Plakoglobin Suppresses Epithelial Proliferation and Hair Growth In Vivo

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Abstract. Plakoglobin regulates cell adhesion by providing a modulatable connection between both classical and desmosomal cadherins and their respective cytoskeletal linker proteins. Both plakoglobin and the related protein β-catenin are posttranscriptionally upregulated in response to Wnt-1 in cultured cells. Uregulation of β-catenin has been implicated in potentiating hyperproliferation and tumor formation. To investigate the role of plakoglobin in these functions we expressed a full-length (PG) and an NH2-terminally truncated form of plakoglobin (ΔN 80PG) in mouse epidermis and hair follicles, tissues which undergo continuous and easily observed postnatal renewal and remodeling. Expression of these constructs results in stunted hair growth, a phenotype that has also been observed in transgenic mice expressing Wnt3 and Dvl2 (Miller et al., 1999). Hair follicles from PG and ΔN 80PG mice show premature termination of the growth phase (anagen) of the hair cycle, an event that is regulated in part by FG F5 (H ebert et al., 1994). The proliferative rate of the epidermal cells was reduced and apoptotic changes, which are associated with entry into the regressive phase of the hair follicle cycle (catagen), occurred earlier than usual.

Key words: plakoglobin • β-catenin • Wnt • cadherin • proliferation

Introduction

Plakoglobin, β-catenin, and Drosophila Armadillo are closely related multifunctional proteins that regulate cell adhesion and participate in signal transduction cascades. All three proteins provide modulatory links in a chain of proteins that connect cadherin cell adhesion molecules to the actin filaments of adherens junctions. Plakoglobin differs from β-catenin and Armadillo in its additional role in desmosomes, where it binds strongly to desmosomal cadherins and weakly to desmoplakin and intermediate filaments (for reviews, see Cowin and Burke, 1996; Kowalczyk et al., 1997; Smith and Fuchs, 1998; Cowin, 1999).

Plakoglobin, β-catenin, and Armadillo are also found in cytoplasmic and nuclear complexes that integrate signals from Wnt/wingless (wg)1 proto-oncogenes and the adenomatous polyposis coli (APC) tumor suppressor protein to direct cell fate and govern aspects of cell proliferation (Miller and Moon, 1996; Ben-Ze’ev and Geiger, 1998; Gumbiner, 1998; Cowin, 1999). By analogy to the wg pathway in flies, one model for vertebrate Wnt signaling posits that secreted Wnts bind to specific members of the Frizzled transmembrane receptor family, resulting in recruitment of cytosolic disheveled (Dvl) proteins to the plasma membrane and inactivation of glycogen synthase kinase (GSK-3β; Bhayot et al., 1996; Wang et al., 1996; He et al., 1997). GSK-3β normally acts as part of a protein complex that promotes a series of posttranslational modifications that target cytoplasmic β-catenin for proteosomal degradation (Rubinfeld et al., 1996; Yost et al., 1996; Berle et al., 1997; Orford et al., 1997; Zeng et al., 1997). Thus, Wnt inactivation of GSK-3β causes β-catenin to accumulate, translocate to the nucleus, bind to Tcf/Lef transcription factors and regulate target genes such as c-myc and cyclin D (Funayama et al., 1995; Karnovsky and Klymkowsky, 1995; Love et al., 1995; Behrens et al., 1996; Molenaar et al., 1996; Van de Wetering et al., 1997; Bauer et al., 1998; Cavallo et al., 1998; He et al., 1998; Roose et al., 1998; Altz and Bier, 1998; Tetsu and McCormick, 1999).}

Experiments in rodent mammary and neuroepithelial cells have shown that Wnt-1 expression upregulates both plakoglobin and β-catenin (Bradley et al., 1993; Hinck et al., 1994). In addition, injection of either β-catenin or plakoglobin mRNA into Xenopus embryos results

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1Abbreviations used in this paper: βG, β-globin sequence; APC, adenomatous polyposis coli; Dvl, disheveled; K14, keratin 14; KGM, keratinocyte growth medium; TDR, [3H]thymidine; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; TX-100, Triton X-100; wg, Wnt/wingless.
in the identical Wnt-1 phenotype, axis duplication (M cM a-
phon and M oon, 1989; M C rea et al., 1993; K arnovsky and 
K lymkowsky, 1995). These results implicate both plako-
globin and β-catenin as effectors of Wnt signals. Whether 
plakoglobin directly transduces Wnt signals or acts to 
modulate β-catenin's functionality is currently an area 
of debate (M erriam et al., 1997; M iller and M oon, 1997; W il-
liams et al., 1998). The late embryonic-lethal phenotype 
of plakoglobin null mice and the inability of endogenous pla-
koglobin to rescue the early embryonic-lethal phenotype of 
β-catenin null mice suggest that plakoglobin does not 
play a significant role in Wnt pathways governing early de-
velopment (H aegel et al., 1995; B ierkamp et al., 1996). 
However, the participation of plakoglobin as a mediator of 
Wnts in tissues that undergo significant postnatal develop-
ment and renewal has not been investigated. Formation of 
the epidermal appendages of hair, feathers, and mammary 
gland are therefore excellent models in which to study this.

In adults, inappropriate activation of elements in this 
type of Wnt signaling cascade has been linked to a number 
of cancers and several studies implicate β-catenin in this 
process. Mutations in the β-catenin gene that result in a 
stabilized protein product occur in colonic, gastric, hepato-
cellular, and hair follicle tumors and melanomas (M un-
eto et al., 1995; R ubinfeld et al., 1997; C han et al., 1999).

Overexpression of β-catenin in vivo increases the prolifer-
ative rate of crypt cells and induces polyp formation in in-
testine and induces hair follicle formation and benign tu-
mors in skin (G at et al., 1998; W ong et al., 1998; H arada et 
al., 1999). The role of plakoglobin in proliferation and can-
cer is less well documented. However, several facts suggest 
that plakoglobin may act as a tumor suppressor. For exam-
ple, the plakoglobin gene lies in the 17q-21 locus, which 
is subject to loss of heterozygosity in human breast tumors 
(A berle et al., 1995). Plakoglobin is absent in a number of 
tumor cell lines, and experimentally induced overexpress-
ion of plakoglobin in highly transformed cells decreases 
their tumorigenicity (N avarro et al., 1991; S ommers et al., 
1994; A berle et al., 1995; S imcha et al., 1996).

To address the questions of whether plakoglobin acts to 
transmit Wnt signals in adult tissues, and whether it pro-
motes or suppresses proliferation and tumor formation in 
normal cells, we expressed two forms of plakoglobin in the 
basal cells of mouse epidermis and hair follicles under the 
control of the keratin 14 (K 14) promoter. Our results show 
that even modest expression of these transgenes reduces 
the proliferative potential of the epidermal cells and sig-
nificantly decreases the length of the growth phase of the 
hair follicle cycle, shortening hairs by 30%. This effect sug-
gests that plakoglobin participates in the transduction of 
growth-suppressive signals to normal epithelial cells.

Materials and Methods

Plasmids and Transgene

Flag-tagged human plakoglobin cDNA (p163/7-PG) was produced by 
PCR using hPG Ca 2.1 as template (G enBank accession number Z 68228; 
F ranke et al., 1989). 20 cycles of standard PCR conditions of 1 min 
at 94°C, 1 min at 54°C, and 3 min at 72°C were carried out with forward oli-
gonucleotide (5'-ccggagatctagccgagctcaagcagcagctgagtggactcaggtca- 
caaagttggataagccctgg-3'), which contains an E co r1 re-
striction site, K ozac's sequence, a sequence encoding the Flag epitope 
(M D Y K D D D D D K) and the first five amino acids of hPG, and reverse oli-
gonucleotide (5'-ccggaattctCTAGGCCAGCATGTGGTC-3') that con-
tains the Eco r1 restriction site at the last 18 bases of the hPG Ca 2.1 cod-
ing sequence including the stop codon. The PCR product was subcloned into the E co r1 restriction site of the p163/7 expression vector (N iehrs et 
al., 1992) containing the M HC class I H-Z promoter, a polylinker embed-
ded in rabbit β-globin sequence (βG; last 20 bp of exon 2, entire intron 2 
and the polylinker dividing exon 3) and both βG and SV-40 polyadenyla-
tion signals, generating p163/7-PG. The transgene construct, pK14-PG-
Flag-PG (see Fig. 1) was generated by introducing a B amH1 linker into the 
NotI site of the p163/7 polylinker downstream of Flag-hPG. This per-
mitted excision and insertion of a 2,935-bp B amH1 fragment containing 5'- 
Flag sequence and Flag-hPG into the B amH1 site of a keratin 14 cassette, 
which was kindly provided to us by Dr. E laine Fuchs (U niversity of Chig-
ago; see Fig. 1).

A cDNA encoding a myc-tagged stabilized form of plakoglobin deleted in 
the first 80 amino acids was generated by PCR. A 531-bp DNA frag-
ment was amplified using hPG Ca 2.1 as template and forward oligonucleo-
tide (5'-ctcggcccctgagatctagctcggcggatccgagctcaagcagcagctgagtggact-
caaagttggataagccctgg-3') and reverse oligonucleotide (5'-GCTGAG- 
CATGGCACCGAGACG-3') which contains NotI, B amH1, and E co r1 re-
striction sites, K ozac's sequence. The PCR product was subcloned into 
the multiple cloning site, K ozac's sequence, the Flag epitope (M E Q K L I S E E D L) and amino acids 
81-87 of hPG, and the reverse oligonucleotide B17 Rev (5'-GCTGAG- 
CATGGCACCGAGACG-3') encompassing the internal Splh at base 
698 of hPG Ca 2.1. The 863-bp NotI-Splh fragment of hPG Ca 2.1 was re-
placed by the 519-bp NotI-Splh PCR fragment to generate pBS-ANPG 
from which the 3,192-bp EcoRI fragment was excised and subcloned into 
pBS-ANPG generating p163/7-PG. A 2,786-bp B amH1 fragment contain-
ing βG-ANPG was excised from the latter construct and subcloned into the 
keraatin 14 transgene vector generating pK14-ANPG. The PCR 
generated regions and newly generated cloning sites of all clones were fully 
sequenced by the dyeoxy termination method (S anger et al., 1977).

Plasmid D NA was prepared by alkaline lysis followed by double band-
ing on CSCI gels. The K14-PG and K14-ANPG transgenes (see Fig. 1) 
were excised from the pGE M 3Z backbone with K pln, which cuts in the 
5' polylinker and N del, which cuts at base 2,083 within the K 14 3' non-
coding sequence, leaving a 327-bp segment after the polyadenylation sig-
nal provided by the hGH sequences.

Southern Blot Analysis of Genomic DNAs

Genomic D NA was prepared by digesting 0.5 cm of mouse tail in 10 mM 
Tris-HCl (pH 8.0), 1 mM EDTA, 20 mM N aci, 1% SDS, and 1 mg/ml of 
proteinase K for 12 h at 35°C followed by two ethanol precipitations.

For Southern blot analysis, 10-20 μg of genomic DNA samples were digested 
with 80 units of B amH1 and separated by electrophoresis in an 1× T AE - 
0.8% agarose gel at 3 V/cm. A titer UV cleavage, denaturation, and neu-
tralization, samples were transferred to Nytran membranes (S chleicher and 
Schuell). Blots were prehybridized for 2 h at 68°C in a solution of 6× 
SSC, 1% SDS, 1× D enhardt's, and 125 μg/ml of denatured IR NA and 
hybridized for 12 h in the same solution followed by washes in 0.1× SSC, 
α-32P-plakoglobin cDNA probe, specific activity 109 cpm/μg (ICN). Blots 
were washed twice in 2× SSC, 0.1% SDS, for 20 min each at 68°C and 
exposed for 12 h to PhosphorImager screens (M olemecular Dynamics).

Transfection, Immunoprecipitation, and Western 
Blot Analysis

Human kidney cells transformed with SV-40 T-antigen (293T) were main-
tained, transfected, and immunoprecipitated as described (W itch et al., 
1996). Forty 30-μm cryosections of tail from 25-4 F1 transgenic and nor-
mal sex-matched littermates were boiled in 200 μl of 25 mM Tris-HCl, pH 
7.4, 4 mM EDTA, 150 mM N aCl, 1% SDS, 10 μg/ml aprotinin, and 1 mM 
PM SF and diluted in 1 ml of 2% Triton X-100 (TX-100) in 50 mM Tris-
HCl, pH 7.4, 1 mM EDTA, 150 mM NaCl, and 10 μg/ml aprotinin, 1 mM 
PM SF. Insoluble debris was pelleted for 15 min at 10,000 g and processed 
for immunoprecipitation as described (W itch et al., 1996). Protein 
concentration was determined by the method of Bradford using a B CA 
(bichinonic acid) protein assay kit (Pierce). Protein samples and immu-
noprecipitations were separated by electrophoresis through 7.5% SDS-
PAGE, electrophoretically transferred to Protran membranes (S chleicher 
and Shuell) and blocked by incubation in PBS containing 0.1% T ween 20 
and 5% nonfat dried milk. The blots were incubated with the following 
mouse monoclonal antibodies: M2 anti-flag (S igna) 9E 10 anti-myc (a 
kind gift of Dr. H arold V armus, N IH, Bethesda, M D), PG5172 anti-plako-
globin (C owin et al., 1986); anti-β-catenin, anti-G S K-3β, and anti-E-cadherin
permitted luciferase and HaCaT keratinocytes (Boukamp et al., 1988) were transfected with one same extracts according to the manufacturer's instructions (Promega).

Sequential Detergent Extraction of Epidermal Proteins

Primary keratinocytes were grown to confluence on 60-mm dishes under low Ca\(^{2+}\) conditions (0.05 mM) to maintain a basal cell phenotype then switched to high (2 mM) Ca\(^{2+}\) for 24 h to permit cell junction formation (Hennings and Holbrook, 1983). Cellular proteins were extracted sequentially in saponin buffer (0.01% saponin, 10 mM Tris, pH 7.5, 140 mM NaCl, 5 mM EDTA, 2 mM EDTA, 1 mM PM SF, and 10 \(\mu\)g/ml aprotinin) and then in TX-100 buffer (1% TX-100, 10 mM Tris-HCl, pH 7.5, 140 mM NaCl, 5 mM EDTA, 2 mM EDTA, 1 mM PM SF, and 10 \(\mu\)g/ml aprotinin) as described (Paik and Green, 1997), with the exception that extracts were centrifuged for 1 h at 100,000 g.

Pulse–Chase

100-mm dishes of transgenic keratinocytes grown as described above were starved for 1 h in methionine/cysteine-free DME then metabolically labeled with methionine/cysteine-free DME containing 0.5 mM \(\text{H}_{2}\text{O}_{2}\)-methionine/cysteine/ml for 20 min. The cultures were rinsed with DME containing 1,000-fold excess of cold methionine and incubated in this medium for the time periods indicated. The cells were harvested, boiled in 1% SDS, and immunoprecipitated as described above.

Determination of Single and Double Pulse-labeled Keratinocytes

The method was carried out as described (Lehrer et al., 1998). 4-d-old mice were injected subcutaneously with 50 \(\mu\)g of BrdU per gram body weight. A 1-hr 24-h chase period, long enough for the BrdU-labeled cells to traverse into a second S phase, the same animals were injected with 10 \(\mu\)Ci/g of [\(\text{H}\)]thymidine (Tdr) and killed 60 min later. Backskin was removed, fixed in 70% ethanol, 30 mM glycine for 24 h, embedded in paraffin and sectioned at 5 \(\mu\)m. A short deparaffinification and rehydration, tissue sections were processed for immunochromogenic staining with anti-BrdU antibody and alkaline phosphatase-conjugated goat anti-mouse IgG (5-bromo-\(\text{Z}-\text{deoxy-uridine labeling and detection kit I, Boehringer Mannheim Corp.}, followed by visualization with the Vector\(\text{R}\) Red alkaline phosphatase substrate kit I (Vector Laboratories Inc.) according to the manufacturer’s instructions. Slides were air dried overnight, coated in nuclear track emulsion (Ilford K-2; Ilford) diluted 1:3 in distilled water at 40°C, air dried again for 2 h, and exposed for 14 d at 4°C in light tight boxes containing antihydrous CaSO\(_4\)(Ammond Dri Ziel). A urotitograms were developed as described previously (Lehrer et al., 1998). Slides were lightly counterstained with hematoxylin. All slides were examined under oil immersion (\(\times\)63) with a Zeiss Axiophot light microscope. A cell was considered double labeled if it contained greater than five silver grains per nucleus over a bright nuclear pattern of red staining. The epidermal labeling index was determined by counting the number of \(\text{H}^{-}\text{Tdr}\)-stained nuclei per 1,000 basal nuclei. The percentage of BrdU-labeled cells that were additionally labeled with \(\text{H}^{-}\text{Tdr}\) was also determined (Lehrer et al., 1998).

Terminal Deoxynucleotidyl Transferase-mediated \(\text{dUTP}\) Nick End Labeling Analysis

The terminal deoxynucleotidyl transferase-mediated \(\text{dUTP}\) nick end labeling (TUNEL) analysis was performed on 5-\(\mu\)m formalin fixed paraffin-embedded mouse backskin sections using the Promega Fluorosecin A pop- tosis Detection System.

Results

Generation of Transgenic Mice

Previous studies on the signaling function of \(\beta\)-catenin employed full-length and NH\(_2\)-terminally deleted forms of...
Figure 1. PG and ΔN80PG transgenes. (a) Luciferase reporter assays. 293T cells and HaCaT keratinocyte cultures were transfected with 1 μg pTOPFLASH (Top) or 1 μg pFOPFLASH (Fop) 1 μg CMV-Lac Z with or without 2 μg PG (P), ΔN80PG (Δ), Lef-1 (L) cloned in pCDNA3 or pCDNA3 (V). Luciferase activity expressed in light units represents an average of three samples, with standard deviations shown in bars after correction for transfection efficiency as measured by β-galactosidase activity. (b) Diagram of the K14-PG and K14-ΔN80PG transgene constructs comprising the K14 promoter (K14P), hGH intron/exon/polyadenylation sequences (int/ex pA) and K14 3’ non-coding sequence. Sequence encoding the NH2- and COOH-terminal domains and 13 central armadillo (arm) repeats of the PG and ΔN80PG cDNA are represented by shaded rectangles. B, BamHI; E, EcoRI; K, KpnI; N, NolI; Nde, NdeI. Southern blot detection of K14-PG and K14-ΔN80PG transgenes in the genome of transgenic lines hybridized with the 32P-labeled PG insert and mouse engrailed gene (en) fragments. Lanes marked 100 and 10 show respective copy equivalents of PG and ΔN80PG cDNA are represented by shaded rectangles. B, BamHI; E, EcoRI; K, KpnI; N, NolI; Nde, NdeI. Lanes marked nos. 21, 24, 4, 9, and 45 show DNA from these respective transgenic mouse lines. Expression of the transgene products detected by indirect immunofluorescence. Frozen sections of 5-d (c) or newborn tail skin (d) and 5-d backskin (e–f) stained with anti-flag (c) or anti-myc (d–f) antibody detect the PG (c) and ΔN80PG (d–f) transgene products. Strongest staining is seen at the intercellular borders of the basal layer of transgenic epidermis and continuous layers of the outer root sheath (ORS) of developing hair follicles (c–d) where the K14 promoter is active. Note the horseshoe staining of the basal cells due to absence of reaction on hemidesmosomes (arrows). Staining, representing the persistence of the transgene product in progeny of former K14-expressing cells, is also observed in suprabasal layers of the epidermis (c–d) and in cells within the hair follicle bulbs lying next to the dermal papilla (DP) (e–f) where the K14 promoter is not active.

b-catenin, which show enhanced stability (Munemitsu et al., 1995). We therefore constructed analogous cDNAs encoding an epitope-tagged full-length (PG) and NH2-terminally deleted (ΔN80PG) form of plakoglobin and tested if the products of these constructs retained transcriptional activity by performing a luciferase reporter assay (Van de Wetering et al., 1997). The products of both plakoglobin constructs transactivated ~100–200-fold a Topflash luciferase reporter in the presence of a putative transcriptional partner Lef-1 (Fig. 1 a). This effect was observed in both 293T cells that make few intercellular junctions and HaCaT keratinocytes that contain many desmosomes (Boukamp et al., 1988). Low levels of transactivation ~10-fold of the Topflash reporter occurred in the absence of Lef-1 and presumably resulted from activation of endogenous Lef/Tcf transcriptional partners. Neither form of plakoglobin transactivated
the control Fopflash luciferase reporter construct which is mutated in the Tcf/lef-responsive elements (Fig. 1 a).

To test the potential role of plakoglobin in regulating cell proliferation, in vivo, we employed a K14 transgene cassette, kindly provided to us by Dr. Elaine Fuchs, which directs expression of transgenes under the control of the K14 promoter to the basal layer of the interfollicular epidermis and the outer root sheath of hair follicles (Guo et al., 1993; Vassar et al., 1989). Both full-length K14-PG and NH₂-terminally deleted K14-ΔN80PG (Fig. 1 b) were used to generate transgenic mice in the Swiss Webster strain.

Two mice (nos. 21 and 24) tested positive by Southern analysis of tail genomic DNA for integration of the K14-PG transgene and gave Mendelian transmission to the F1 generation. Seven mice tested positive for integration of the K14-ΔN80PG transgene. Of these, nos. 1, 4, 45, 46 showed Mendelian transmission of the transgene to the F1 generation, nos. 5 and 9 showed <10% transmission, suggesting mosaicism, and no. 31 gave 75% transmission to the F1 progeny, suggesting that the transgene inserted at multiple sites in the genome. Immunofluorescence detection of the epitope tags on frozen sections of tail epidermis indicated that expression of the transgene protein products occurred in one K14-PG founder (no. 21) and three K14-ΔN80PG founders (nos. 4, 9, and 45). Significantly only these four mice displayed hair phenotypes. Their F1 progeny were studied further in the experiments described below.

Expression of the ΔN80PG Transgene

Southern analysis of tail genomic DNA from F1 mice with probes derived from plakoglobin cDNA and a fragment of the engrailed gene showed that lines nos. 21, 4, 9, and 45

![Figure 2. Expression of the transgene products detected by indirect immunofluorescence. Frozen sections of tail skin from newborn sex-matched ΔN80PG transgenic (a–d) and control (a’ and d’) F1 littermates. A ntt-myc antibody detects the transgene product in transgenic skin (a and c) but gives no reaction on normal skin (a’ and c’). Strong staining is seen at the intercellular borders of the basal layer of transgenic epidermis and contiguous outer root sheath (ORS) of the hair follicle (a and c) where the K14 promoter is active. Note the horseshoe staining of the basal cells due to absence of reaction on hemidesmosomes (arrows). Staining representing the persistence of the transgene product in progeny of former K14-expressing cells is observed in suprabasal layers of the epidermis (a and c) where the K14 promoter is not active. Note that the cellular localization of endogenous plakoglobin (b and b’), detected with an antibody H-80 directed against NH₂-terminal epitopes not present in the transgene, or of endogenous β-catenin (d and d’) is the same in transgenic (b and d) skin as in normal skin (b’ and d’). DP, dermal papilla.](image-url)
carried 50, 40, 10, and 15 copies, respectively, of the transgene, based on normalization and calibration to a series of titrated plasmid controls (Fig. 1 b). Immunofluorescence microscopy with anti-flag and anti-myc antibody on frozen sections of F1 tails detected the epitope tags in transgenic epidermis (Fig. 1, c and d) but not normal littermate epidermis showing the strongest expression within the basal layer. Staining was most obvious at the intercellular borders of keratinocytes reflecting the distribution of desmosomes and adherens junctions but was absent from the basal borders where the cells contact the basal lamina via hemidesmosomes (see arrows in Fig. 1, c and d). In transgenic mice from all four lines, expression was also seen strongly in outer root sheath cells of the hair follicles (Figs. 1 d and 2 c). Staining was also observed in layers of the epidermis and hair follicle, which do not express the K14 promoter. For example, staining was seen in suprabasal epidermal cells (Figs. 1 d and 2, a and c), and in epithelial cells of the hair follicle bulb including those surrounding the dermal papilla (Fig. 1, e and f). This likely reflects per-
sistence of the transgene protein product in progeny of previously K14 positive stem cells. Weak staining was seen in the cytoplasm of all positive cells, nuclear localization was not generally observed in the transgenic tissue sections.

It has been argued in other systems that plakoglobin signaling may be an indirect result of competitive displacement of $\beta$-catenin from the membrane. To address this possibility we compared the localization of endogenous $\beta$-catenin plakoglobin in transgenic (Fig. 2, a–d) and normal skin (Fig. 2, a′–d′). Staining for endogenous plakoglobin with an NH$_2$-terminal antibody (Fig. 2, b and b′) and for endogenous $\beta$-catenin with a COOH-terminal antibody (Fig. 2, d and d′) showed essentially similar patterns in transgenic (Fig. 2, b and d) and normal (Fig. 2, b′ and d′) epidermis and hair follicles.

Examination of mouse skin under the electron microscope showed that transgenic skin contained desmosomes with normal appearance and frequency demonstrating that the transgene expression had no deleterious effect on formation or stability of these cell junctions (Fig. 3, a and b). Immunofluorescent staining of primary keratinocyte cultures from the epidermis of transgenic mice showed strong localization of the epitope tag at cell–cell borders and faint cytoplasmic staining (Fig. 3 c). Strong reaction was also observed in the nucleus of some but not all transgenic cultured keratinocytes (Fig. 3 c). These staining patterns were specific for transgenic keratinocytes and were not observed in cells derived from normal littermates (Fig. 3 c′). As predicted from previous studies, which showed that the first 80 amino acids of plakoglobin are dispensable for its interactions with all known partners (desmoglein, desmocollin, E-cadherin, $\alpha$-catenin, APC, and Lef/Tcf), no perturbations were seen in the patterns of localization of several major junctional proteins such as desmoplakins, $\beta$-catenin, endogenous plakoglobin or E-cadherin (Fig. 3, d–f and d′–f′) (for review, see Cowin and Burke, 1996).

Confirmation that the epitopes recognized by the antibodies in the immunofluorescence assays above represented the PG and $\Delta N$80PG products encoded by the transgene was obtained by cross-blotting anti-plakoglobin immunoprecipitates with anti-flag or anti-myc antibody (Fig. 4 a). In each case, a protein of the appropriate mass, ~86 kD for PG and 75 kD for $\Delta N$80PG, could be detected in transgenic animals (lanes 2, 4, 6, and 8) but not in their normal littermates (lanes 1, 3, 5, and 7). Primary keratinocytes from normal and transgenic F1 mice were cultured under low Ca$^{2+}$ conditions, which enrich for basal cells and hence for cells expressing the K14 promoter (Hennings et al., 1980; D'otto, 1998). After 2–3 d in culture the cells were switched for 12 h to high Ca$^{2+}$ to permit cell-junction formation (Hennings and Holbrook, 1983; D'otto, 1998). Under these conditions, 100% of the cells express the transgene product as judged by immunofluorescence microscopy. Equal amounts of protein from normal and transgenic keratinocytes, as judged by protein estimation, were processed for immunoblotting. Protein loading was controlled by monitoring the expression level of ribophorin, a resident protein of the endoplasmic reticulum (Fig. 4 b). The level of transgene expression was found to be ~50% that of the endogenous protein in line no. 4. No significant downregulation of the endogenous pool of plakoglobin or $\beta$-catenin was observed in the transgenic keratinocytes when compared with normal keratinocytes.
partitioning of els in transgenics (Fig. 4 b). To determine the biochemical 
However, we observed a slight increase in E-cadherin lev-
quentially extracted first in saponin and then in TX-100 
fractions as the endogenous plakoglobin. No differences were de-
product 
chased for the hours indicated above the lanes. Note that in pri-
epidermal keratinocytes, its stability relative to full-length 
expression of endogenous pla-
D 
D 
D 
D 
N80PG keratinocytes were metabolically 
time span during which the first two highly synchro-
Transgenic PG and 
interaction of endogenous plakoglobin or β-catenin among these 
subcellular fractions when normal mice were compared 
with transgenics (Fig. 4 c).

In other studies, NH2-terminally deleted ΔN89 forms of β-catenin have been shown to have increased stability due to 
removal of critical phosphoserines that are required for 
targeting for degradation (M.unemitsu et al., 1995). In 
 transient expression experiments in 293T cells, we consist-
tently saw higher steady-state expression of ΔN80PG than 
PG, suggesting that removal of this analogous domain may 
increase the steady-state level of plakoglobin in a similar 

**Figure 4. Western blot analysis of PG and ΔN80PG protein ex-
pression in skin and keratinocytes.** (a) Proteins from tail skin of 
25-d sex-matched F1 normal mice (N) nos. 4, 9, 45, 21 (lanes 1, 3, 
5, and 7, respectively), and their transgenic littermates (lanes 2, 4, 
6, and 8, respectively) were immunoprecipitated then immuno-
botted with anti-myc (lanes 1-6) and anti-flag (lanes 7 and 8) an-
tibodies. The arrowheads denote the migration of the 86-kD PG 
and 75-kD ΔN80PG and transgene products. (b) Equal amounts 
of total proteins, extracted from primary keratinocytes were blot-
ted with anti-ribophorin antibody as a loading control; anti-pla-
Antibodies used to detect the endogenous proteins and anti-myc antibody to 
de- 

**Transgenic Mice Have Stunted Hair Growth**

The striking feature that distinguished transgenic animals of 
PG line no. 21 and all three ΔN80PG lines from their 
normal littermates was a pronounced short hair phenotype giving the mice a less fluffy appearance to their coat and a 
pronounced pink hue (Fig. 5, a and b). Three founders (nos. 21, 4, and 45) and the 50% of their offspring that car-
ried the transgene had dramatically shorter hair uniformly 
throughout their pelt. Founder no. 9, however, showed 
both short and long hair in banded and patchy fashion but 
the 10% of F1 progeny from this line that were transgenic 
showed the uniform short hair phenotype. These observa-
tions are consistent with the presence of the transgene 
causing the shortening of the hair and with founder no. 9 
having mosaic expression of the transgene within its epi-
dermis.

Transgenic F1 mice had a taut facial appearance and 
prominent ears, features resulting in part from the short-
ness of the hair tufts found abundantly in the cheeks and 
around the ears of normal mice. It was possible to accu-
ately identify transgenic mice from day 11 on the basis of 
coat morphology. The short hair phenotype was most ob-
vious once the coat was well formed, between days 15-40, 
the time span during which the first two highly synchro-
nized hair cycles occur in the mouse. A dull transgenic 
mice remained distinguishable from wild-type littermates, 
having a close-cut smooth coat, although the phenotype 
became less obvious in the older mice probably due to the
asynchrony of subsequent hair cycles. Heterozygous and homozygous transgenic mice showed no increase in morbidity or mortality or impairment of fertility up to one year of age. Heterozygous and homozygous pups often looked smaller than their normal littermates, but, as there was no consistent correlation in weight differences between these groups, this observation reflected a difference in coat appearance. The identical phenotype elicited by both the full-length and the NH₂-terminally deleted form of plakoglobin argues strongly that the latter acts as an active form of plakoglobin and not by interfering with the function of endogenous plakoglobin.

Analysis of Hair

Hairs plucked from the heads of 25-d sex-matched F1 transgenic and control littermates were examined under a dissecting microscope. We first asked whether the appearance of the transgenic mice resulted from lack of the longer outer coat hairs. The percentage of each of three major types of hair (guard, awl, and zigzag), however, were represented to the same degree in both the transgenic animals and their normal littermates. All hair types of the transgenic mice showed a striking reduction in size, being 30–40% shorter than hairs of normal littermates of the same age (Fig. 5 c and Table 1). The root and tapered ends of the hairs were clearly visible, ruling out the possibility that the hairs were shorter due to fragility and breakage. In zigzag hairs, all four segments were present but each segment was shorter in the transgenic than in the wild-type hairs. Moreover, the segments became progressively more affected, with segments that formed first being reduced in length by ~20% and those formed last being reduced by ~50%. By scanning electron microscopy, the structure of the cuticle of the coat hairs appeared normal. However, transgenic hairs were composed of ~20% fewer cells than normal hairs and the cells were 14% smaller in diameter. No significant differences were found between the length of hairs in the different lines, suggesting that the transgene dose was irrelevant beyond a critical threshold. This was also suggested by the fact that homozygotes could not be phenotypically distinguished from heterozygotes.

Mechanistic Studies: PG and ΔN80PG Expression Results in Early Withdrawal from the Growing Phase (Anagen) of the Hair Follicle Cycle, Reduction in Cycling Cells, Premature Apoptosis, and Follicle Degeneration but Not from Changes in the Differentiation Program

Histology. To search for a basis for the unusual length of hairs from the transgenic mice, we examined skin from the mid-back region of two to three pairs of normal and transgenic littermates from each line at multiple time intervals throughout the first 41 d encompassing the first two hair cycles. Up to day 11 there was no discernible difference in the histological appearance of the transgenic epidermis or primordial follicles from those of normal littermates. The length and width of the follicular bulb, number, spacing,
and angling of the hair follicles was similar and in cross-
sections of epidermis the follicle density appeared identi-
cal in both groups. However, remarkable differences were
observed during later stages of anagen. In this strain of
mice (Swiss Webster) anagen begins late in gestation
$\sim \text{E17}$ and peaks around day 14 as estimated by follicle
length (see below). At day 13 normal mouse skin shows
the classical features of the anagen phase of the hair cycle
with the epithelial component of the follicle bulb contain-
ing large numbers of cells in mitosis (see arrows in Fig. 6
a). In contrast, by day 13 transgenic hair follicles have al-
ready entered into catagen and display large numbers of
involuting follicles trailing compact dermal papilla (Fig. 6
b). A result the dermis of the transgenic skin becomes
much thinner than the normal. Hair follicles from normal
skin do not enter catagen until day 15 (Fig. 6 c) at which
time the follicles of the transgenic skin have already fully
regressed and entered the quiescent telogen phase (Fig. 6
d). Follicle lengths were measured, from the base of the
follicle bulb to the epidermis, to quantify the first two hair
cycles (Fig. 7). A similar but more attenuated scenario is
seen in the second cycle. Transgenic mice enter the second
cycle slightly ahead of their normal counterparts but again
leave earlier (Fig. 7). All transgenic lines showed this
trend.

**Proliferation.** To determine the effects of $\Delta N80PG$
expression on cellular proliferation in epidermis and hair fol-
licles we undertook a double (pulse) labeling procedure.
Double (pulse) labeling gives information both on the
number of cells cycling at any one time and the number of
cells that have undergone two rounds of DNA synthesis
(Lehrer et al., 1998). Mice were first injected with BrdU
then 24 h later injected with $\text{H}^{-}\text{TdR}$. A statistically signif-
icient decrease was observed in the number of labeled cells
in the transgenic group of mice (8.9 $\pm$ 6 4.5) as compared
with their wild-type littermates (11.6 $\pm$ 6 3.1; $P < 0.001$

### Table I. Lengths (mm) ± Standard Deviations of Hairs

| Line   | No. of mice | Awl N ± SD  | Guard N ± SD | Zigzag N ± SD |
|--------|-------------|-------------|--------------|--------------|
| $\Delta NPG$ no. 4 | 6 | 5.6 ± 0.43  | 8.1 ± 0.99   | 5.0 ± 0.32   |
| $\Delta NPG$ no. 9 | 6 | 5.7 ± 0.46  | 8.4 ± 0.93   | 4.9 ± 0.45   |
| $\Delta NPG$ no. 45 | 3 | 5.5 ± 0.42  | 8.2 ± 1.16   | 4.9 ± 0.37   |
| PG no. 21 | 3 | 5.5 ± 0.34  | 7.7 ± 0.58   | 4.9 ± 0.34   |

Pelage hairs of three (line nos. 45 and 21) to six (lines nos. 4 and 9) pairs of 25-d sex-matched F1 normal littermate (N) and transgenic mice (TG). Values represent means ± standard deviations of the total lengths of five hairs of guard type and ten hairs of awl and zigzag types from 3–6 mice. The $P$ value was $< 0.001$ for awl and zigzag hairs and $0.002 < P < 0.01$ for guard hairs. Each type of hair from transgenic mice was 30–40% shorter compared with the normal littersmates.
cells within the inner root sheath of the hair follicle. A apoptotic nuclei were not observed in normal matrix keratino-cytes of the hair follicle until day 15. The earliest signs of catagen became obvious. TUNEL positive nuclei reached a maximum at day 17 and were clustered around the dermal papilla cells next to the hair matrix and in the bulge isthmus. These results are similar to previous observa-tions on normal epidermis (Lindner et al., 1997). In contrast we observed a small number of apoptotic nuclei at day 11 in the inner root sheath of the transgenic hair folli-cles, a few apoptotic nuclei were seen in the matrix keratino-cytes at day 13 reaching a peak at day 15. A apoptotic changes occur two days earlier in the transgenic than in normal hair follicles, with a time course in both groups that reflects the stage of involution. In both groups apop-totic nuclei first appear in the early stages of catagen as the lower portion of the follicles begin to degenerate and reach a maximum at mid catagen. Thus, in both groups apoptotic changes detected by TUNEL accompany but do not precede the changes observed histologically in the hair folli-cles.

Differentiation. Changes in K10 and K16 expression have recently been reported to lead to altered prolifera-tion potential of keratinocytes and hair follicles in vivo and in vitro (Paladini and Coulombe, 1998; Paramio et al., 1999). To determine whether the transgenic mice exhib-it changes in the normal differentiation program of the epidermis or hair follicles frozen sections of epidermis were stained with a panel of antibodies. These included antibodies against: keratins 5 and 14, which are synthe-sized in the basal layers; keratin 1, which is expressed in the suprabasal layers; keratin 6, which is expressed under conditions of hyperproliferation; high sulfur hair proteins, which are restricted to cells of the hair cuticle and loricrin which is expressed in the granular layer. No detectable changes were observed in response to transgene expres-sion (Fig. 10).

Discussion

Our results show that modest expression of either PG or a truncated ΔN80 form of plakoglobin in the basal cells of the epidermis and outer root sheath of the hair follicle stunts hair growth. The principal findings are that hair folli-cles of these mice leave anagen prematurely, prolifera-tion of epidermal and hair follicle keratinocytes is reduced and apoptotic changes occur earlier than usual. These re-sults are consistent with the hypothesis that plakoglobin regulates aspects of cell proliferation as well as cell adhe-sion in normal cells in vivo and raises the interesting possi-bility that plakoglobin may assist in coordinating these cel-lular functions.

The identity of the PG and ΔN80 phenotype argues that the latter acts as an active rather than dominant nega-tive form of plakoglobin. This is reminiscent of the situa-tion with β-catenin where the full-length and NH₂-termi-nally deleted forms act similarly in many experimental signaling assays. The lack of dose dependency in the phe-notype between four independent transgenic lines strongly suggests that plakoglobin exerts its full effect on prolifera-tion at a critical threshold and that expression above that threshold is irrelevant. Such a feature suggests that there

where the number [n] of mice in each group = 10. Moreover a highly significant difference was seen in the number of cells undergoing two S phases within a 24-h period (Fig. 8). In transgenic epidermis, 8.5 ± 4.5% of cells initially la-beled with BrdU were also labeled with 3H-TdR indicating they had undergone two rounds of DNA synthesis as com-pared with 13.4 ± 4.8% of cells in normal epidermis (P < 

0.001 where n = 10 mice in each group). Thus, a significant decrease in cycling cells results from ΔN80PG expression.

Apoptosis. The factors governing the extremely rapid and synchronous involu-tion of hair follicles during catagen are poorly understood, but are thought to involve an apoptotic pathway (Lindner et al., 1997). We therefore investigated whether the early involution observed by day 13 in the hair follicles of mice expressing ΔN80PG results from or is associated with premature onset of such a mecha-nism. TUNEL analysis was performed to detect DNA fragmentation as a marker of apoptotic nuclei in sections of three pairs of transgenic and normal littermate epidermis. Representative pictures are shown (Fig. 9). In normal epidermis, no apoptotic nuclei were observed until day 13 and at this stage they were restricted to a few clusters of cells within the inner root sheath of the hair follicle.
are a limited number of binding sites to be occupied on downstream effectors. This would be expected if plakoglobin is acting, in a manner similar, or perhaps antagonistic to the transcriptional role played by its close relative β-catenin, by saturating the binding sites on promoters of a limited set of target genes. That plakoglobin is capable of this type of role is suggested by its ability to transactivate a luciferase reporter gene. This transactivation capability was observed even in keratinocytes, which make numerous desmosomes that sequester the majority of plakoglobin at the membrane.

**Plakoglobin and Proliferation**

Follicle stem cells reside in the bulge, a structure that lies at the bottom of the permanent part of the hair follicle and is continuous with the outer root sheath and basal layer of epidermis (Cotsarelis et al., 1990; Morris and Potten, 1999). Bulge stem cells have been postulated to respond to signals from the closely adjacent telogen dermal papilla by transiently proliferating and producing a population of transit amplifying cells that migrate downwards to regenerate the hair follicle (Cotsarelis et al., 1990; Sun et al., 1991). Thus progenitor cells residing in the epidermal basal layer and in the bulge structure and their immediate transit amplifying progeny express the K14 promoter and the transgene product. The principal effect of plakoglobin expression is seen in the hair follicles and hairs rather than the epidermis. This can be explained by the fact that follicular cells divide at six times the rate of epidermal cells (12 h for follicular keratinocytes versus 3 d for epidermal keratinocytes), and so factors that regulate proliferation will be more apparent in these structures (Lehrer et al., 1998). It should be noted, however, that the proliferative capacity of the basal epidermal keratinocytes was also reduced. Due to the much slower cell cycle time a morphological effect on the epidermis was not observed. However, we noted that the transgenic mice had taut skin suggesting that the transgene effect on the proliferative rate of epidermal cells might have restricted the surface area of the epidermis rather than the vertical thickness, which in haired animals consists essentially of only 2–3 layers of cells. The reduction in both the single labeling as well as in the double labeling index demonstrates that the transgene expression has reduced the number of transit amplifying cells cycling at any given time and slowed their rate of proliferation (Lehrer et al., 1998). We propose that this reduction in proliferative capacity of transit amplifying cells limits their capacity to regenerate full size hair follicles or full-length hairs, which is seen most obviously at later stages of anagen due to the exponential nature of the proliferative process. This is entirely consistent with studies showing that overexpression of plakoglobin reduced the tumorigenicity and growth rate of highly transformed cell lines (Simcha et al., 1996). Our results extend these observations to show that even modest levels of expression of either full-length or NH2-terminally deleted forms of plakoglobin restricts the proliferative potential of normal cells in vivo. This suggests that negative regulation of proliferation may be a generalized function of plakoglobin. These results contrast strikingly to the effects of overexpressing ΔN89-β-catenin in the same cells under the
same promoter (Gat et al., 1998). Expression of a ΔN89-β-catenin induces de novo hair follicles in mature haired skin, suggesting that it may cause epidermal cells to retain pluripotent stem cell potential and promote their ability to proliferate in response to unknown factors, present in haired regions of skin, that induce hair follicle formation (Gat et al., 1998). Furthermore, ΔN89-β-catenin expression eventually induces two types of follicular tumor in epidermis (Chan et al., 1999; Gat et al., 1998). Plakoglobin appears therefore to act in the opposite fashion to ΔN89-β-catenin by restricting the hair follicle's potential for proliferation.

ΔN80PG Does Not Act by Downregulating β-Catenin

Two studies have suggested that plakoglobin expression modulates β-catenin levels and that plakoglobin’s signaling capabilities relate to this action. In Xenopus, plakoglobin expression was proposed to sequester APC and, thus, protect β-catenin from degradation and promote its signaling function (Miller and Moon, 1997). This is clearly not the case in epidermis, as full-length plakoglobin and ΔN80PG do not produce a β-catenin phenotype. In the second study, plakoglobin overexpression in HT1080 cells downregulated β-catenin levels, and was hypothesized to do this by competitively displacing β-catenin from the low

Figure 9. Apoptotic changes detected by TUNEL staining occur earlier in the matrix cells of transgenic hair follicles. Paraffin embedded sections from the backskin of K14-ΔN80PG and control littermates were subjected to TUNEL staining to determine DNA fragmentation during apoptotic cell death. No apoptotic nuclei are seen in the matrix keratinocytes of the wild-type day 13 hair follicles which are at the peak of anagen. In transgenic littermates of the same age, the hair follicles are in catagen and a few apoptotic nuclei can be seen (arrows). Of day 15 wild-type hair follicles begin to enter catagen and a few apoptotic nuclei are found close to the trailing dermal papilla. Day 15 transgenic hair follicles are in the last stages of catagen and contain many apoptotic nuclei. Apoptotic nuclei reach a maximum by day 15 in the transgenic and by day 17 in normal mice. Bar, 25 μm.
levels of cadherin present in these cells thereby exposing the \( \beta \)-catenin to the cytosolic degradation machinery (Salamon et al., 1997). However, in keratinocytes \( \Delta N80PG \) does not appreciably decrease the levels of endogenous \( \beta \)-catenin or plakoglobin leading us to conclude that the effect of \( \Delta N80PG \) on proliferation does not involve down-regulation of \( \beta \)-catenin. This raises the possibility that plakoglobin and \( \Delta N80PG \) may antagonize \( \beta \)-catenin's ability to transactivate/derepress genes such as \( \text{myc} \) and \( \text{cyclin D} \) that are involved in growth promotion, and/or modulate the cell's response to \( \beta \)-catenin signaling by stimulating intersecting cadherin and/or growth factor mediated pathways at the membrane.

Plakoglobin and Wnt

The dramatic difference between the effects of plakoglobin/\( \Delta N80PG \) and \( \Delta N89-\beta \)-catenin expression raises the question as to which of these Armadillo homologues is the downstream effector of Wnts in epidermis and its appendages. This is a complex issue as many Wnt genes (Wnt-3, 4, 7a, 10a, 10b and 11) as well as proteins involved in Wnt signal transduction (Dvl-1, 2, Lef-1, and Tcf-3) have been described in embryonic or adult epidermis and its appendages (Bitgood and McMahon, 1995; Cygan et al., 1997; Gat et al., 1998; Millar, 1997; Millar et al., 1999; Wang and Shackleford, 1996). Some of these Wnts are known, in other systems, to act through alternative and possibly antagonistic pathways, and Wnt cascades are subject to a highly complex system of negative regulation at each step (van Genderen et al., 1994; Wong et al., 1994; Du et al., 1995; Zhou et al., 1995; Zhou et al., 1995; Katochwil et al., 1996; Brown and Moon, 1998). Significantly, the K14-PG and K14-\( \Delta N80PG \) mice show a strikingly similar phenotype to that observed in mice expressing K14-Wnt-3 or K14-Dvl-2 (see Millar et al., 1999; Fig. 4). Specifically, all four mice display an identical short hair phenotype. K14-Wnt-3 mice display additional cyclical balding that is not seen in K14-Dvl-2, K14-PG, or K14-\( \Delta N80PG \) mice. This additional phenotype results from paracrine activation of Wnt cascades in more distant cells of the hair follicle that produce hair shaft structural defects. The short hair phenotype, however, that is common to all four mice, is independent of this process, and, as Dvl2, PG, and \( \Delta N80PG \) are, by their cytoplasmic nature, restricted to the cells that express them must result from an initial effect on cells of the outer root sheath. Taken together this suggests that plakoglobin may function downstream of Dvl-2 in an endogenous Wnt pathway operating in outer root sheath cells, a cell type that has previously been shown to play a role in limiting hair growth (Hbert et al., 1994). While premature withdrawal from anagen and changes in proliferative rate were not observed in the K14-Wnt-3 or K14-Dvl-2 mice it is not clear that a sufficient sample size of mice were investigated in order to detect the subtle changes seen in the K14-PG mice (Millar et al., 1999).

Figure 10. Expression of differentiation-specific markers in epidermis of transgenic compared with normal mice. 5-\( \mu m \) frozen sections of newborn tail skin (a, b, a' and b') or 7-d backskin (c-e and c'-e') from sex-matched normal and K14-\( \Delta N80PG \) transgenic F1 littermates were stained with anti-K1 (K1) and anti-K5 (K5); anti-desmoplakin (DP), anti-loricrin (LOR), anti-K6 (K6) and anti-high sulphur protein (HS). Note that the differentiation markers tested are expressed normally in the transgenic epidermis. Bar: (a’-b) 30 \( \mu m \); (c’-e) 50 \( \mu m \).
Potential Effects of ΔN80PG at the Plasma Membrane

Our EM, immunofluorescence and biochemical studies found no evidence that cell junctions were deleteriously affected by expression of PG or ΔN80PG. However, as the transgene products are found in all three subcellular fractions of transgenic keratinocytes, we cannot rule out that plakoglobin may additionally, or alternatively, exert its effects via enhancing the function of a transmembrane or cytoskeletal partner protein. Cytokeratin 10 and 16 have been shown to lead to changes in keratinocyte proliferation and reduced number of hair follicles (Paladini and Coulombe, 1998; Paramio et al., 1999). No significant changes were seen in the patterns of intermediate filament proteins ruling out their possible involvement in the plakoglobin phenotype. No changes were seen in desmosomes or in the expression levels of junctional proteins beyond a slight upregulation of E-cadherin again suggesting that the plakoglobin phenotype does not result from a deleterious effect on cell adhesion. The marginal changes in E-cadherin expression may contribute to the proliferative and apoptotic changes that are elements of the plakoglobin phenotype: E-cadherin has been shown to decrease cell proliferation (St. Croix et al., 1998). However, E-cadherin has been variously reported to promote and prevent apoptosis (Wong et al., 1998; Carmeieit et al., 1999; Hermsmon et al., 1996; Pece et al., 1999). Moreover, genetic experiments in flies show that apoptosis is induced by the transcriptional functions rather than the adhesive functions of Armadillo (A hamed et al., 1998). Transgenic expression of several cytokines and growth factors in skin produce shortened hair as part of their phenotype and both EGF and FGF5 are known to regulate the timing of the end of anagen (Vassar and Fuchs, 1991; Turksen et al., 1992; Hebert et al., 1994; Murillas et al., 1995). Both plakoglobin and β-catenin are known to bind to and be substrates for the EGF receptor (Hoschuetzky et al., 1994). However, the EGF receptor is only transiently expressed during the early stages of mouse hair development and the plakoglobin phenotype is persistent a connection between these proteins is unlikely to account for the plakoglobin phenotype (Vassar and Fuchs, 1991). There is no reported connection between plakoglobin and FGF5 expression. However, as cadherins have been shown to stimulate FGF5 activity in other systems (Doherty et al., 1991; Doherty and Walsh, 1996), the possibility that plakoglobin enhancement of E-cadherin expression might simulate an FGF5 pathway must be considered. Development of ΔN80PG mice and their derived keratinocytes now permits these potential models to be tested both genetically and biochemically.

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