Links between signal transduction, transcription and adhesion in epithelial bud development

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Abstract

The morphogenesis of organs as diverse as lungs, teeth and hair follicles is initiated by a downgrowth from a layer of epithelial stem cells[1,2]. During follicular morphogenesis, stem cells form this bud structure by changing their polarity and cell–cell contacts. Here we show that this process is achieved through simultaneous receipt of two external signals: a Wnt protein to stabilize β-catenin, and a bone morphogenetic protein (BMP) inhibitor to produce Lef1. β-Catenin then binds to, and activates, Lef1 transcription complexes that appear to act uncharacteristically by downregulating the gene encoding E-cadherin, an important component of polarity and intercellular adhesion. When either signal is missing, functional Lef1 complexes are not made, and *E-cadherin* downregulation and follicle morphogenesis are impaired. In *Drosophila*, E-cadherin can influence the plane of cell division and cytoskeletal dynamics[3]. Consistent with this notion, we show that forced elevation of E-cadherin levels block invagination and follicle production. Our findings reveal an intricate molecular programme that links two extracellular signalling pathways to the formation of a nuclear transcription factor that acts on target genes to remodel cellular junctions and permit follicle formation.

During skin development, signals from adjacent epithelial and mesenchymal cells instruct select ectodermal cells to form hair follicle buds. In turn, each bud signals to a small group of underlying mesenchymal cells to condense[1,2]. Once the bud proliferates to form a larger bulb (matrix), it encases this dermal condensate (papilla), and further differentiates into the cells of the hair shaft (Fig. 1a)[1]. Recent evidence suggests that Wnt signalling is involved in this process at a time that correlates with bud-specific patterns of upregulation of P-cadherin and downregulation of E-cadherin[4–9]. Cadherins form the transmembrane core of adherens junctions (AJs) by bridging to α-catenin and the cytoskeleton through β-catenin, a protein which on its own is prone to degradation[10,11]. β-Catenin's degradation machinery is transiently suppressed by Wnt signalling. This renders β-catenin a new-found stability and function, binding to and activating members of the Lef1/Tcf family of DNA binding proteins[11–13]. Whether there is underlying functional significance to β-catenin's link between adhesion and transcription is an issue that we now address.

Wnts are expressed in ectodermal buds[14,15], and are prime candidates to stabilize β-catenin at these sites. We confirmed this by testing the ability of the canonical skin Wnt3a to generate nuclear β-catenin in mouse keratinocytes. Keratinocytes exposed to Wnt3a-conditioned media displayed an ~7 times increase in β-catenin, as judged by immunoblot and densitometry analysis (Fig. 1b). This increase was paralleled by accumulation of β-catenin in ~85 ± 5% of the nuclei of treated cells (Fig. 1c).

Wnt-treated cultures did not express appreciable Lef1, suggesting the need for additional signalling molecules to induce a DNA binding protein for β-catenin activation. As bud formation *in vivo* requires a mesenchymal cue[1], we searched for candidates expressed by developing dermal condensates. Epithelial cells and mesenchymal cells within follicle buds express BMP2 and BMP4 (refs 16, [17]), while only mesenchymal cells express their inhibitor, noggin[18]. Polymerase chain reaction with reverse transcription (RT–PCR) and western analyses revealed that *in vitro*, keratinocytes express BMPs, but not noggin (Fig. 1d). When exposed to noggin-conditioned media, Lef1 was induced, and localized to the nucleus (Fig. 1b, c).

Together, Wnt and noggin promoted transactivation of the *TOPFLASH* reporter gene, which regulated luciferase expression through Lef/Tcf binding sites in the *TOP* promoter[12] (Fig. 1e). When the Tcf/Lef1 binding sites were mutated (*FOPFLASH*), promoter activity was abolished, as it was when noggin was absent and BMPs were present. Additionally, although some stabilization of cytoplasmic β-catenin occurred naturally in keratinocyte cultures, transactivation was clearly enhanced by Wnt3a. Thus, *in vitro*, noggin and Wnt3a appeared to act in concert to generate transcriptionally competent Lef1 complexes.

Noggin and Wnts also appeared to be essential for this process *in vivo* (Fig. 1f). In skin from embryonic day (E) 16.5, nuclear Lef1 and β-catenin could be seen in most follicles except for the mature guard follicles developing at E13.5 independently of Lef1. Lef1-positive hair buds also expressed β-galactosidase under the control of the *TOP* promoter[7]. In contrast, *noggin*-null (*Nog*^{−/−}) mice lacked two-thirds of E16.5 buds[18], and those that formed exhibited cytoplasmic β-catenin and little or no Lef1 or TOPGAL activity (Fig. 1f). When *Nog*^{−/−}/*TOPGAL* mice were mated on a background of *K14-Lef1*[5] to force Lef1 expression, nuclear β-catenin and *TOPGAL* expression were restored in ~80% of the E16.5 buds (Fig. 1f). This said, follicles were not restored to wild-type levels, indicating that some of the altered genes and morphology associated with *Nog*^{−/−} follicles are not under control of Lef1/β-catenin.

When cells of embryonic epidermis (epi) reorient to form an epithelial bud, they switch from E-cadherin to P-cadherin based AJs (Fig. 2a, b). This timing coincided with Lef1 and *TOPGAL* activation (Fig. 2c, d). At the mesenchymal–epithelial interface, the cadherin switch persisted throughout follicle development, and was still in Lef1-positive, stem cell progeny of adult follicles (Fig. 2e–g). As in buds, adult hair precursor cells were not only E-cadherin dim and P-cadherin bright, but also active for nuclear Lef1, β-catenin and *TOPGAL*[7,19]. These results were interesting in light of findings by Shimamura *et al.*[20], who noted that in developing mouse brain, E-cadherin was absent wherever Wnt-1 was expressed. A correlation between reduced E-cadherin and elevated nuclear β-catenin has also been observed *in vitro* and in human cancers[21].

In situ hybridization revealed that the cadherin switch was regulated at the messenger RNA level (Fig. 2h, i). Both *cadherin* promoters harbour multiple sequence motifs corresponding to the optimal Lef1/Tcf binding site, and the *E-cadherin* promoter has been shown to bind Lef1[22]. Although P-cadherin appeared unaffected by a *Nog*-null or *Lef1*-null background, *E-cadherin* mRNA and protein failed to be downregulated (Fig. 2j–m), a feature which was restored when mice were bred on the *K14-Lef1* background (Fig. 2n). These data reveal the *E-cadherin* gene as a candidate for negative repression by noggin-induced Lef1.

To further assess the extent to which *E-cadherin* downregulation correlated specifically with follicle downgrowth, we analysed E16.5 skin null for *sonic hedgehog* (*Shh*), which when absent impairs follicle development soon after Lef1/β-catenin activation[14]. Arrested *Shh*^{−/−} hair buds were positive for P-cadherin, and suppressed E-cadherin at both protein (shown) and mRNA

(not shown) levels (Fig. 2o, p). Taken together, these data place *noggin* involvement earlier than *Shh*, and specifically required for *E-cadherin* downregulation.

A priori, the negative effects of Lef1 on *E-cadherin* mRNA expression could be independent of β-catenin, a view consistent with how Tcf/Lef family members are known to repress target genes[12]. To test this possibility, we examined E-cadherin expression in skin from a mouse expressing a constitutively stable β-catenin. In interfollicular epidermis, the transgene (*K14ΔNβ-catenin*) elicits *de novo* follicle-like downgrowths[6], which display evidence of Wnt-responsive gene transactivation on a *TOPGAL* background[7]. E-cadherin downregulation was consistently observed at Lef1-positive sites of *K14ΔNβ-catenin* induced epithelial invaginations (Fig. 2q, r). A complementary study on *Wnt-1*-null mice reported a broadening of the E-cadherin pattern in developing brain tissue[20]. Our findings now uncover a dual and atypical importance of both stabilized β-catenin and Lef1 in repressing *E-cadherin* expression.

To begin to address whether the changes we observed in *E-cadherin* mRNA expression are reflected at the transcriptional level, we first cloned 6.5 kilobases (kb) of 5′ murine *E-cadherin* gene sequence, and engineered a mutation in the promoter that had previously been shown by gel shift assays to bind recombinant Lef1[22]. When tested in primary mouse keratinocytes, both wild-type and mutant promoters yielded comparable β-galactosidase reporter gene expression, indicating that the Lef1/Tcf site is not required for promoter activity (Fig. 3a).

Transfection of either stable β-catenin or Lef1 on their own did not appreciably affect the activity of the *E-cadherin* promoter. In contrast, Lef1 and stabilized β-catenin in combination markedly suppressed *E-cadherin* promoter activity in a manner dependent upon the Lef1/Tcf binding site (Fig. 3a). Similar repression was observed when noggin and Wnt 3a together were added to the keratinocyte cultures (Fig. 3b).

To test whether Wnt-activated Lef1 complexes bind to the endogenous *E-cadherin* promoter, we conducted chromatin immunoprecipitation analyses (ChIP) on Lef1-expressing keratinocytes. Only when these cells were exposed to Wnt3A were we able to specifically precipitate 120-base-pair (bp) chromatin fragments of the E-cadherin promoter containing the Lef1 binding site (Fig. 3c). In contrast to the repressive effects on the E-cadherin promoter, Wnt and noggin together activated the murine *HK1-hair keratin* promoter, previously identified as a bona fide Lef1/β-catenin responsive target in keratinocytes (Fig. 3d)[19].

Our findings suggest that Lef1's ability to function as repressor and activator in the same Wnt-treated cells depends upon the context of responsive promoter elements. Located 3′ from the Lef1 motif in the *E-cadherin* promoter is an E-box sequence, which in various cell lines serves as the binding and regulatory site for the Snail family of transcriptional repressors[23,24]. Mutation of the Lef1 binding site did not interfere with Snail's ability to repress the *E-cadherin* promoter (Fig. 3a). Moreover, these two sites together functioned additively to repress wild-type *E-cadherin* promoter activity by up to 70% of its normal activity in keratinocytes (Fig. 3a).

Despite the presence of functional *in vitro* binding sites for Snail and Lef1, the *E-cadherin* promoter might still be regulated *in vivo* by indirect pathways involving these factors. Irrespective of mechanism, the independent repressor action of Snail was intriguing given that the large guard hair follicles that develop on both the *noggin*-null and *Lef1*-null genetic backgrounds still exhibited *E-cadherin* downregulation (not shown). These data reveal *E-cadherin* down-regulation as a common thread among the waves of follicle morphogenesis and expose the existence of multiple mechanisms to govern this downregulation.

To test the functional importance of E-cadherin downregulation in hair follicle morphogenesis, we engineered transgenic mice expressing elevated levels of an epitope-tagged E-cadherin. Previous studies have shown that the addition of a carboxy-terminal tag does not interfere with E-cadherin's ability to form intercellular junctions[25]. Several of the newborn animals harbouring the *K14-Ecad-herin-HA* transgene were sickly, and were killed shortly after birth. Immunofluorescence analyses of frozen newborn skin sections revealed a mosaic pattern of anti-HA negative (Fig. 4a) and positive (Fig. 4b) domains. Signs of the characteristic E-cadherin down-regulation were seen in the non-transgenic (Tg −) epithelium, but were not detected in transgene positive (Tg +) areas. Haematoxylin and eosin staining confirmed that the most notable morphological difference between Tg − and Tg + regions was the paucity of hair follicles in Tg + skin (Fig. 4c, d).

Skin conditionally null for *a-catenin* (by *K14-Cre*) also lacked proper AJs[26]. At E16.5, developing placodes were visible (Fig. 4e, f), but they were severely arrested by birth (Fig. 4g, h). Correspondingly, sebaceous glands failed to develop. These findings demonstrate the deleterious consequences of too few as well as too many AJ proteins in hair follicle morphogenesis, and provide compelling evidence that dynamic changes in *AJ* gene expression may be a key step in morphogenesis. In this regard, it seems particularly relevant that either complete loss or overexpression of E-cadherin can also impair formation of intestinal epithelia and mammary glands[27,28]. In contrast, a reduction in AJs is characteristic of epithelial cancers, whose cellular masses bear some resemblance to aberrant bud formation[21]. The extent to which these effects go beyond simple adhesion is at present unknown.

Our findings shed new light on the molecular mechanism underlying the early steps of epithelial bud development, a process that is anticipated to have implications that extend beyond the hair follicle. Inverse correlations between E-cadherin and Wnt signalling have been noted in various tissues and organs, during development and in cancers. In some human epithelial cancers, *E-cadherin* gene mutations seem to promote not only malignant invaginations but also Wnt signalling, as judged by the detection of nuclear β-catenin in the tumour tissue[21]. Our studies now provide evidence for a converse mechanism, namely nuclear β-catenin/Lef1 mediated suppression of E-cadherin expression during normal follicle development. The downregulation of E-cadherin may in turn perpetuate the Wnt signalling pathway by increasing the pool of transcriptionally competent β-catenin[10].

Although Lef/Tcf factors are known to function as repressors, β-catenin involvement is typically associated with converting these proteins from repressors to activators[12]. In this regard, our *in vitro* results on the *E-cadherin* promoter are surprising, as they suggest a role for β-catenin in mediating the repressive effects of Lef1 on the *E-cadherin* promoter. One possibility is that the atypical context of the Lef1 binding site in the *E-cadherin* promoter may recruit other cofactors, such as Snail, to endow β-catenin stimulated Lef1 with this unusual repressor activity. Although we have shown that the Snail and Lef1 binding sites can function independently, Lef1's ability to bend DNA still affords a means of bringing these complexes into contact. Additionally, β-catenin's ability to seemingly act through both chromatin-dependent[12] and independent[13] mechanisms to modulate Lef1's action hints at unexplored avenues for how these negative activities might be coordinated *in vivo*. But our results do not preclude the possibility that *in vivo*, the ability of Lef1 and β-catenin to downregulate *E-cadherin* mRNA expression may be indirect, perhaps through transcriptionally activating one of the E-box repressors, such as Snail, that bind to this promoter[29]. Once transcriptionally downregulated, E-cadherin's relatively short half-life and multiple modes of posttranslational regulation[10] may facilitate the ensuing changes that accompany follicle morphogenesis.

We have found that in noggin-regulated waves of Lef1-expressing follicles, Wnts appear to collaborate by stabilizing β-catenin so that it can bind and activate a transcription factor that

participates in finely balancing the levels of E-cadherin to sculpt the epithelial bud. The sources of noggin and Wnt offer an additional level of regulation in the process. Whereas canonical Wnts have been found in skin epithelium[14,15], noggin is mesenchymal[18], and BMPs and their surface receptors are expressed by both mesenchyme and epithelium[16,17]. Our findings suggest a model in which embryonic skin epithelial stem cells require simultaneous inputs of stimulatory and inhibitory signals from multiple neighbouring cell types for the purpose of producing an activated transcription factor able to remodel AJ gene expression and form a follicle bud (see Supplementary Information). Such a mechanism furnishes an exquisite level of governance by converging signals to specify stem cell lineage.

Methods

Plasmid construction

TOPFLASH and *FOPFLASH* were gifts (H. Clevers). The *E-cadherin* promoter was generated by PCR with primers (Celera Genomics database) and a *BAC* clone as template. Mutation of the Lef1 binding site (−242 to −233) was achieved using the QuickChange Site-Directed Mutagenesis Kit (Stratagene). Promoter fragments were subcloned into pGL3-basic (Promega; luciferase) or pNASSβ(M) (β-galactosidase)[7]. *HA-tagged Snail* cDNA (A. Garcia de Herreros) was subcloned into the *Bam*HI/*Not*I sites of the *K14-cassette*[6]. *E-cadherin-HA* cDNA was generated by PCR amplification of pKS + UM plasmid (R. Kemler) with a forward primer at the *Xho*I site (3′ end), and a reverse primer containing the HA tag, a stop codon and *Xba*I site. E-cadherin-HA localized to intercellular junctions in keratinocytes.

Nuclear Lef1 and β-catenin generated by noggin and Wnt3a conditioned media

Keratinocytes from newborn mouse skin were cultured in low calcium E-media[7], and then either treated for (1) 12 h with control-media (Nog(−)) or conditioned-media (Nog(+)) from a noggin-secreting cell line (R. Harland), or (2) 5 h with control-media (Wnt(−)) or conditioned-media (Wnt(+)) from a Wnt3A-secreting cell line (S. Takada). In some cases, 7 h noggin/control-media was replaced with Nog/Wnt conditioned or Nog(−)/Wnt(−) media for 5 h. In all cases, EGTA was added to 5 mM and control/conditioned media was at a final dilution of 1:5.

For harvesting, cells were washed twice with PBS and lysed in RIPA buffer (1% Triton X100, PBS, 10 mM EDTA, 150 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, protease inhibitors). After determining protein concentrations (BCA, Pierce) for equal loadings, SDS–PAGE and immunoblot analyses were performed with mouse anti-tubulin (Sigma), rabbit anti-Lef1 (UC72), and mouse anti-β-catenin (Sigma). HRP-conjugated secondary-antibodies were followed by enhanced chemiluminescence (Amersham).

BMP/Noggin expression analyses

RNAs were purified by Trizol-extraction of keratinocytes (Invitrogen) and spin-column chromatography (Qiagen). Reverse transcription was performed with a Superscript kit (Invitrogen). *BMP2*, *BMP4*, *noggin*, and *GAPDH* mRNAs were detected by RT–PCR. For BMP immunodetection, 1 ml of spent-keratinocyte or control media were precipitated with two volumes of acetone, while keratinocytes were pelleted directly by centrifugation. Samples were subjected to 10% SDS–PAGE and immunoblotting with anti-BMPs (R&D Systems).

Transfections

FUGENE6 (Roche) was used to transfect newborn skin keratinocytes with expression vectors. For β-galactosidase reporters, *CMV-luciferase* was used to control for transfection efficiency, and for luciferase reporters, *CMV-β-galactosidase* was used. After 24 h, cells were treated with Wnt and/or noggin media as described. β-Galactosidase activity was measured with the

Galacto-Lite-Assay Kit (Tropix Inc.) and luciferase activity by the Dual-Luciferase Kit (Promega). For standardizations, the activity in the transfected lysate of control cells was assigned an arbitrary value.

Animals

noggin and *Shh* null mice (A. McMahon) and *Lef1* null mice (R. Grosschedl) were gifts. *α-Catenin* conditional null, *TOPGAL*, *K14-Lef1* and *K14-ΔNβcat* animals have been described[5–7,27]. *K14-EcadherinHA* transgenic mice were generated in the laboratory.

In situ hybridization

Digoxygenin-labelled probes were synthesized with the DIG-RNA labelling kit and detected with Anti-Digoxigenin AP (both from Roche). Antisense *E-cadherin* cRNA was generated from T7 RNA polymerase transcription of a *Bgl*II-*Xho*I extracellular domain fragment that had been subcloned into pCRII (Invitrogen) and linearized with *Hind*III. Sense probe was generated by *Xho*I digestion and Sp6 RNA polymerase transcription. 3′UTR *P-cadherin* cRNAs were generated by subcloning a 379 bp PCR product into pCRII. Probes were applied to 15-μm sections of frozen, optimal cutting temperature (OCT) compound-embedded tissue and processed as described[7].

Immunohistochemistry and tissue analysis

Sections (10 μm) of frozen embryos or derived-keratinocytes were fixed in 4% paraformaldehyde and processed for indirect immunofluorescence. Primary antibodies used were: rabbit anti-Lef1 (UC54), rat anti-E-cadherin (M. Takeichi), rat anti-P-cadherin (Zymed), guinea pig anti-K5, rat anti-HA (Roche) and rabbit anti-laminin5 (8LN5; R Burgeson). Secondary FITC (fluorescein isothiocyanate) or Texas Red conjugated antibodies (Jackson Labs) were diluted 1:100. For detection of nuclear β-catenin, whole embryos were fixed overnight in 4% PFA, dehydrated, embedded in paraffin, and rehydrated before probing with mouse anti-β-catenin antibody (Clone 15B8, Sigma). Expression of *TOPGAL* was determined by X-gal staining of frozen embryo sections (7.5 μm) embedded in OCT and fixed in 0.1% glutaraldehyde[7]. Histological analysis of embryonic skin was performed by staining with haematoxylin and eosin (Richard Allan Scientific).

ChIP and PCR

Protein-DNA complexes were crosslinked in whole cells with 1% formaldehyde, followed by sonication to fragment genomic DNA to a mid-range of 600 bp (protocols from H. Singh). DNA from anti-Lef1 immunoprecipitation was subjected to PCR using Taq-polymerase (Promega) and primers specific for a 123-bp sequence encompassing the Lef1 binding site (Lef1 site; 5′CAAAGAAAATAAAAACATAAGAAAC3′; 5′ TCCTATTCCACGGTCGTTCG3′)[22] and a site ~2 kb upstream (5′ site; 5′ AGCACCTCTATAGATGAGGC3′, 5′TACTAAGGC-CAAAACAATCACTG3′). PCR was performed with 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, 72 °C for 30 s. PCR products were separated on 1.5% agarose gels and visualized with ethidium bromide.

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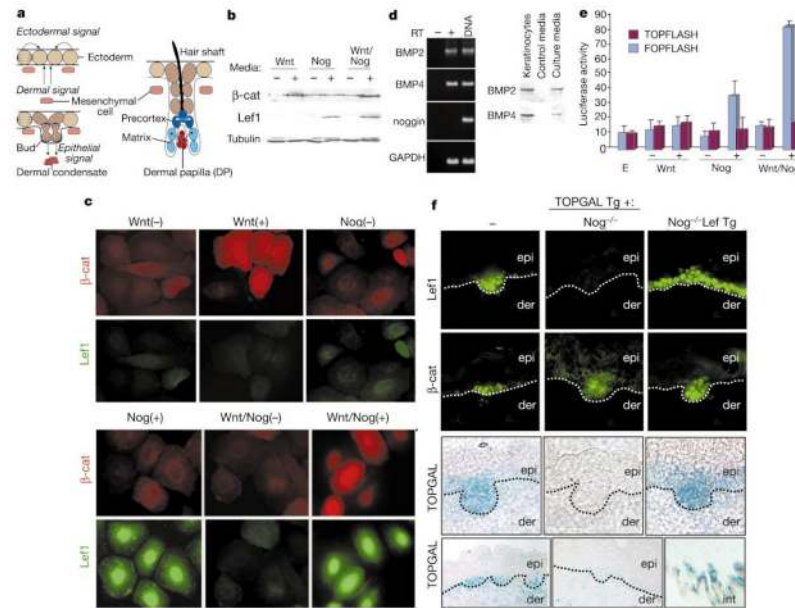
Figure 1.
Wnt and noggin induce a transcriptionally competent Lef1 complex that is absent in *Nog* $^{-/-}$
mice. **a**, Cell–cell signalling (denoted by arrows) in developing follicles: noggin is expressed
by mesenchyme and Wnts by ectoderm. **b**, SDS–PAGE immunoblots showing Wnt3a-induced
β-catenin upregulation and noggin-induced Lef1 induction in lysates from keratinocytes treated
with control (−) or conditioned (+) media.
c, Immunofluorescence of keratinocytes, revealing nuclear Wnt3a-induced β-catenin and
noggin-induced Lef1. **d**, RT–PCR and immunoblot analyses revealing BMP2 and BMP4, but
not noggin, in keratinocytes. **e**, Effect of Wnt3a and/or noggin on *TOPFLASH* (test) and
FOPFLASH (control) in transiently transfected keratinocytes. E, low calcium media.
f, Dependency of nuclear β-catenin, Lef1 and *TOPGAL* expression on *noggin in vivo*. E16.5
skins from the knockout/transgenic (Tg) mice as indicated at top. Note that in epithelial buds
lacking noggin, neither Lef1 nor TOPGAL are expressed and β-catenin is diffuse; these features
are rescued on the K14-Lef1 transgenic background. Dotted lines denote mesenchymal–
epithelial boundaries between epidermis (epi) and dermis (der). Bottom row are low-
magnification views; lower right is of *Nog* $^{-/-}$ intestinal epithelium, which uses Tcf4, rather
than Lef1, to regulate Wnt signalling[7]. For **b**, **c**, **f**, antibodies or *TOPGAL* activity assays are
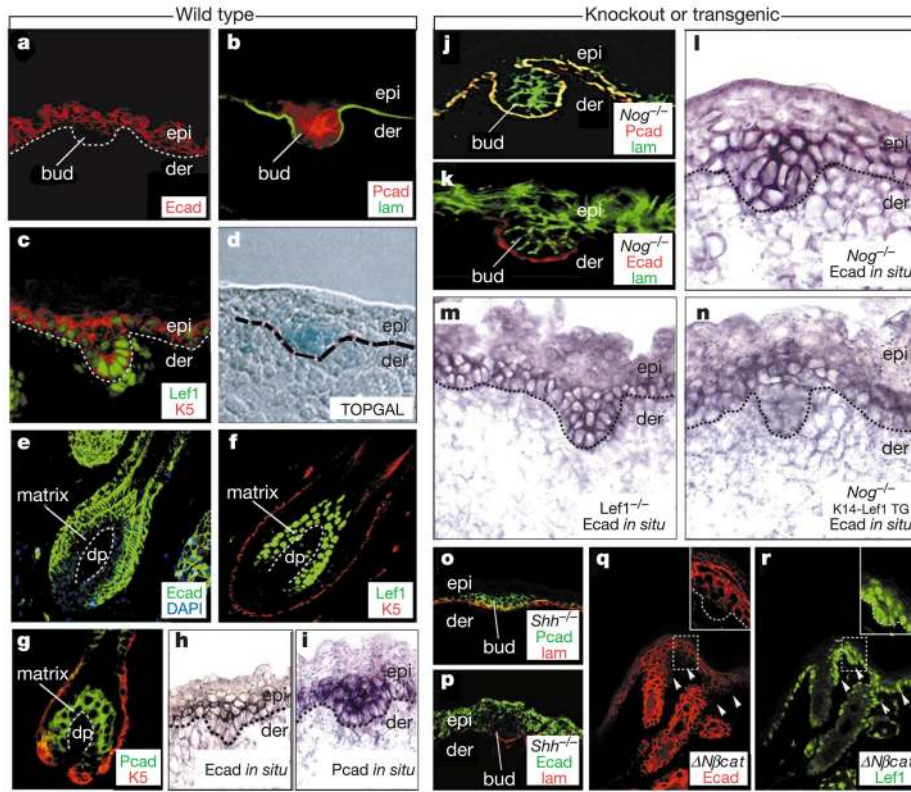denoted at left.

Figure 2.
During follicle morphogenesis and cycling, skin stem cells change cadherin expression in a fashion dependent upon noggin and Wnt signalling. Wild-type skins from *TOPGAL* reporter mice at E16.5 (**a**–**d**, **h**, **i**) or adult (**e**–**g**) (left) or mutant E16.5 skins from the genetic backgrounds indicated (**j**–**r**) (right) were processed for double immunofluorescence, *TOPGAL* (β-galactosidase) activity or *in situ* hybridizations, using the markers indicated (colour coding denotes secondary antibodies). Wherever noggin and Wnt signalling were active, *E-cadherin* was downregulated, and nuclear Lef1 was present, indicating β-catenin activated Lef1 complexes. This was true even for *Shh*-null skin (**o**, **p**), arrested at the bud stage, and for the Lef1-positive epithelial invaginations (arrowheads) of *K14-ΔNβcat* transgenic (Tg) skin, expressing stable β-catenin (**q**, **r**). Without *noggin*, few buds formed and *E-cadherin* remained high (**j**–**m**), but this was rescued by *K14-Lef1* (**n**). lam, laminin 5 antibody, demarcating the epithelial–mesenchymal boundary (elsewhere delineated by dotted lines); K5 antibody, marking the basal layer of epidermis and outer root sheath of the hair follicle. epi, embryonic epidermis; der, dermis; DP, dermal papilla.
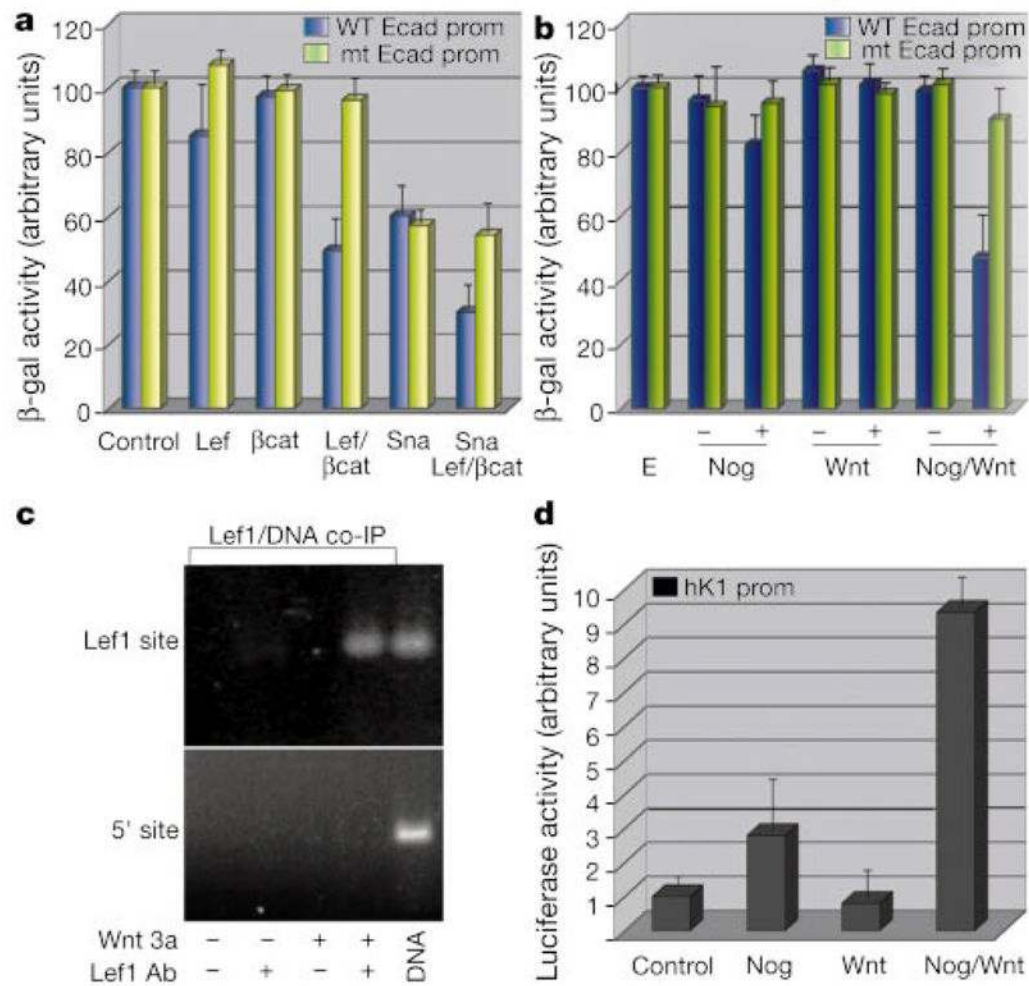
Figure 3.
Lef1 and β-catenin transcriptionally downregulate *E-cadherin in vitro*. **a**, **b**, **d**, Promoter activity assays on extracts from keratinocytes treated without (**a**) or with (**b, d**) control (E) or conditioned media as indicated. Cells were co-transfected with *E-cadherin-β-galactosidase* (WT (wild-type) or mt (mutant) Lef1-site; **a**, **b**) or *HK1-luciferase* (**d**)[19] ± K14-expression vectors encoding Lef (Lef1), βcat (ΔNβcat), Sna (Snail), or control (empty vector). **c**, Chromatin IP analyses of *Lef1*-expressing keratinocytes ± Wnt-conditioned medium. Fragmented, crosslinked DNA from chromatin immunoprecipitated with Lef1 or control antibody was subjected to PCR with primers encompassing the known Lef1 site or control sequence in the *E-cadherin* promoter. DNA, genomic control.
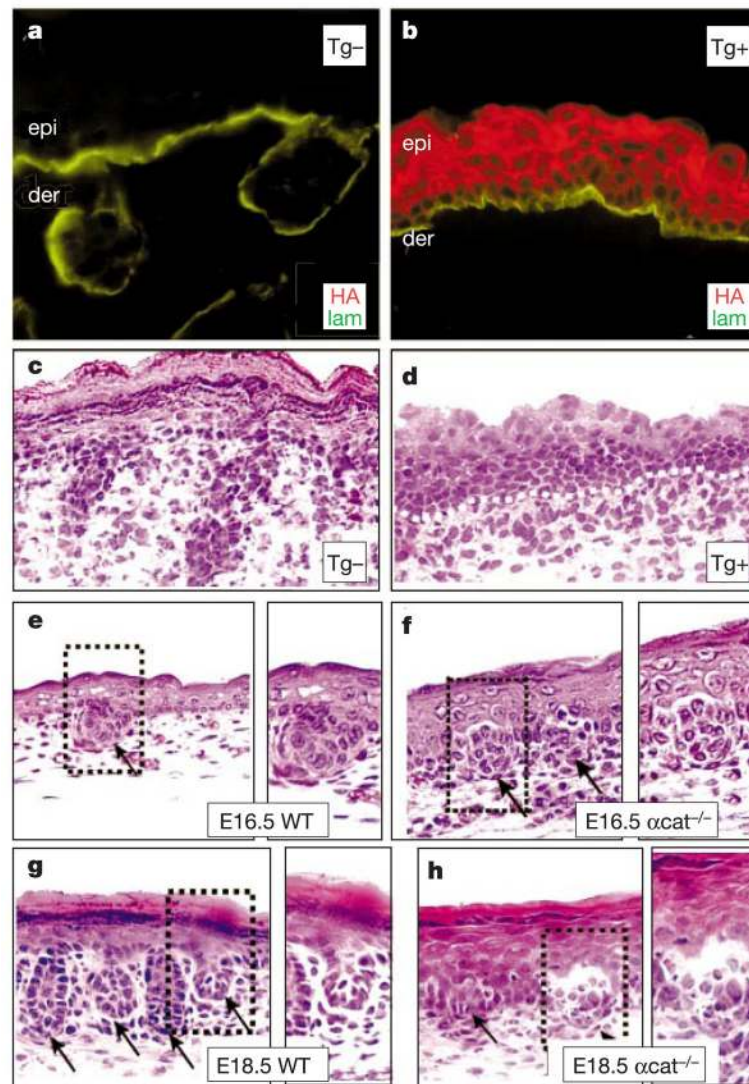
Figure 4.
Levels of adherens junction proteins influence follicle morphogenesis. **a–d,**
Immunofluorescence and haematoxylin-eosin staining of frozen back-skin sections from a
representative newborn mouse mosaic for the *K14-Ecadherin-HA* transgene. Transgene-
negative (Tg −) and positive (Tg +) regions were identified by anti-HA (HA), and anti-laminin
5 (lam) delineated the basement membrane. **e–h,** Haematoxylin-eosin staining of back-skin
sections from wild-type and *α-catenin K14-Cre* conditional-null (*αcat* $^{-/-}$) embryos. Boxed
areas are shown at higher magnification to right of each frame. Note that in *α-catenin*-null skin,
follicle buds (arrows) begin to form at E16.5, but cellular disorganization arrests their
downgrowth by E18.5.