows point to the specific nuclear labeling with the anti-*Ela* mAbs. Note in *A* the heterogeneity of *Ela* staining between adjacent epidermal keratinocytes. Bar, 90 μm.

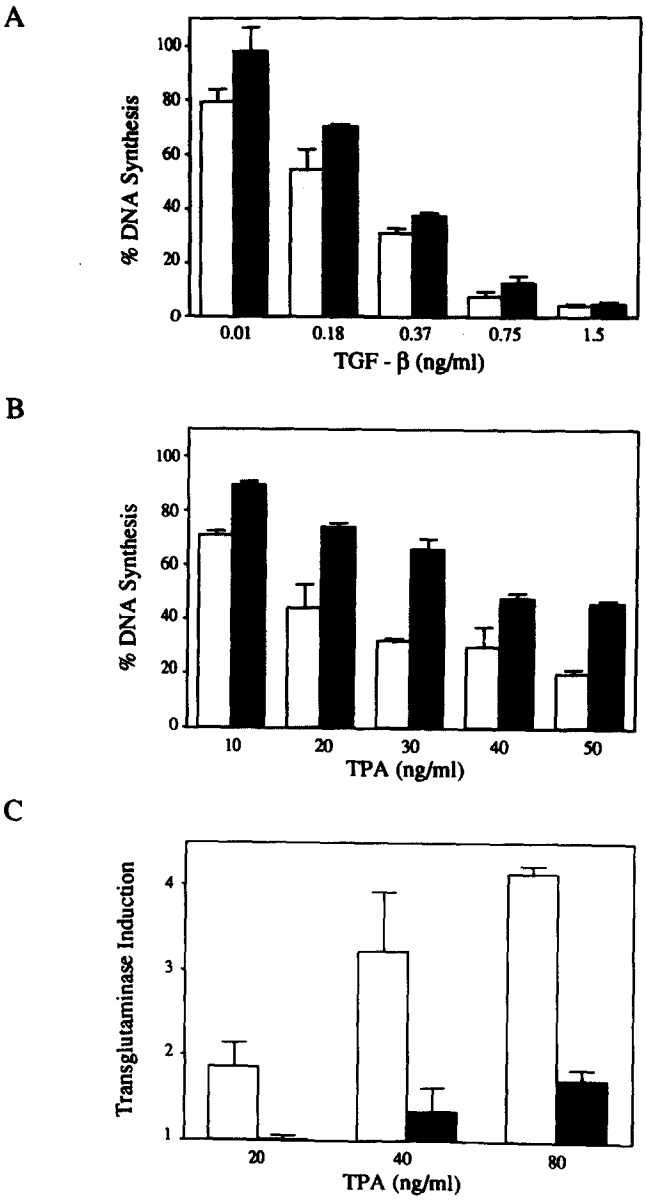

Figure 7. In vitro response of transgenic primary keratinocytes to TGF-β and TPA. Primary keratinocytes derived from 906T *Ela*-positive mice (■) and *Ela*-negative littermates (□) were tested for their sensitivity to growth inhibition by TGF-β and TPA (*A* and *B*, respectively) and epidermal transglutaminase induction by TPA (*C*). Cells were tested in triplicate wells and standard deviation was as indicated. Cells were incubated with fresh medium plus/minus TGF-β 1 or TPA for 20 h. DNA synthesis was measured by a 1-h pulse with [Methyl-^{3}H]thymidine and expressed as percentage values relative to untreated controls. Epidermal transglutaminase activity was measured by [^{3}H]-putrescine incorporation and expressed as fold induction of treated versus untreated controls.

primary keratinocytes to the growth inhibitory effects of TPA. DNA synthesis of *Ela*-positive cells was found to be inhibited by this agent but to a significantly lesser extent than that of their negative counterparts, and this difference was maintained even at high TPA concentrations (Fig. 7 *B*). In parallel with these results, epidermal transglutaminase, an enzymatic marker of keratinocyte differentiation, was induced by TPA to substantially lower levels of *Ela*-positive keratinocytes than in the negative controls (Fig. 7 *C*).

To test whether there was a decreased responsiveness to TPA in vivo, groups of *Ela*-positive and -negative mice were treated with this agent or acetone and sacrificed 72 h later (Fig. 8). Serial sections of the skin of each of these animals revealed a strong and homogeneous hyperplastic response in the *Ela*-negative mice (Fig. 8 *C*). In contrast, the epidermis

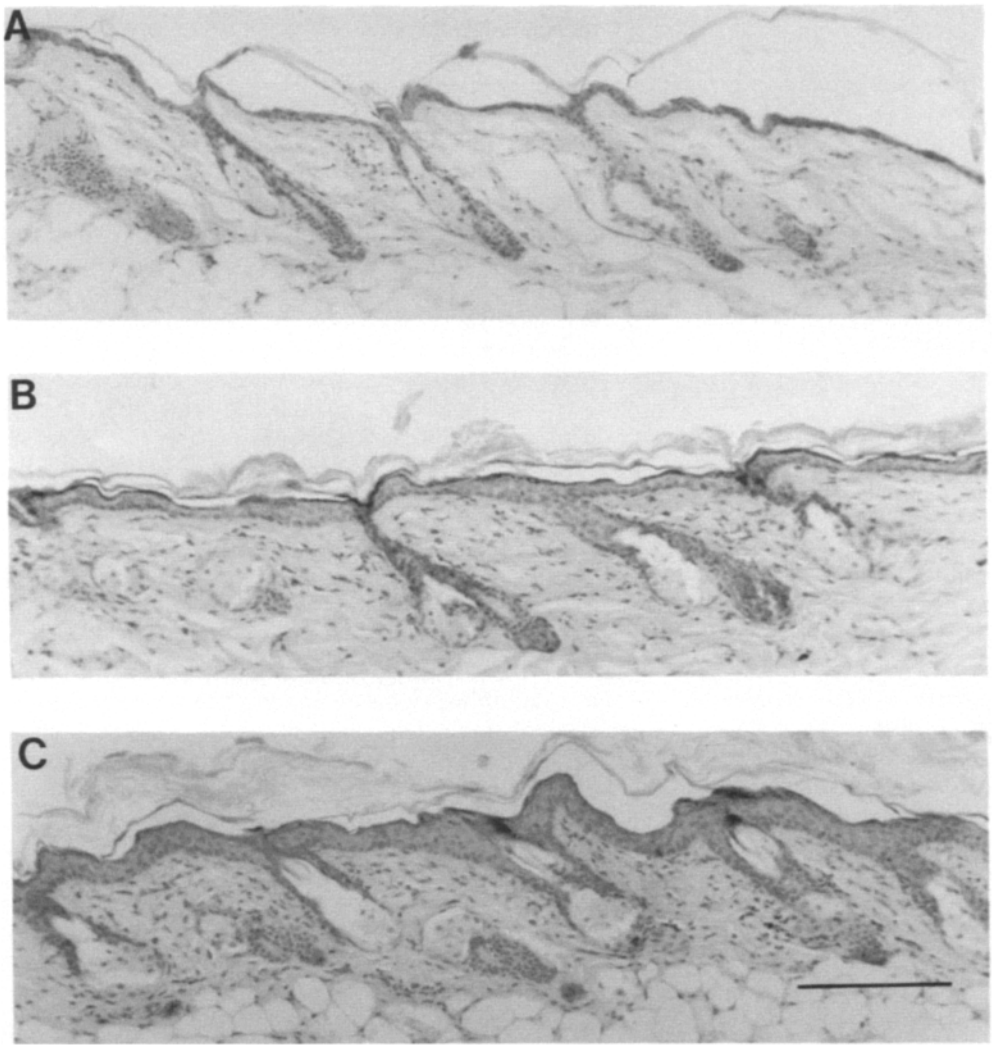

Figure 8. In vivo response of transgenic mice to acute TPA treatment. A group of six *Ela*-negative and six *Ela*-positive littermates (*1* mo old) were shaved and 24 h later topically treated with TPA (5×10^{-5} M, 400 μl, in acetone) and sacrificed 3 d later. Shown is a representative picture of the skin of an *Ela*-positive mouse treated with acetone (*A*) or TPA (*B*) or of an *Ela*-negative sibling treated with TPA (*C*). Bar, 152 μm.

of the *Ela*-positive animals showed only regions of mild hyperplasia, which alternated with others where the hyperplastic response was totally absent (Fig. 8 *B*).

Thus the *Ela*-expression keratinocytes are substantially less responsive to the biological effects of TPA under both in vitro and in vivo conditions.

Tumor Induction in Ela-positive and -negative Mice

Spontaneous skin tumor development has not been detected so far in our *Ela* transgenics (at 15 mo from the beginning of the experiments). Similarly, initiation of mice with DMBA (7,12-Dimethylbenz[a]anthracene) without subsequent tumor promotion elicited no skin tumor development in either *Ela*-positive or -negative mice (Table I).

A complete initiation-promotion protocol with DMBA and TPA resulted in efficient papilloma formation in both positive and negative mice (Table I). There were no significant differences between the two groups in either time of appearance, size, and rate of tumor growth. The numbers of papilloma-bearing animals and of papillomas per mouse were somewhat lower with the *Ela*-positive mice than with the negative controls. The histological characteristics of the papillomas obtained in the two cases were very similar (data not shown). Only one carcinoma developed so far from one *Ela*-negative mouse (at 7 mo from the beginning of the experiment). We conclude from these studies that expression of the truncated *Ela* NTdl646 gene is unlikely to favor keratinocyte tumor development (at least as tested in the Sencar genetic background), nor does it influence its histological type.

Table I. Chemically Induced Tumors in E1a-positive and E1a-negative Mice

	DMBA*	DMBA + TPA‡	
	Mice with tumors	Mice with papillomas	Papillomas per mouse
E1a-positive	0/15	28/38 {*33/37*}	5.6 {*9.7*}
E1a-negative	0/12	13/13 {*13/13*}	10.8 {*16.1*}

* Mice were exposed to a single dose of DMBA either 1–3 d after birth via intraperitoneal injection (20 μg/injection, as described in Materials and Methods) or at 7 wk of age by topical application (4 μg/200 μl acetone). Duration of the experiment: 9 mo.

‡ Mice were exposed to a single dose of DMBA (4 μg/200 μl acetone) at 7 wk of age followed by biweekly treatments with TPA (10^{-4} M, 200 μl, in acetone). Values are given after 2 and 4 (*brackets*) mo. Tumor size varied significantly between and within individual animals, with no consistent differences observed between the *E1a*-positive and -negative groups.

Discussion

An increasing number of negative growth regulatory genes are being identified which are deleted and/or functionally suppressed in tumor cells (Stanbridge, 1990). The specific consequences of loss of these genes for normal tissue homeostasis and neoplastic development are currently being investigated (Donehower et al., 1992; Lee et al., 1992; Jacks et al., 1992). We have attempted to address this question in mouse skin, by using a transformation-deficient *Ela* mutant (NTdl646) which interferes with a specific set of cell negative regulatory proteins of which p105-Rb, p107, and p60-cyclin A are probably the best characterized ones (as reviewed by Boulanger and Blair, 1991).

Expression of the *Ela* NTdl646 transgene did not lead to detectable alterations of the epidermis while hair follicles were substantially abnormal. The main defect appeared to be an absent or markedly dystrophic IRS, with an impaired function as a guide and mold for the nascent hair shaft. A fine balance must exist between growth of keratinocytes in the bulbar part of the hair follicle and their differentiation and organization into the upper structure. In vivo [^{3}H]thymidine incorporation experiments with *Ela*-positive and -negative siblings, revealed no differences in the number of dividing cells below the Auber's line of the bulb and along the base of the ORS (data not shown). Given this similar growth kinetic, the significantly shorter hair shafts found in the *Ela*-positive mice are probably due to a shorter hair cycle, with follicle cells entering catagen sooner than in the controls.

Maturation of bulbar cells into Huxley's and Henle's layers of the IRS was particularly affected. Immunohistochemical data indicated that expression of the *Ela* transgene, similar to that reported for keratin 5 (Heid et al., 1988), was limited mostly to the bulb and the ORS, while it was rarely found in the IRS (and never in the hair shaft). It has been previously proposed that IRS development depends on interacting ORS signals (Straile, 1962), which could be transmitted through processes (Flugelzellen) connecting cells of the ORS to those of Henle's and Huxley's layers (Clemmensen et al., 1991). Thus, expression of the *Ela* transgene, besides having a direct effect on bulbar cells maturation, may also alter the ability of ORS cells to support IRS formation and function.

Within the same mouse, hair follicle abnormalities showed a very interesting pattern of spatial localization with horizontal stripes and more extensive areas of baldness sharply limited by the midline. Transgenic mice with a striped hair coat phenotype have been previously described, with aberrant keratin expression causing breaking of the hair shaft and cyclic hair loss (Powell and Rogers, 1990). The discrete localization of hair follicle abnormalities found in our mice was independent of the hair cycle and constant over time. This pattern could be explained by parallel spatial variations in *Ela* expression within the skin. This situation would be reminiscent of a recently described strain of transgenic mice characterized by different expression levels of the transgene in clones of genetically identical melanocytes (phenoclones) (Mintz and Bradl, 1991). Similarly, our immunohistochemical data indicate that levels of *Ela* expression are not homogeneous, but differ between neighboring hair follicles and groups of keratinocytes in the epidermis. However, direct measurements of *Ela* RNA expression in transgenics with a strong phenotype failed to reveal any significant differences between well defined areas of "baldness" and neighboring, less affected regions (data not shown). Thus, besides variations in *Ela* expression, the discrete hair follicle abnormalities observed in our transgenics may be due to different *Ela* effects, depending on the lower or higher concentrations of some as yet undefined determinant(s) of hair follicle function.

Very little is known about the molecular signals which control hair follicle formation. A possible role for TGF-β factors has been suggested by immunohistochemical methods which have revealed high concentrations of these molecules in developing hair follicles and surrounding mesenchyme (Heine et al., 1987; Pelton et al., 1991). Transgenic primary keratinocytes expressing the *Ela* NTdl646 gene showed only a partial reduction in TGF-β sensitivity, as measured by in vitro growth inhibition assays. However, *Ela* NTdl646 may interfere significantly with other aspects of TGF-β action, especially in a complex in vivo situation such as that of the hair follicle. Furthermore, stronger interference with TGF-β signaling could occur in vivo due to the higher expression of the *Ela* NTdl646 gene in the intact skin. Besides TGF-β, hair follicle function may be under the control of other diffusible messengers such as cAMP, retinoids and corticosteroids. Expression of the *Ela* NTdl646 gene in established Pam212 keratinocytes can interfere with these agents (Florin-Christensen et al., 1992; unpublished observations) and it may have similar effects in keratinocytes of the skin.

In contrast to the substantial alterations found in hair follicles, very little changes were observed in growth/differentiation of interfollicular epidermal cells under basal conditions. However, their hyperplastic response to acute TPA treatment was significantly reduced, with spatial variations that are reminiscent of those of hair follicle abnormalities. The in vivo findings were corroborated by in vitro studies. Primary keratinocytes derived from the *Ela* transgenics responded normally to the growth/differentiating effects of increased extracellular calcium (data not shown). In contrast, their sensitivity to the effects of TPA was significantly reduced. Future studies will be required to determine which specific aspects of the keratinocyte response to TPA are compromised. The function of the TPA-responsive transcription factor AP-1 will be worth investigating, as the c-*fos* protein, an integral AP-1 component (Chiu et al., 1988; Sassone-Corsi et al., 1988), is highly expressed in the hair follicle (Fisher et al., 1991).

Expression of an *Ela* gene able to interfere with the Rb tumor suppressor protein, did not lead to epidermal hyperplasia, nor increased skin tumor formation, or, if anything, seemed to reduce it. The histological type of chemically induced tumors was similar in the *Ela*-positive and -negative mice, suggesting that the target cell population and its growth-differentiation behavior is not influenced by *Ela* expression. Greenhalgh and Yuspa (1988) reported that carcinogenic conversion of *ras*-transformed papilloma cells is not increased by an intact *Ela* oncogene. Even in our transgenics, assuming that the carcinogenic effects of DMBA are due to activation of the Ha-*ras* gene (Balmain et al., 1984), expression of the truncated *Ela* oncogene does not appear to exert a *ras*-cooperating activity. However, it remains to be tested whether tumor-facilitating effects of this transgene can

be manifested in a genetic background less prone to skin carcinogenesis, such as that of the Balb/c strain (Hennings et al., 1981). In any case, it has to be stressed that many negative regulatory loops may act in vivo to maintain skin homeostasis, some of which are totally unaffected by *Ela* NTdl646 activity. For instance, gap junctional intercellular communication, which is an important mechanism to coordinate control of keratinocyte growth and differentiation (Yamasaki, 1990), is not changed in keratinocytes expressing this truncated oncogene (our own unpublished results).

In conclusion, expression of an *Ela* gene which interferes with known negative growth regulators, such as p105-Rb and p60-cyclin A, does not cause gross abnormalities in keratinocyte growth and differentiation, nor facilitates neoplastic outgrowth. Rather, expression of this gene interferes with the regulatory mechanisms involved in keratinocyte hair follicle maturation. Besides p105/p107/p60, expression of this *Ela* gene may affect other unrelated regulatory proteins, for instance through its intact transactivation domain (domain 3; Moran and Mathews, 1987). An elucidation of the specific interactions involved should prove invaluable to understand how a complex epithelial structure such as the hair follicle is organized and maintained.

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References

Argyris, T. S. 1980. Tumor promotion by abrasion-induced epidermal hyperplasia in the skin of mice. *J. Invest. Dermatol.* 75:360–362.

Bailleul, B., M. A. Surani, S. White, S. C. Barton, K. Brown, M. Blessing, J. Jorcano, and A. Balmain. 1990. Skin hyperkeratosis and papilloma formation in transgenic mice expressing a *ras* oncogene from a suprabasal keratin promoter. *Cell.* 62:697–708.

Balmain, A. 1978. Synthesis of specific proteins in mouse epidermis after treatment with the tumour promoter TPA. *In* Carcinogenesis, 2: Mechanisms of Tumour Promotion and Cocarcinogenesis, T. J. Slaga, A. Sivak, and R. K. Boutwell, editors. Raven Press, New York. 153–172.

Balmain, A., M. Ramsden, G. T. Bowden, and J. Smith. 1984. Activation of the mouse cellular Harvey-ras gene in chemically induced benign skin papillomas. *Nature (Lond.).* 307:658–660.

Barrandon, Y., J. R. Morgan, R. C. Mullingan, and H. Green. 1989. Restoration of growth potential in paraclones of human keratinocytes by a viral oncogene. *Proc. Natl. Acad. Sci. USA.* 86:4102–4106.

Berenblum, I., N. Haran-Ghera, and N. Trainin. 1959. An experimental analysis of the "hair cycle effect" in mouse skin carcinogenesis. *Br. J. Cancer.* 12:402–413.

Bhisey, R. A., B. Iyengar, and S. M. Sirsat. 1982. Effect of the active tumor promoter 12-O-tetradecanoylphorbol-13-acetate on hair follicular growth and development of hair anlage tumors in the mouse skin: a comparison with human adnexal lesions. *J. Cancer Res. Clin. Oncol.* 102:245–252.

Billingham, R. E., and W. K. Silvers. 1967. Studies on the conservation of epidermal specificities of skin and certain mucosas in adult mammals. *J. Exp. Med.* 125:429–446.

Boulanger, P. A., and Blair, G. E. 1991. Expression and interactions of human Adenovirus oncoproteins. *Biochem. J.* 275:281–299.

Cao, L., B. Faha, M. Dembski, L.-H. Tsai, E. Harlow, and N. Dyson. 1992. Independent binding of the retinoblastoma protein and p107 to the transcription factor E2F. *Nature (Lond.).* 355:176–179.

Chellappan, S. P., S. Hiebert, M. Mudryj, J. M. Horowitz, and J. R. Nevins. 1991. The E2F transcription factor is a cellular target for the RB protein. *Cell.* 65:1053–1061.

Chiu, R., W. J. Boyle, J. Meek, T. Smeal, T. Hunter, and M. Karin. 1988. The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription from AP-1 responsive genes. *Cell.* 54:541–552.

Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.

Clemmensen, O. J., B. Hainau, and B. Hansted. 1991. The ultrastructure of the transition zone between specialized cells ("flugelzellen") of Huxley's layer of the inner root sheath and cells of the outer root sheath of the human hair follicle. *Amer. J. Dermatopath.* 13:264–270.

Cotsarelis, G., T.-T. Sun, and R. M. Lavker. 1990. Label-retaining cells reside in the bulge area of the pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell.* 61:1329–1337.

Coulomb, B., C. Lebreton, and L. Dubertret. 1989. Influence of human dermal fibroblasts on epidermalization. *J. Invest. Dermatol.* 92:122–125.

Donehover, L. A., M. Harvey, B. L. Slagle, M. J. McArthur, C. A. Montgomery, Jr., J. S. Butel, and A. Bradley. 1992. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature (Lond.).* 356:215–221.

Ewen, M. E., B. Faha, E. Harlow, and D. M. Livingston. 1992. Interaction of p107 with cyclin A independent of complex formation with vital oncoproteins. *Science (Wash. DC).* 255:85–87.

Filvaroff, E., D. F. Stern, and G. P. Dotto. 1990. Tyrosine phosphorylation is an early event involved in primary keratinocyte differentiation. *Mol. Cell. Biol.* 10:1164–1173.

Fisher, C., M. R. Byers, M. J. Iadarola, and E. A. Powers. 1991. Patterns of epithelial expression of Fos protein suggest important role in the transition from viable to cornified cell during keratinization. *Development (Camb.).* 111:253–258.

Florin-Christensen, M., C. Missero, G. P. Dotto, and J. Florin-Christensen. 1992. The *E1a* gene prevents inhibition of keratinocyte proliferation by dexamethasone. *Exp. Cell Res.* 203:285–288.

Florin-Christensen, M., C. Missero, J. Florin-Christensen, P. Tranque, S. Ramon y Cajal, and G. P. Dotto. 1993. Counteracting effects of *Ela* transformation on cAMP growth inhibition. *Exp. Cell Res.* In press.

Fuchs, E. 1990. Epidermal differentiation: the bare essentials. *J. Cell Biol.* 111:2807–2814.

Fuchs, E., and H. Green. 1980. Changes in keratin gene expression during terminal differentiation of the keratinocyte. *Cell.* 19:1033–1042.

Greenhalg, D. A., and S. H. Yuspa. 1988. Malignant conversion of murine squamos papilloma cell lines by transfection with the *fos* oncogene. *Mol. Carcinog.* 1:134–143.

Heid, H. W., I. Moll, and W. W. Franke. 1988. Patterns of expression of trichochytic and epithelial cytokeratins in mammalian tissues. Human and bovine hair follicles. *Differentiation.* 37:137–157.

Heine, U. I., E. F. Munoz, K. C. Flanders, L. R. Ellingsworth, H.-Y. P. Lam, N. L. Thompson, A. B. Roberts, and M. B. Sporn. 1987. Role of transforming growth factor-beta in the development of the mouse embryo. *J. Cell Biol.* 1105:2861–2876.

Hennings, H., D. Michael, C. Cheng, P. Steinert, K. Holbrook, and S. H. Yuspa. 1980. Calcium regulation of growth and differentiation of mouse epidermal cells in culture. *Cell.* 19:245–254.

Hennings, H., D. Devor, M. L. Wenk, T. J. Slaga, B. Former, N. H. Colburn, G. T. Bowden, K. Elgjo, and S. H. Yuspa. 1981. Comparison of two-stage epidermal carcinogenesis initiated by 7,12-dimethylbenz(a)anthracene or N-methyl-N′-nitro-N-nitrosoguanidine in newborn and adult SENCAR and BALB/c mice. *Cancer Res.* 41:773–779.

Hirai, Y., K. Takebe, M. Takashina, S. Kobayashi, and M. Takeichi. 1992. Epimorphin: a mesenchymal protein essential for epithelial morphogenesis. *Cell.* 69:471–481.

Hogan, B., F. Costantini, and E. Lacy. 1985. Manipulating the Mouse Embryo: A Laboratory Manual. Cold Spring Laboratory Press, Cold Spring Harbor, NY.

Inaba, M., J. Anthony, and C. McKinstry. 1979. Histologic study of the regeneration of axillary hair after removal with subcutaneous tissue shaver. *J. Invest. Dermatol.* 72:224–231.

Jacks, T., A. Fazeli, E. M. Schmitt, R. T. Bronson, M. A. Goodell, and R. A. Weinberg. 1992. Effects of an Rb mutation in the mouse. *Nature (Lond.).* 359:295–300.

Koike, K., S. H. Hinrichs, K. J. Isselbacher, and G. Jay. 1989. Transgenic mouse model for human gastric carcinoma. *Proc. Natl. Acad. Sci. USA.* 86: 5615–5619.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

Lee, E. Y.-H. P., C.-Y. Chang, N. Hu, Y.-C.J. Wang, C.-C. Lai, K. Herrup, W.-H. Lee, and A. Bradley. 1992. Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. *Nature (Lond.).* 359: 288–294.

Lenoir, M-C., B. Z. Bernard, G. Pautrat, M. Darmon, and B. Shroot. 1988. Outer root sheath cells of human hair follicle are able to regenerate a fully differentiated epidermis in vitro. *Dev. Biol.* 130:610–620.

Lersch, R., V. Stellmach, C. Stocks, G. Giudice, and E. Fuchs. 1989. Isolation, sequence and expression of a human keratin K5 gene: transcriptional regulation of keratins and insights into pairwise control. *Mol. Cell. Biol.* 9:3685–3697.

Limat, A., D. Breitkreutz, T. Hunziker, C. Boillat, U. Wiesmann, E. Klein, F. Noser, and N. E. Fusenig. 1991. Restoration of the epidermal phenotype by follicular outer root sheath cells in recombinant culture with dermal

fibroblasts. *Exp. Cell. Res.* 194:218–227.
Mintz, B., and M. Bradl. 1991. Mosaic expression of a tyrosinase fusion gene in albino mice yields a heritable striped coat color pattern to transgenic homozygotes. *Proc. Natl. Acad. Sci. USA.* 88:9643–9647.
Missero, C., E. Filvaroff, and G. P. Dotto. 1991*a*. Induction of TGF-β1 resistance by the *E1a* oncogene requires binding to a specific set of cellular proteins. *Proc. Natl. Acad. Sci. USA.* 88:3489–3493.
Missero, C., S. Ramon y Cajal, and G. P. Dotto. 1991*b*. Escape from TGF-β control and oncogene cooperation in skin tumor development. *Proc. Natl. Acad. Sci. USA.* 88:9613–9617.
Moll, R., W. W. Franke, and D. L. Schiller. 1982. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell.* 31:11–24.
Moran, E., and M. Mathews. 1987. Multiple functional domains in the Adenovirus *E1a* gene. *Cell.* 48:177–178.
Mudryj, M., S. H. Devoto, S. W. Hiebert, T. Hunter, J. Pines, and J. R. Nevins. 1991. Cell cycle regulation of the E2F transcription factor involves an interaction with cyclin A. *Cell.* 65:1243–1251.
Nevins, J. R. 1992. E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. *Science (Wash. DC).* 258:424–429.
Oliver, R. F. 1966. Whisker growth after removal of the dermal papilla and lengths of follicle in the hooded rat. *J. Embryol. Exp. Morphol.* 15:331–347.
Oliver, R. F., and C. A. B. Jahoda. 1988. The dermal papilla and maintenance of hair growth. *In* The Biology of Wool and Hair. G. E. Rogers, P. J. Reis, K. A. Ward, and R. C. Marshall, editors. Chapman and Hall, London, 51–67.
Pelton, R. W., B. Saxena, M. Jones, H. L. Moses, and L. I. Gold. 1991. Immunohistochemical localization of TGF-β1, TGF-β2, and TGF-β3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development. *J. Cell Biol.* 115:1091–1105.
Pietenpol, J. A., R. W. Stein, E. Moran, P. Yaciuk, R. Schlegel, R. M. Lyons, M. R. Pittelkow, K. Munger, P. M. Howley, and H. L. Moses. 1990. TGF-β inhibition of c-myc transcription and growth in keratinocytes is abrogated by viral transforming proteins with pRB binding domains. *Cell.* 61:777–785.
Powell, B. C., and G. E. Rogers. 1990. Cyclic hair-loss and regrowth in transgenic mice overexpressing an intermediate filament gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:1485–1493.
Reiners, J. J., Jr., and T. J. Slaga. 1983. Effects of tumor promoters on the rate and commitment to terminal differentiation of subpopulation of murine keratinocytes. *Cell.* 32:247–255.
Rogers, G. E., M. J. Fietz, and A. Fratini. 1991. Trichohyalin and matrix proteins. *Ann. N Y Acad. Sci.* 642:64–81.
Saiag, P., B. Coulomb, C. Lebreton, E. Bell, and L. Dubertret. 1985. Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model in vitro. *Science (Wash. DC).* 230:669–672.
Sassone-Corsi, P., W. W. Lamph, M. Kamps, and I. M. Verma. 1988. *fos*-associated cellular p39 is related to nuclear transcriptional factor AP-1. *Cell.* 54:553–560.
Schweizer, J., and F. Marks. 1977. Induction of the formation of new hair follicles in mouse tail epidermis by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res.* 37:4195–4201.
Southern, P. J., and P. Berg. 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early promoter. *J. Mol. Appl. Genet.* 1:327–341.
Stanbridge, E. J. 1990. Human tumor suppressor genes. *Annu. Rev. Genet.* 24:615–657.
Steinert, P., and S. H. Yuspa. 1978. Biochemical evidence for keratinization by mouse epidermal cells in culture. *Science (Wash. DC).* 200:1491–1493.
Stenn, K. S., A. G. Messenger, and H. P. Baden. 1991. The Molecular and Structural Biology of Hair. *Ann N Y Acad. Sci.* 642:1–519.
Straile, W. E. 1962. Possible functions of the external root sheath during growth of the hair follicle. *J. Exp. Zool.* 150:207–223.
Tso, J. Y., X.-H. Sun, T.-H. Kao, K. S. Reece, and R. Wu. 1985. Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. *Nucleic Acid. Res.* 13:2485–2501.
Vassar, R., and E. Fuchs. 1991. Transgenic mice provide new insights into the role of TGF-α during epidermal development and differentiation. *Genes Dev.* 5:714–727.
Whyte, P., N. M. Williamson, and E. Harlow. 1989. Two regions of the Adenovirus early region 1A proteins are required for transformation. *Cell.* 56:67–75.
Wilson, J. B., W. Weinberg, R. Johnson, S. Yuspa, and A. J. Levine. 1990. Expression of the BNLF-1 oncogene of Epstein-Barr virus in the skin of transgenic mice induces hyperplasia and aberrant expression of keratin 6. *Cell.* 61:1315–1327.
Yamasaki, H. 1990. Gap junctional intercellular communication and carcinogenesis. *Carcinogenesis.* 11:1051–1058.
Yuspa, S. H., P. Hawley-Nelson, B. Koeehler, and J. R. Stanley. 1980. A survey of transformation markers in differentiating epidermal cell lines in culture. *Cancer Res.* 40:4694–4703.
Yuspa, S. H., T. Ben, H. Hennings, and U. Lichti. 1982. Divergent responses in epidermal basal cells exposed to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res.* 42:2344–2349.