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The chromatin remodeler Mi-2β is required for establishment of the basal epidermis and normal differentiation of its progeny

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Using conditional gene targeting in mice, we show that the chromatin remodeler Mi-2β is crucial for different aspects of skin development. Early (E10.5) depletion of Mi-2β in the developing ventral epidermis results in the delayed reduction of its suprabasal layers in late embryogenesis and to the ultimate depletion of its basal layer. Later (E13.5) loss of Mi-2β in the dorsal epidermis does not interfere with suprabasal layer differentiation or maintenance of the basal layer, but induction of hair follicles is blocked. After initiation of the follicle, some subsequent morphogenesis of the hair peg may proceed in the absence of Mi-2β, but production of the progenitors that give rise to the inner layers of the hair follicle and hair shaft is impaired. These results suggest that the extended self-renewal capacity of epidermal precursors arises early during embryogenesis by a process that is critically dependent on Mi-2β. Once this process is complete, Mi-2β is apparently dispensable for the maintenance of established repopulating epidermal stem cells and for the differentiation of their progeny into interfollicular epidermis for the remainder of gestation. Mi-2β is however essential for the reprogramming of basal cells to the follicular and, subsequently, hair matrix fates.

KEY WORDS: Mi-2β (Chd4), Chromatin, Epidermis, Stem cells

INTRODUCTION

The stratified epithelium of the epidermis provides the barrier between the organism and the outside world (Fuchs and Raghavan, 2002; Watt, 1998). The cells of the basal layer of the epidermis are comparatively undifferentiated and proliferate to generate progeny that detach from the basement membrane and progressively differentiate as they are displaced through the successive layers of the epidermis before being shed from the surface. Homeostasis of the mature epidermis is thought to depend on the cycling of stem cells in the basal layer, although it remains unclear whether there are dedicated stem cells that self renew while giving rise to more committed 'transient amplifying' (TA) basal cells, or whether basal cells are more generally equipotent. The differentiation of the epidermis of the mouse begins at E8.5, as the embryonic ectoderm begins to express epithelial markers such as the nuclear factor p63 (also known as Trp63 – Mouse Genome Informatics) and keratins 8 and 18 (Jackson et al., 1981; Koster et al., 2004). Ectodermal commitment to the epidermal lineage occurs between E9.5 and E12.5, producing a basal layer expressing keratins 5 and 14 and a second, transitory layer known as the periderm (Byrne et al., 2003). Further differentiation commences at E14.5, as the intermediary layer and then the spinous and granular layers of the mature epidermis are formed. The final stages of differentiation include the formation of a cornified layer and the acquisition of barrier function, events that occur just prior to birth. This progression of development in embryonic epidermis is demarcated by the expression of structural proteins in the newly formed layers, a pattern that is recapitulated by individual keratinocytes as they transit from the basement membrane to the surface in mature epidermis.

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During skin development, a subset of keratinocytes is recruited to form hair follicles. This fate decision is guided by inductive interactions with an underlying population of mesenchymal cells, some of which eventually form the dermal papilla (DP) (Hardy, 1992). These epithelial-mesenchymal interactions lead to the downgrowth of a hair peg and to the formation of a hair bulb, in which keratinocytes proliferate and differentiate into distinct concentric layers of epithelial cells that constitute the inner root sheath and the hair shaft (Sengel and Mauger, 1976). In the mouse, pelage hairs consist of different types of hair follicles that are formed in successive waves during embryogenesis (Hardy, 1992). Primary (tylotricht) follicles initiate development at E14.5 and are characterized by two sebaceous glands and a large hair bulb that gives rise to a long straight hair. Induction of secondary hair follicles that produce awls hairs begins at E16.5. A final wave of follicle formation in late gestation and after birth gives rise to the zigzag and auchene hairs.

Gene expression changes associated with the partially characterized genetic hierarchy that guides follicle development serve as markers of specific steps in follicle development (Millar, 2002). Activation of the canonical Wnt/β-catenin pathway is required for the initial formation of hair placodes in all three waves (Andl et al., 2002). Another early step in placode development is a local increase in the expression of the ectodysplasin-A receptor (Edar), which is expressed at low levels throughout the basal epidermis before placode formation. The local increase in Edar expression is followed rapidly by a decrease in E-cadherin (cadherin 1) expression and induction of both sonic hedgehog (Shh) and P-cadherin (cadherin 3) expression in the epithelial cells in contact with the forming DP (Hardy and Vielkind, 1996; Headon and Overbeek, 1999; Jamora et al., 2003). Expression of bone morphogenetic proteins 2 and 4 (Bmp2 and Bmp4) in the mesenchyme indicates the formation of the DP, and subsequent Wnt5a expression in the DP reflects its further maturation that is dependent on Shh expression in the epidermal placode (Reddy et al., 2001; Wilson et al., 1999). Although the

disruption of components of these signaling pathways might have preferential effects on specific waves of follicle formation, this sequence of gene expression is shared by follicles in all three waves.

Commitment to the epidermal lineage and subsequent decisions between interfollicular and follicular cell fates rely on a balance between positive and negative events in gene expression. Sequence-specific transcription regulators have been implicated in lineage decisions and function in part by targeting genes whose expression supports lineage progression. p63, a member of the p53 family of DNA-binding factors, is a key regulator of epidermal differentiation as its ectopic expression in simple epithelia induces expression of epidermal keratins and presumably induces the squamous cell fate (Koster et al., 2004). The abilities of lineage-determining DNA-binding factors to either access their chromosomal sites and/or provide permanence to the regulation of the associated locus is central to lineage commitment. Chromatin regulators function in concert with lineage-specific factors to provide long-term epigenetic regulation (Kim et al., 1999). These include ATP-dependent remodelers, histone deacetylases (HDACs), histone acetyltransferases (HATs) and methylases, which are enzymes that can transiently or permanently change the accessibility of genes to transcriptional machineries. Chromatin regulators can generate epigenetic markings on chromatin that underlie the cell's memory and allow for the stable propagation of lineage-specific expression profiles through multiple divisions during development (Georgopoulos, 2002).

Mi-2α and Mi-2β (also known as Chd3 and Chd4, respectively – Mouse Genome Informatics) are closely related genes encoding ATP-dependent chromatin remodelers (Seelig et al., 1996). Mi-2β is expressed at significantly higher levels than Mi-2α in developing and adult tissues and is observed in the skin, mucosal epithelia, the thymus, the kidney, specific areas of the brain, and in the hemopoietic foci of the liver of the mouse embryo (Kim et al., 1999). Mi-2 proteins contain two PHD (plant homeodomain) zinc-finger domains, two chromo domains and a SWI2/SNF2-type helicase/ATPase domain. They reside in the nucleosome remodeling histone deacetylase (NURD) complex that includes the histone deacetylases Hdac1 and Hdac2, two histone-binding proteins RbAp46 and RbAp48 (also known as Rbbp7 and Rbbp4, respectively – Mouse Genome Informatics), and the metastasis-associated proteins Mta1 and Mta2 (Hassig et al., 1998; Xue et al., 1998; Zhang et al., 1998). Because of the Mi-2 association with HDAC, it was thought to be involved primarily in establishing a repressive chromatin environment by cooperating with the HDACs of the NURD complex. However, recent reports indicate that Mi-2 can participate in other regulatory activities that relate to transcriptional elongation, termination (Alen et al., 2002; Krogan et al., 2003), chromatid cohesion (Hakimi et al., 2002), and positive regulation of gene expression (Hirose et al., 2002; Williams et al., 2004).

In the immune system, much of the Mi-2β is found in a stable complex with members of the Ikaros family of lymphoid-lineage-determining factors and HDACs in the NURD complex (Kim et al., 1999; O'Neill et al., 2000). However, during T-cell development, Mi-2β also acts in association with the E-box DNA-binding protein HEB (also known as Tcf12 – Mouse Genome Informatics) and the HAT p300 as a positive regulator of the *Cd4* gene, a hallmark in the differentiation of the helper T-cell lineage. Conditional inactivation of Mi-2β during T-cell development revealed that it is required to generate a chromatin environment at positive-acting regulatory elements that is conducive to Cd4 expression, thereby setting the stage for lineage-specific transcription factors to drive this developmental decision in the T lineage. These studies also revealed a key role for Mi-2β in the transition from the double negative to the double positive stage of thymocyte differentiation, and in mature T cells in promoting antigen-mediated proliferative responses (Williams et al., 2004).

The high levels of Mi-2β expression in the embryonic ectoderm and its preferential expression in the hair placode and the matrix of the hair follicle (Fig. 1A) prompted us to examine the role of Mi-2β in skin development. The conditional allele of Mi-2β was inactivated in keratinocytes at distinct stages of epidermal development and new insights into the regulatory events that control development and homeostasis of the epidermis and its appendages were revealed.

MATERIALS AND METHODS

Mice

The generation and characterization of the Mi-2β$^{LoxPF/LoxPF}$ mice have been described previously (Williams et al., 2004). K14-Cre transgenic mice obtained from Dr P. Chambon (Li et al., 2001) were mated to Mi-2β$^{LoxPF/LoxPF}$ mice to generate mice homozygous for loss of Mi-2β function in the epidermis. PCR genotyping was performed using the genomic DNA isolated from P1 dorsal epidermis. Briefly, epidermis was separated from dermis by treating with 0.05% collagenase overnight at 4°C. PCR primers are as follows:

For WT or *LoxPF* allele: Mi-2β^{+}F, 5′-CTCCAAGAAGAAGACGGCA-GATCT-3′ and Mi-2 INR, 5′-GTCCTTCCAAGAAGAGCAAG-3′;

For Δ F allele: Mi-2β^{+}F and KG4R, 5′-CTTCCACAGTGACGTCCA-GACGCA-3′;

For K14-Cre: K14Cre-5′-2, 5′-ACAGACATGATGAGGCGGAT-3′ and K14Cre3′, 5′-CGACCGGTAATGCAGGCAAAT-3′.

Cycling conditions for all the reactions were as follows: 35 cycles of 30 seconds at 94°C, 1 minute at 56°C and 1 minute at 72°C.

Histology

For histopathology, tissue samples were frozen in OCT or fixed in 3.7% formalin and embedded in paraffin. Sections (4 μm) were stained with Hematoxylin and Eosin. For immunofluorescence, TUNEL, and in situ hybridization, tissue samples were frozen in OCT and sectioned at 6 μm.

In situ analysis

Digoxygenin (DIG) probe synthesis was performed according to the manufacturer's instructions (Roche), using probes transcribed from a plasmid containing Mi-2β cDNA (4264-5668 bp, NM145979), Mi-2α cDNA (C-terminal 1401 bp, BC030435), β-catenin (1830-2456 bp, NM007614), Bmp2 (825-1524 bp, NM007553), Shh (120-760 bp, X76290), and Wnt5a (520-870 bp, NM009524). Sections were fixed in 4% formaldehyde, permeabilized by proteinase K digestion, refixed in 4% formaldehyde, acetylated in 0.1 M triethanolamine/25% acetic anhydride, and hybridized to respective probes at 55-65°C for 16 hours. After hybridization, the sections were washed sequentially with 6×SSC, then 2×SSC containing 50% formamide and 10 mM EDTA, then 2×SSC, and finally 0.2×SSC. The DIG-label was detected by anti-DIG Fab (Roche) coupled to alkaline phosphatase using NBT/BCIP (Roche).

Immunofluorescence

Sections were fixed in 4% formaldehyde and subjected to indirect immunofluorescence. When staining with mouse mAbs, the MOM Fluorescent Kit (Vector) was used. Primary antibodies used were: mouse monoclonal Mi-2β [16G4 (Kim et al., 1999)], rabbit polyclonal K5 (BAbCo), rabbit polyclonal K1 (BAbCo), rabbit polyclonal loricrin (BAbCo), mouse monoclonal Pcna (PC10, Santa Cruz), goat polyclonal Edar (R&D Systems), rat monoclonal P-cadherin (PCD-1, Zymed), rat monoclonal E-cadherin (ECCD-2, Zymed), mouse monoclonal p63 (4A4, Santa Cruz). Fluorescence-conjugated secondary antibodies for primary antibodies developed in rabbit, rat, or goat were obtained from Jackson ImmunoResearch Laboratories. DAPI was used to stain nuclei.

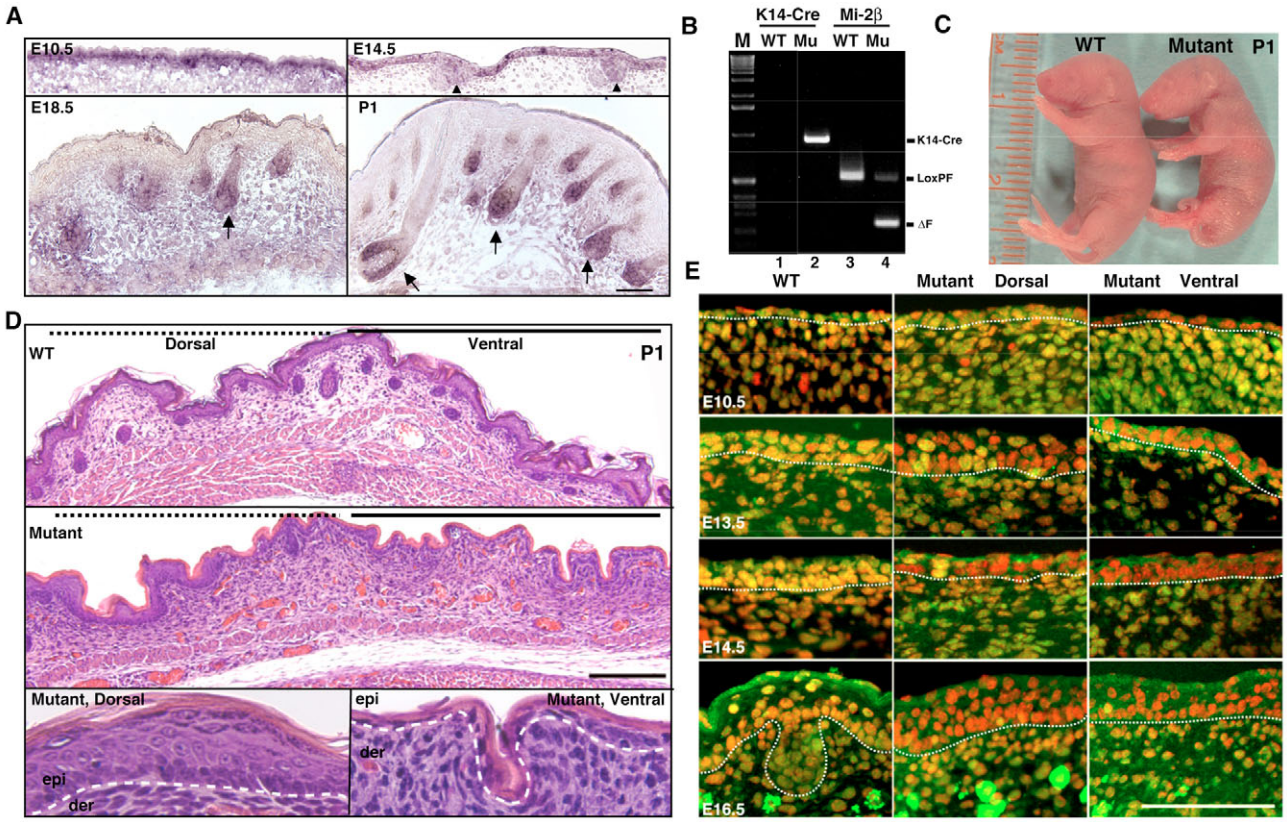


Fig. 1. Expression of Mi-2β mRNA during epidermal development and conditional inactivation of Mi-2β in the skin. (**A**) In situ hybridization studies reveal Mi-2β mRNA expression at E10.5, E14.5, E18.5 and P1. Mi-2β is uniformly expressed in the embryonic ectoderm (E10.5). Mi-2β transcripts are also detected in E14.5 epidermis in the basal and stratum intermediate layers. In the differentiating hair follicle, increased levels of Mi-2β mRNA are first detected in the placode (arrowhead) and then in the matrix (arrow). Scale bar: 50 μm. (**B**) Cre-dependent conversion of the floxed allele (LoxPF) to the mutant allele (ΔF) in the P1 dorsal epidermis revealed by PCR of genomic DNA (lanes 2 and 4). (**C**) Wild-type (WT) and mutant littermates at P1. (**D**) Hematoxylin and Eosin-stained cross-sections of WT and mutant skin at P1. The dotted line demarcates the dorsal region and the unbroken line the ventral region. The mutant skin exhibits an exacerbation of phenotypes from the dorsal to the ventral side that includes thinning of the epidermis and reduction in hair follicles. Scale bar: 100 μm. A further magnification of the mutant skin is provided beneath to show the absence of the basal layer and the thinning of the suprabasal and cornified layers in the ventral region (right), and the more normal epidermal differentiation in the dorsal region (left). (**E**) The presence of Mi-2β protein (green) in WT (left) and in the dorsal (middle) and ventral (right) mutant skin was evaluated by immunofluoresence at successive stages of development. DAPI-stained nuclei are shown in red, and the white dotted line demarcates the dermal-epidermal junction. Expression of Mi-2β protein is indicated by the presence of yellow nuclei, depletion of Mi-2β by red nuclei. Depletion of Mi-2β protein occurs earlier (E10.5) in the ventral skin, and later (E13.5 and later) in the dorsal skin. Scale bar: 50 μm.

TUNEL analysis

TUNEL was performed according to the manufacturer's protocol (Promega). Briefly, sections were fixed in 3.7% formalin, permeabilized by proteinase K digestion, and subjected to a TdT reaction. The TdT label was detected by DAB.

Skin permeability assay

Skin permeability assay was performed as described previously (Hardman et al., 1998). Briefly, mice were sacrificed by terminal anesthesia, incubated for 5 minutes in methanol, rinsed in PBS, followed by incubation in 0.1% Toluidine Blue. After extensive washing, dye penetration reveals barrier status – white skin indicating an intact barrier, stained skin the lack of a barrier.

Quantitative histomorphometry

The number of hair follicles per unit length of epidermis was counted in frozen and paraffin sections of Mi-2β mutant dorsal skin (n=5) at E18.5 and P1, and was compared with that of age-matched wild-type (WT) skin (n=3). The percentage of hair follicles at different stages of morphogenesis was assessed. These stages were defined on the basis of accepted morphological criteria (Hardy, 1992). At least 151 longitudinal hair follicles in 63 microscopic fields derived from five Mi-2β mutant animals were compared with those of 290 hair follicles from five age-matched WT mice at E18.5. At least 412 longitudinal hair follicles in 66 microscopic fields derived from five Mi-2β mutant animals were compared with those of 436 hair follicles from three age-matched WT mice at P1.

RT-PCR

RNA was extracted from P1 dorsal epidermis with Trizol (Invitrogen) according to the manufacturer's protocol. cDNA was generated using random primers and the Superscript II Kit (Invitrogen). The cDNA was amplified by PCR using the following conditions: 28-35 cycles of 30 seconds at 94°C, 45 seconds at 57°C and 45 seconds at 72°C. The PCR products were ligated into the pCRII TA vector (Invitrogen) and verified by sequencing. The primers used for PCR were:

dl (Edar) S, 5′-GTGCTGGTGGTGTCTCTGAT-3′ and dl (Edar) AS, 5′-GCTGTCAGCTTCTGGAACTC-3′;

β-catenin S, 5′-ACATCCTTGCTCGGGACGTT-3′ and β-catenin AS, 5′-TACTAAGGACGATTTACAGG-3′;

Lef1 S, 5′-CACGGACAGTGACCTAATGC-3′ and Lef1 AS, 5′-GAAACAACCGTTTTCGGCTT-3′;
Bmp2 S, 5′-CAGGAAGCTTTGGGAAACAG-3′ and Bmp2 AS, 5′-CCTCCACAACCATGTCCTGA-3′;
Shh S, 5′-GACCCCTTTAGCCTACAAGC-3′ and Shh AS, 5′-AGAAGACCTTCTTGGCGCCT-3′;
Patched S, 5′-CCTTCGCTCTGGAGCAGATT-3′ and Patched AS, 5′-GGCATTCTTGACGGTACCAC-3′;
Mi-2β(E11-13) S, 5′-CCTTTCCAGTTTCCGTAGCTTCAC-3′ and Mi-2β(E11-13) AS, 5′-CAGCGGAAGAATGATATGGACGAC-3′;
Mi-2β (E30-34) S, 5′-CCCGAACTGGCTGAAGTAGAGGAAAAC-3′ and Mi-2β (E30-34) AS, 5′-GGAGTTTCTCCATTCTGAAGCATCACG-3′;
Mi-2α S, 5′-GTGTTGACCCGCATTGG-3′ and Mi-2α AS, 5′-TCTGTATCCAGGTTCAGG-3′; and
GAPDH S, 5′-AAGGTCGGTGTGAACGGATT-3′ and GAPDH AS, 5′-TGGTGGTGCAGGATGGATTG-3′.

RESULTS

Mi-2β expression during epidermal differentiation

To gain insight into the role of Mi-2β during epidermal morphogenesis, we examined the pattern of Mi-2β mRNA expression during development. At E10.5, the embryonic ectoderm consists of a single-cell layer in which Mi-2β was uniformly and highly expressed (Fig. 1A, E10.5). At E14.5, the ectodermal layer had begun to differentiate into the basal and stratum intermediate layers, which express Mi-2β (Fig. 1A, E14.5). At this stage of development, hair placodes expressing Mi-2β were also observed. Increased levels of Mi-2β mRNA were detected in the growing tip of the hair peg and persisted in the cells that form the matrix of the differentiating hair follicle (Fig. 1A, E14.5, E18.5 and P1). In contrast to the distinctive pattern of expression in the hair follicle, Mi-2β mRNA was expressed at relatively low levels in the mature interfollicular epidermis (Fig. 1A, E18.5 and P1).

Mi-2β inactivation in the epidermis causes abnormal development of the integument

The role of Mi-2β during development of the epidermis and hair was evaluated using a conditional inactivation strategy. Mice containing an Mi-2β allele with loxP sites flanking the ATPase domain were crossed to a *K14cre* transgenic line that expresses cre recombinase in the basal epidermis and the outer root sheath of the hair follicle (Li et al., 2001). As previously shown (Williams et al., 2004), cre recombinase removes sequences encoding the ATPase domain required for Mi-2β remodeling activity, resulting in a mutant mRNA that does not produce stable protein. As revealed by genomic PCR, the floxed Mi-2β alleles (Mi-2β$^{loxPF/loxpF}$) were efficiently disrupted by K14cre in the epidermis (Fig. 1B).

Mice homozygous for the *Mi-2β*^{LoxPF} allele and carrying the *K14cre* transgene die within 24 hours of birth. The skin of these mutant mice is shiny and flaky (Fig. 1C), exhibits a severe reduction in hair follicles and shows abnormal whisker hairs (data not shown). A curly tail is another morphological phenotype of the *K14cre* deletion of Mi-2β (Fig. 1C). Histological analysis of the mutant skin demonstrated a marked difference in phenotypes between the dorsal and ventral areas (Fig. 1D). In the dorsolateral region, a relatively normal multilayered epidermis was detected, but the number and length of hair follicles were drastically reduced. In most of the ventral region, the structure of the epidermis was greatly affected, with most layers reduced in size. Hair follicles were also absent. A stratum corneum was present in both areas (Fig. 1D). Occasional patches of multilayered skin lacking hair follicles were observed among the severely depleted ventral skin of the mutant (see Fig. S1 in the supplementary material).

The difference in phenotype between the dorsal and ventral skin could be explained either by a fundamental difference in the requirement for Mi-2β in the development of dorsal and ventral skin, or by a difference in the timing of Mi-2β protein depletion. Examination of Mi-2β protein at E10.5 revealed its dramatic depletion in most of the ventral skin, while expression persisted in dorsal skin (Fig. 1E). Mi-2β was still detectable at E13.5 in dorsal epidermis (Fig. 1E). However, by E14.5, extensive depletion of Mi-2β protein was also observed in dorsal skin, although some areas of persistent expression were apparent at this and later stages of development (Fig. 1E and data not shown). Thus, the more severe phenotype in the ventral region of the skin is associated with depletion of Mi-2β during the earliest stages of epidermal development, whereas the distinct phenotypes seen in dorsal skin occur when Mi-2β is removed after the initiation of epidermal differentiation.

Effects of Mi-2β depletion prior to epidermal differentiation

Although Mi-2β depletion in the ventral epidermis occurs at E10.5, ventral skin from embryos at E14.5 was histologically normal (Fig. 2A, mutant E14.5). The effects of Mi-2β depletion were not detected until E16.5, when expansion and maturation of the stratified layers became apparent (Fig. 2A, mutant E16.5). At this and subsequent stages, the epidermis was markedly reduced in thickness and no appendages were detected (Fig. 2A, mutant E16.5-P1). A thin, cornified layer could be discerned in ventral skin even in the most severely affected areas (Fig. 1D; Fig. 2A, mutant P1).

Keratin 5 (K5), keratin 1 (K1) and loricrin serve as markers of the basal, suprabasal and granular layers, respectively, in mature skin and are also indicative of the formation of these layers during embryonic development. K5 was expressed in a wild-type (WT) pattern in the innermost layer of the mutant epidermis up to E14.5-16.5 (Fig. 2B). However, after E16.5, a progressive reduction in the K5-expressing layer was seen. Strikingly, by P1, some areas of the skin failed to express any K5 (Fig. 2B, mutant P1-2). K1 expression in the suprabasal layer of WT skin was readily detected by E13.5 (Fig. 2C, WT). In the mutant skin, induction of K1 expression was delayed to E14.5 (Fig. 2C, mutant). A progressive reduction in the K1-expressing layer was seen from E16.5 to P1, with some areas completely lacking the suprabasal layer (Fig. 2C, mutant P1-2). Finally, loricrin, which demarcates the granular layer, is normally detected by E14.5 and its induction was not influenced by the absence of Mi-2β (Fig. 2D, mutant). Nonetheless, by P1, the loricrin-expressing layer was greatly reduced in the mutant skin with very little staining detected (Fig. 2D, mutant).

Thus, Mi-2β depletion at an early stage of development, prior to or during ectodermal commitment to the epidermal lineage, severely affects epidermal differentiation. The initially normal induction and expression of squamous-cell-layer markers indicates that there is no major defect in the differentiation of the cell types of the epidermis. However, the progressive depletion of the lower layers of the skin during development suggests either a defect in the ability of epidermal stem cells to renew themselves or to continue to generate their more differentiated progeny. Defects in proliferation, survival or differentiation of the cells of the basal layer of the epidermis could give rise to this general thinning of the epidermis and depletion of the lower layers. However, at both E14.5 and E16.5, a similar

number of Pcna-positive cells were detected in the basal layer of the ventral mutant as compared with WT skin (Fig. 3A,B), indicating that there is no initial defect in the proliferation of the basal cells. By contrast, from E18.5 through P1, a significant reduction of Pcna-positive cells was seen in the basal layer of ventral mutant skin (Fig. 3A,B). A possible effect of Mi-2β depletion on apoptosis during epidermal differentiation was also examined. A process akin to apoptotic cell death normally occurs during terminal differentiation in the uppermost layer of the WT skin, but little or no apoptosis is normally observed in the basal layer (Fig. 3C, WT ventral). In the mutant skin, the persistence of nuclei in the uppermost layers of the skin accounts for the increased number of TUNEL-positive cells observed (Fig. 3C, mutant ventral). However, no increase in apoptosis was observed in the basal or immediate suprabasal layers of many of those areas where the depletion of Mi-2β lead to a dramatic thinning of the epidermis. Thus, the abnormal differentiation of the skin can be attributed in part to a defect in renewal of the basal epidermis, rather than to the death of this cell population.

Effects of Mi-2β depletion during epidermal differentiation

Mi-2β depletion occurred later in development in the dorsal epidermis, beginning at E13.5 during the onset of epidermal differentiation (Fig. 1E). In contrast to ventral skin, the dorsal mutant epidermis appeared relatively normal and multilayered throughout development, although the number and length of hair follicles was drastically reduced (Fig. 1D). With the exception of a modest delay in the onset of K1 expression (Fig. 4B, E13.5 and E14.5), the initial appearance and subsequent maturation of the basal, intermediate and

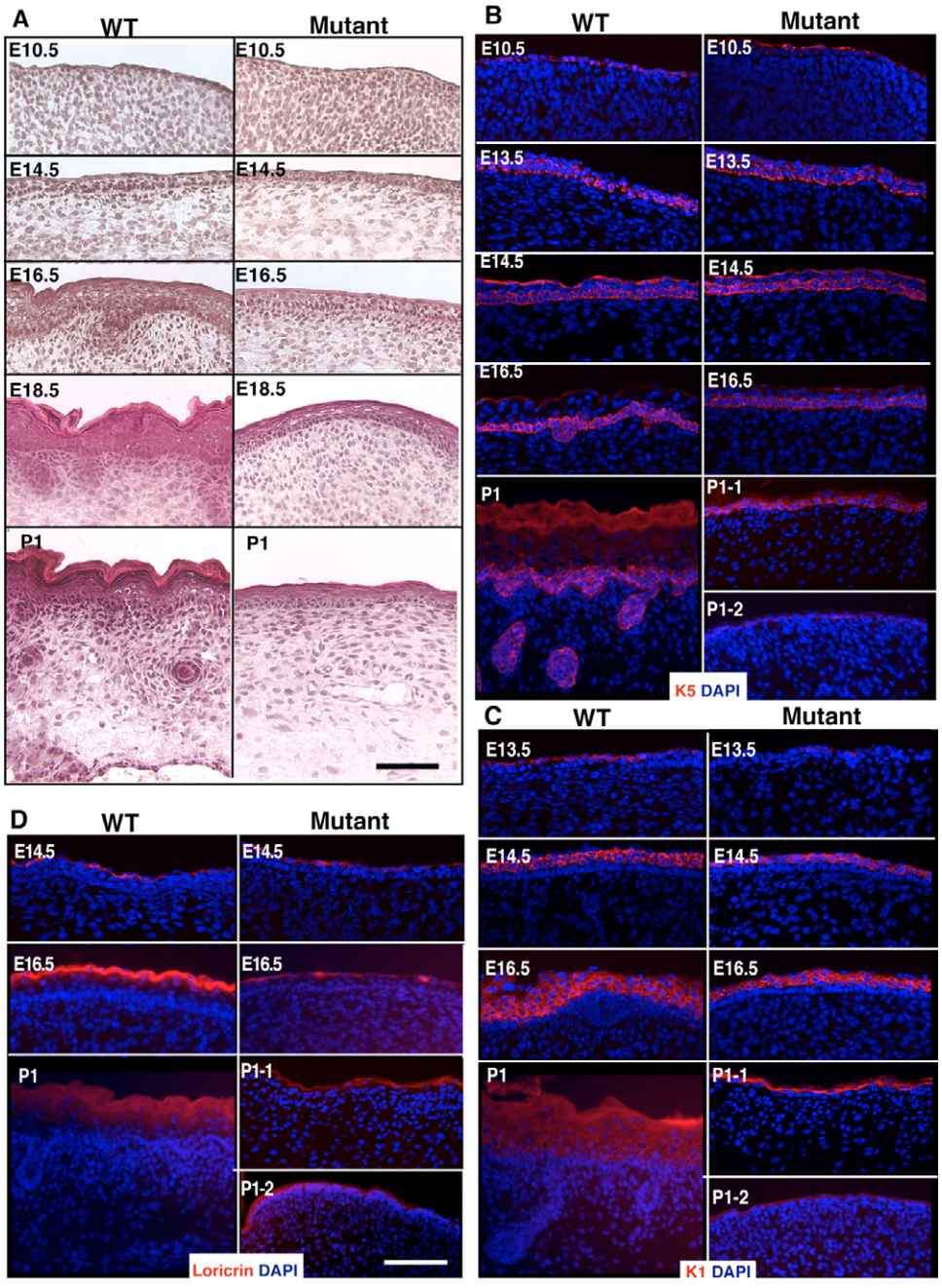


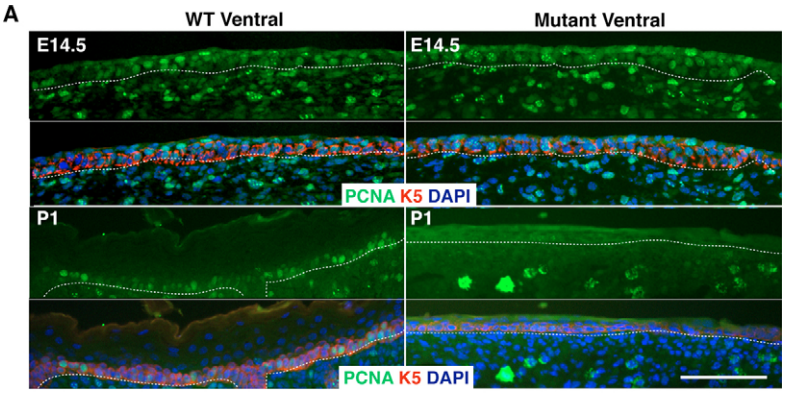
Fig. 2. Early depletion of Mi-2β in the ventral epidermis results in late depletion of the basal and suprabasal layers. (**A**) Hematoxylin and Eosin-stained sections of WT and mutant ventral skin isolated from E10.5-P1 stages of development. A reduced cellularity in the ventral epidermal layers was apparent from E16.5 to P1. (**B-D**) Expression of keratin 5 (K5) (basal epidermis), keratin 1 (K1) (suprabasal epidermis), and loricrin (granular epidermis) was examined by immunofluoresence. DAPI-stained nuclei are shown in blue. The timing of induction and expression of these epidermal differentiation markers is not initially affected. Their progressive reduction later in development reflects a progressive depletion of basal and suprabasal layers. Scale bars: 50 μm.

granular layers of the epidermis were largely indistinguishable from WT. Even in regions where Mi-2β had been deleted throughout the epidermis, K5, K1 and loricrin staining were similar to that in WT skin (Fig. 4A-C).

A notable defect in the dorsal epidermis is the expression of keratin 6 (K6). K6 is expressed in the periderm that overlies the developing epidermis (McGowan and Coulombe, 1998). After the cornified layer has fully developed around E17.5, the periderm sloughs off and K6 expression is confined to the hair follicle. In both the WT and Mi-2β-depleted skin, a K6-expressing periderm was detected at E16.5 and was shed by E18.5 (Fig. 4D, E16.5 and E18.5). In sharp contrast to the situation in the WT, cells in the suprabasal layers that lacked Mi-2β expressed K6 starting at E18.5 (Fig. 4D, mutant). A barrier defect could explain the K6 induction in the suprabasal layers (Paladini et al., 1996). However, the barrier was largely intact on the dorsal side (Fig. 4E). Analysis of regions where mosaic depletion of Mi-2β had occurred demonstrated that K6 expression was confined to cells that lacked Mi-2β (Fig. 4F). Mi-2β-deficient cells interspersed among WT cells specifically expressed K6, whereas cells that retained Mi-2β in largely deleted areas lacked K6 despite its expression in immediately adjacent cells. Furthermore, aberrant K6 expression was confined to the suprabasal layers. Although the induction of K6 might depend on a signal induced in response to a subtle defect in epidermal structure or barrier function (Fig. 4E), this signal is only adequate to induce K6 in suprabasal cells that lack Mi-2β.

Mosaic Mi-2β depletion severely affects hair follicle morphogenesis

Although differentiation of the dorsal interfollicular epidermis was for the most part intact in the mutants, the development of hair follicles was severely compromised in the absence of Mi-2β. Hair follicle induction normally occurs during the period that Mi-2β is being deleted in a mosaic fashion in the dorsal epidermis. Despite the variable nature of this deletion pattern, dramatic differences in hair follicle development were evident when whole skin was



B

Percentage of PCNA positive cells in the total K5-positive basal cells

		E14.5	E16.5	E18.5	P1
Ventral	WT	35%	46%	50%	45%
	Mutant	36%	44%	20%	4%
Dorsal	WT	30%	40%	49%	43%
	Mutant	32%	46%	41%	38%

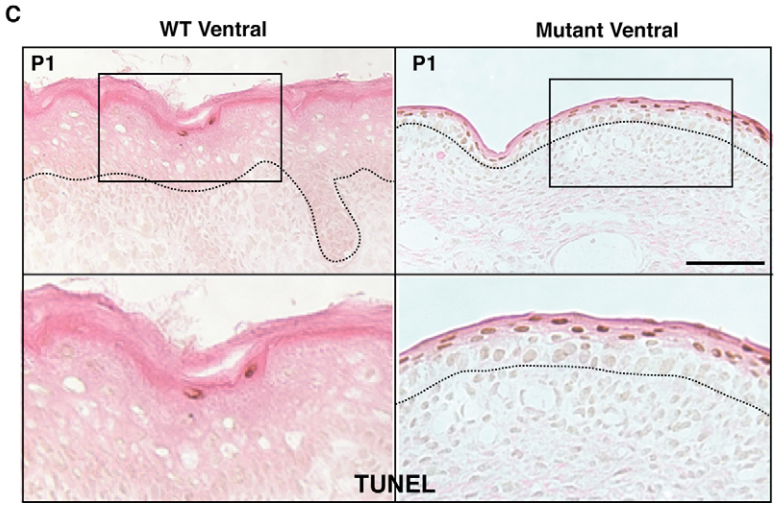


Fig. 3. Late effects on proliferation and apoptosis in the Mi-2β-depleted ventral epidermis. (**A**,**B**) The percent of Pcna-positive cells within the basal layer (K5-positive) of WT and mutant skin was estimated during development. Sagittal sections were co-labeled with antibodies against the proliferation marker Pcna and K5. Nuclei were counterstained with DAPI (A). The data (B) represent the mean percentage of Pcna-positive cells within the basal layer (K5- and DAPI-positive) from five independent animals. From E14.5 through E16.5, a similar number of Pcna-positive basal cells were seen in both the mutant and WT skin. However, starting at E18.5 and through P1, a significant reduction of Pcna-positive cells was detected in the ventral, but not the dorsal, mutant regions. (**C**) Apoptotic cell nuclei (brown) were detected by TUNEL analysis on ventral skin at P1. In both the WT and mutant, TUNEL-positive cells were detected in the uppermost layer of the epidermis, but the dramatic increase in their number seen in the mutant suggests persistence of nucleated dead cells and a defect in terminal differentiation. Scale bars: 50 μm.

evaluated (Fig. 5). Such effects on hair follicle development were even more dramatic when the analysis was confined to areas where Mi-2β depletion was complete.

At E18.5, a later wave of hair follicle morphogenesis is occurring and tylotrich follicles have reached stage 3-4, while awl follicles have reached stage 1-2 in the WT (Fig. 5A). At this time, only half the number of hair follicles seen in the WT were detected in the mutant (Fig. 5A,B). Furthermore, hair follicles at advanced stages of morphogenesis were greatly reduced: about 20% of hair follicles in the WT had reached stage 3b and above, whereas only 5% were this mature in the mutant (Fig. 5C, E18.5). By P1, the total number of follicles in the mutant was still 50% of WT, and the distribution of developmental stages remained distinct (Fig. 5B,C, P1). In the WT, many tylotrich follicles and awl follicles had reached stage 4-6. In addition, newly forming hair follicles at stage 1-2 were readily detected (Fig. 5A,C, P1 WT). By contrast, in the

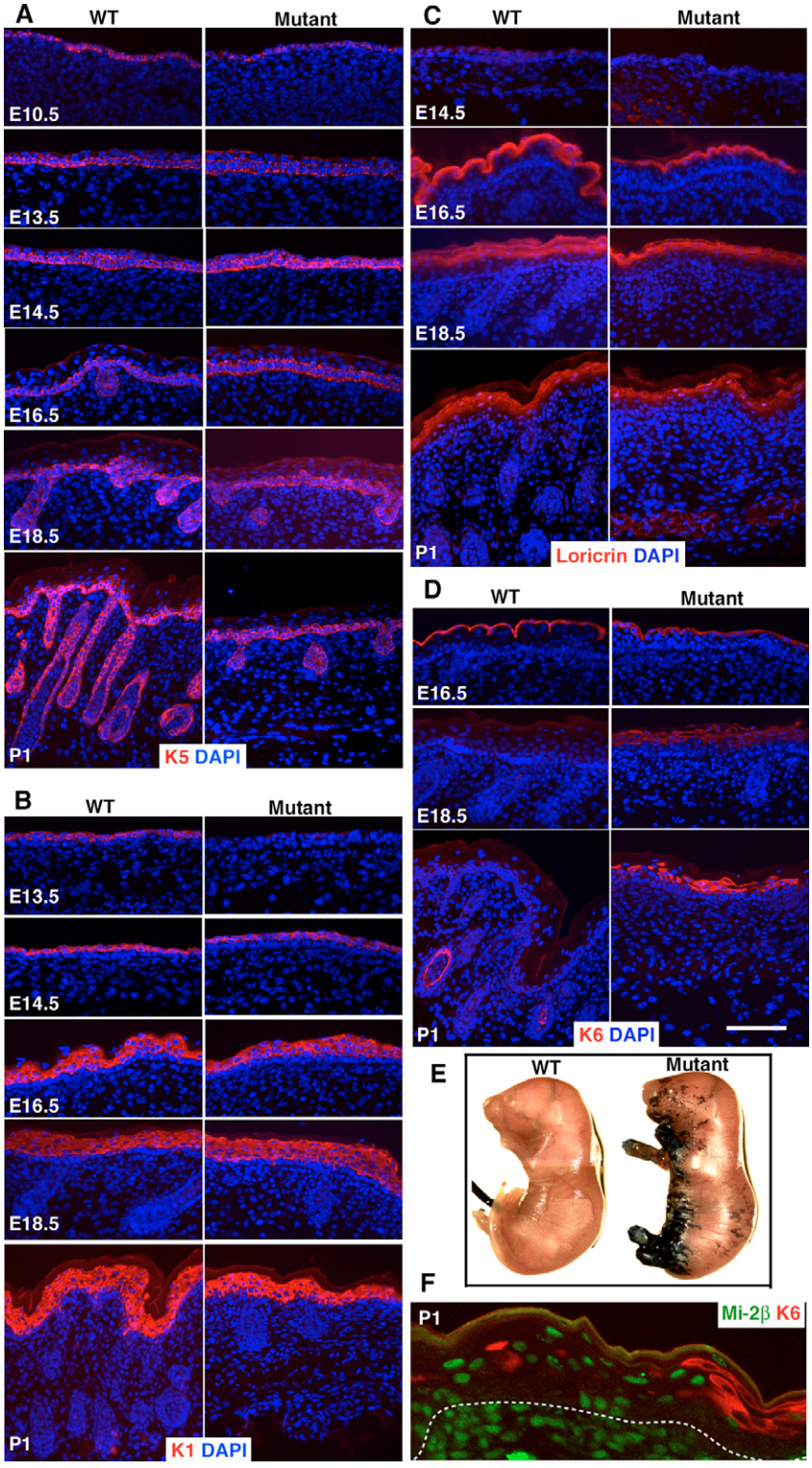


Fig. 4. Effects of Mi-2β depletion in the dorsal epidermis. (**A-D**) Development of the basal and suprabasal layers was examined by immunofluoresence using antibodies to K5, K1, loricrin and keratin 6 (K6). DAPI-stained nuclei are shown in blue. Expression of K5, K1 and loricrin was similar in WT and mutant throughout development (A-C). The K6-positive periderm detected at E16.5 was shed at E18.5 in both WT and mutant (D). From E18.5 through P1, K6 induction was observed in the suprabasal layers of the mutant but not WT skin (D). (**E**) Skin barrier function was analyzed by a barrier-dependent dye exclusion assay at E19.5. The WT and, for the most part, the mutant dorsal epidermis prevented dye penetration indicating intact barrier function. By contrast, the ventral part of the mutant epidermis was readily penetrated by the dye indicating lack of a barrier. (**F**) K6 expression (red) is confined to cells that lack Mi-2β (green). Scale bar: 50 μm.

mutant, follicles at stage 1 and at stages 4-6 (stage 4 and above) were greatly reduced or even absent. The majority of the follicles (79%) were distributed between stages 2 and 3b, whereas only 45% of the follicles were in this range in WT skin (Fig. 5A,C, P1). This distinct developmental stage distribution of hair follicles in the mutant versus the WT epidermis is suggestive of discrete defects occurring during follicle initiation and during the later stages of follicular morphogenesis, rather than of a general delay in appendage development.

Effects of Mi-2β deletion on follicular gene expression

The general reduction in hair follicle initiation and development was confirmed by semi-quantitative RT-PCR. Transcripts of genes preferentially expressed in the hair follicle epithelium – *Edar*, *β-catenin*, *Lef1*, *Shh*, *Patched1* and *Bmp2* – were consistently reduced in the mutant skin (see Fig. S2A in the supplementary material). Furthermore, no increase in the normally low levels of Mi-2α expression was detected in the mutant skin by either RT-PCR or in situ hybridization (see Fig. S2 in the supplementary material). Augmented expression of this closely related gene does not ameliorate the consequences of deleting Mi-2β in embryonic skin.

Examination of marker gene expression on tissue sections revealed more dramatic defects in follicle formation. At E18.5 and P1, Edar was detected at low levels in the basal layer prior to placode formation and expressed more highly in the developing hair placode. This higher level of expression persisted in cells at the leading edge of the hair peg as it invaded the dermis, whereas cells in the rest of the peg exhibited a lower level similar to that in the interfollicular epidermis (Fig. 6A,B, WT). At E18.5, many stage-0 and stage-1 follicles exhibiting bright Edar expression were observed in WT skin (Fig. 6A,B). By contrast, although the levels of Edar in the basal epidermis were similar to those in the WT, no patterned expression of Edar indicative of the initiation of hair placodes was seen in epidermal regions lacking Mi-2β (Fig. 6A, mutant). Analysis of other early molecular markers of follicle induction, including the downregulation of E-cadherin, induction of P-cadherin, or expression of *Bmp2* or *Shh*, confirmed the absence of stage-0 or stage-1 follicles in the Mi-2β-depleted regions (Fig. 6A mutant, and data not shown). More mature follicles at stage 2-3 of development were observed in regions lacking Mi-2β, but these were assumed to be tylotricht follicles that initiated prior to depletion of Mi-2β.

In areas of mosaic Mi-2β depletion, nascent follicular structures expressing Edar were seen (Fig. 6B,Cb). In these incipient follicles, most of the cells of the placode expressed Mi-2β but no nascent follicles were forming in adjacent regions completely devoid of Mi-2β expression. In early follicles with mosaic Mi-2β depletion, Shh was readily detected at the growing tip of the follicular epithelium (Fig. 6Ce), whereas in early follicles without any Mi-2β, Shh was greatly reduced (Fig. 6Cd). By contrast, in more mature follicles (stage 2/3 or 3a), expression of Shh (and Edar) was observed, albeit at lower levels, even when Mi-2β was completely absent (Fig. 6Db and data not shown). This argues that although Mi-2β activity is required for the induction of genes involved in follicular morphogenesis, it is not required for maintenance of their patterned expression at later stages of development.

The growth of a follicle is dependent on the continued inductive interactions between DP and the follicular epithelium. Wnt5a was examined as a marker of the DP that is dependent on expression and signaling of Shh in the follicular epithelium. Significantly, a marked reduction in Wnt5a expression was seen in the DP of Mi-2β-depleted follicles, whereas normal levels of Wnt5a were associated with Mi-2β-expressing follicles in the same animal (Fig. 6Dd,De).

Taken together, these observations indicate that Mi-2β is required for the initial patterning of the expression of signaling molecules involved in follicular morphogenesis. Once committed to a follicular fate, epidermal cells lacking Mi2β can sustain some follicular development. However, inductive signaling to and from the DP is impaired and follicular development is ultimately arrested.

DISCUSSION

Here, we examine the role of the ATP-dependent chromatin remodeler Mi-2β in the development of skin and its appendages. Our experimental approach results in loss of Mi-2β in keratinocytes of the developing integument over the period ranging from before overt epidermal differentiation through to late gestation. Although Mi-2β depletion occurs over a developmental continuum, the phenotypes observed suggest that three discrete steps in the development of the epidermis and its appendages are critically dependent on Mi-2β activity (Fig. 7). When Mi-2β is depleted during the early commitment of an ectodermal progenitor to the epidermal lineage in the ventral regions of the mouse, no immediate effect is observed. The initial differentiation of the epidermis, several days later, occurs

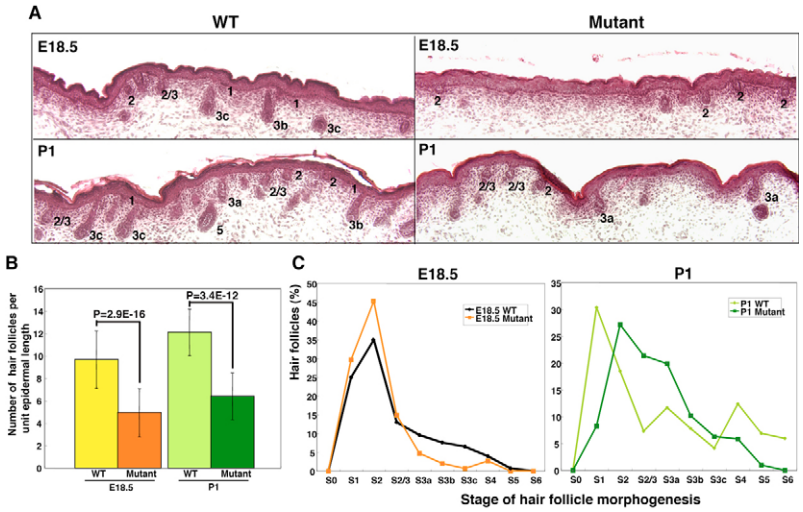


Fig. 5. Mi-2β depletion causes severe effects on hair follicle morphogenesis. (**A**) Hematoxylin and Eosin-stained sagittal sections of dorsal skin from WT and mutant at E18.5 and P1. The number next to the follicles designates their developmental stage according to Hardy (Hardy, 1992). (**B**,**C**) The number of hair follicles per unit length (B) and the percentage of follicles at distinct developmental stages (C) were evaluated from E18.5 through P1. The number of hair follicles in the mutant was reduced by approximately 50% relative to WT from E18.5 through P1. At E18.5 in the WT, primary follicles have developed to stage 3 or 4, whereas secondary follicles have reached stage 1-2. In the mutant, a reduction was detected from stage 3a onwards. By P1 in the WT, primary, secondary and tertiary follicles have developed to stages 5-6, 3-4, and 1-2, respectively. However, by P1 in the mutant, follicles at stage 1 and after stage 3c were severely reduced relative to WT.

normally. However, epidermal development is not sustained, suggesting the capacity of the basal epidermis to self renew is compromised. When Mi2β is deleted in the dorsal epidermis at a later stage, after a differentiated basal epidermal cell is established, epidermal differentiation is largely normal and is sustained throughout embryogenesis and the perinatal period. However, the conversion of a basal epidermal cell to a progenitor of the pilosebaceous unit does not occur. Finally, when follicular anlagen are established in the presence of Mi-2β, they can continue to develop in its absence. However, depletion of Mi-2β after follicular specification results in arrest of follicular development at stage 3, a period when follicular progenitors are transformed to progenitors of the hair shaft and inner root sheath in the forming hair bulb.

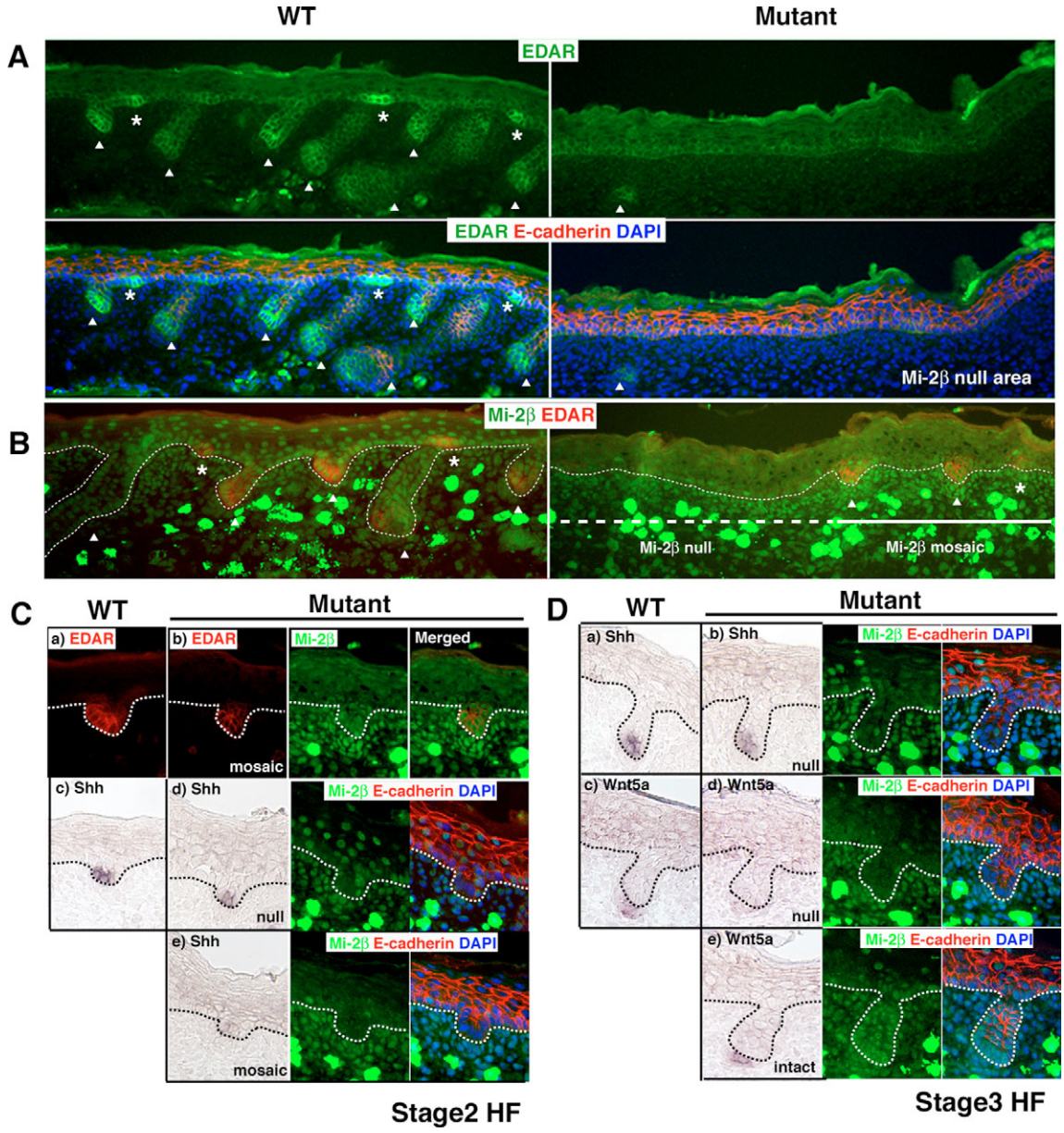


Fig. 6. **Effects of Mi-2β depletion on the signaling network that controls hair follicle morphogenesis.** (**A-D**) Immunofluorescence of E18.5 dorsal skin labeled with antibodies against Edar or Edar and E-cadherin (A), or against Mi-2β and Edar (B,C), or against Mi-2β and E-cadherin (D). (C,D) In situ hybridization of E18.5 dorsal skin with probes against Shh and Wnt5a. The depletion of Mi-2β in follicles was confirmed by immunofluoresence staining of adjacent serial sections using antibodies to Mi-2β and E-cadherin. DAPI-stained nuclei are blue. (A,B) In the WT, a local increase in Edar expression was detected among basal epithelial cells (asterisks) that give rise to the hair placode, as well as within the hair follicle (arrowhead). Edar upregulation was followed by a decrease in E-cadherin expression (A, WT). In the mutant, no Edar upregulation or E-cadherin downregulation was seen in areas of the mutant skin in which Mi-2β was absent (A, mutant). By contrast, in areas with mosaic Mi-2β depletion, follicular structures expressing Edar were detected in the Mi-2β mosaic area in the mutant (arrowhead in B mutant, Cb). Shh expression was seen at the tip of stage-2 and stage-3a follicles in the WT (Cc and Da). In the mutant, Shh transcript was seen in stage-2 follicles with mosaic Mi-2β depletion (Cd), but was significantly reduced in the Mi-2β-null counterparts (Ce). By contrast, Shh was seen in Mi-2β-null stage-3a follicles (Db). Expression of Wnt5a was observed in the dermal condensate of stage-3a follicles in the WT and in Mi-2β-positive stage-3a follicles in the mutant (Dc and De). Wnt5a was, however, significantly reduced in the Mi-2β-null counterparts (Dd).

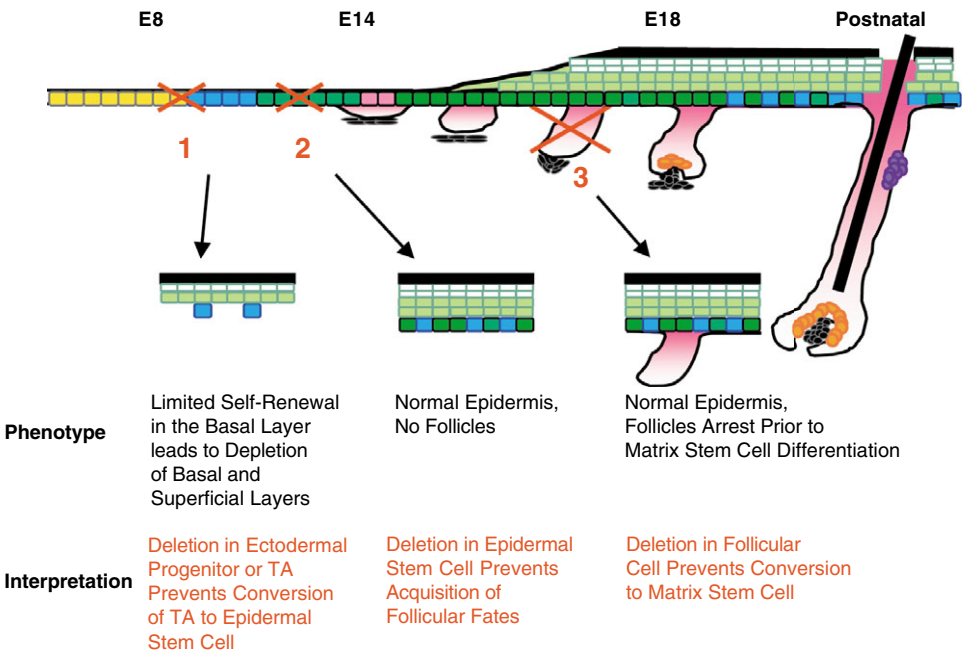


Fig. 7. The role of Mi-2β in skin development: a model for the development of the skin and its appendages and the role of Mi-2β in this process. Successive stages in the development of wild-type skin are depicted on the upper time line. We propose that ectodermal cells (yellow) are first committed to an epidermal TA cell (blue) that can make epidermis but has limited proliferative potential and developmental plasticity. This cell type is then converted to an epidermal stem cell (green) with extensive proliferative capacity and plasticity to adopt alternative fates. Starting at E14.5, some of these cells are induced to become follicular progenitor cells (pink), and sometime thereafter other epidermal stem cells give rise to TA cells of the epidermis with more restricted proliferative and developmental potential. Follicular progenitors proliferate to make the hair peg, while epidermal stem and TA cells generate the stratified epidermis. Finally, a subset of follicular progenitors at the base of the follicle are specified as the matrix stem cells (orange) that give rise to the hair shaft and inner root sheath over the anagen phase of the hair cycle (right). These matrix stem cells are distinct from the follicular bulge stem cells (purple) that regenerate the lower follicle in the adult. The three phenotypes resulting from deletion of Mi-2β at different stages of development are shown (1-3, indicated by a red cross) and interpretations of these phenotypes in the context of the model are shown beneath.

The most severe phenotype associated with early deletion of Mi-2β is observed in ventral epidermis, whereas the phenotypes associated with later deletion of Mi-2β predominate in dorsal epidermis. It is formally possible that the difference in phenotype reflects a fundamental difference in the physiology of the keratinocytes of the dorsal and ventral compartments, rather than the timing of Mi-2β deletion. However, the timing of deletion in the ventral region is also somewhat variable. Patches of epidermis exhibiting the intermediate phenotype of relatively normal epidermis but lacking hair follicles, are also observed on the ventrum of some embryos. We interpret these to be regions where Mi-2β is deleted later in the ventral region, and conclude that although the different phenotypes observed on the dorsum and ventrum may be influenced in part by differences in these keratinocyte populations, the timing of deletion appears to play a more central role in the phenotype observed. However, the timing of the deletion in the patches that give rise to the 'dorsal' phenotype in ventral epidermis has not been directly assessed, and a more significant role for differences in the physiology of dorsal and ventral keratinocytes in the observed differences in phenotype remains a possibility.

Distinct transitions in the differentiation of a hair follicle are dependent on Mi-2β

Of the three transitions in keratinocyte behavior that reveal a crucial requirement for Mi-2β, only the formation of the epidermal placode of the hair follicle represents an empirically defined transition in developmental fate. The follicular epithelium is a lineage compartment that segregates from the surrounding epidermis at the time of epidermal placode formation (Levy et al., 2005). In the dorsal epidermis, Mi-2β is progressively depleted from E14 through birth, when hair follicles normally form in successive waves. The general reduction in hair follicle number, and the more specific perturbation of gene expression and structure within the follicles that do form, must be interpreted in the context of this mosaic and progressive depletion. Newly initiating placodes were not observed in the Mi-2β-depleted regions at the early and later stages of embryogenesis examined in this study. The absence of locally increased Edar expression, of decreased expression of E-cadherin, or of induction of Shh expression in these regions, demonstrates that placode induction is blocked at the very first steps of this process. The fact that isolated groups of cells expressing Mi-2β within a mosaically deleted epithelium can form epidermal placodes demonstrates that the development of inductive signals in the dermis is not impaired, and that Mi-2β is required in the keratinocytes that must respond to these inductive signals. Although Mi-2β-deficient basal cells cannot initiate follicle formation, they can nonetheless sustain the development and differentiation of the epidermis. Thus, the apparent defect in the basal mutant epidermis is in the plasticity of these cells to assume the pilosebaceous (follicular) fate (Fig. 7, phenotype 2).

Although Mi-2β is required for the activation of a battery of markers in the context of follicle initiation, it does not seem to be directly required for the maintenance of gene expression patterns once they have been established. Edar, Shh, Bmp2 and β-catenin are all found in cells lacking Mi-2β in follicles that presumably formed and activated gene expression in its presence. In a similar fashion, the suppression of E-cadherin expression is stable in the absence of Mi-2β. Although the levels of Shh expression are apparently decreased in less mature follicles lacking Mi-2β, this is likely to be an indirect effect of a failure in inductive signaling. A more consistent and presumably previous decline in Wnt5a expression despite normal levels of Mi-2β in the DP of Mi-2β-depleted follicles suggests that inductive signaling to the papilla is compromised in the mutant follicles. This might in turn reduce the levels of expression of genes in the follicular epithelium that are dependent on inductive signaling from the DP. The principal exception to the observed

maintenance of gene expression in the absence of Mi-2β is the behavior of P-cadherin, which appears to decline rapidly in Mi-2β-depleted cells (data not shown).

During follicle neogenesis, the follicular bulge stem cells arise from within the follicular epithelium (Levy et al., 2005), but the timing of this event remains unknown. In a similar fashion, the segregation of transient matrix stem cells from the cells that will constitute the permanent portions of the follicular epithelium during this first hair cycle, is ill defined. It is assumed to occur during stage 3, when differentiated cell types begin to appear within the follicular epithelium (Hardy, 1992). During this stage, the hair matrix is generated and the follicle begins the transition to an organized structure of concentrically arranged, differentiated cell types. It is thus noteworthy that a preponderance of Mi-2β-depleted follicles is arrested in mid-stage 3. As observed in the initial formation of the epidermal placode, Mi-2β appears to be preferentially required during the specification of a progenitor population with a characteristic developmental potential, rather than for the expansion of cell populations with common developmental potential. Finally, follicles lacking Mi-2β at later stages of development are observed. These comparatively rare follicles are likely to represent those that completed the establishment of the hair matrix stem cells before Mi-2β was depleted.

Determination of the self-renewal capacity of epidermal precursors by Mi-2β

Skin development begins from a single layer of embryonic ectoderm that gives rise to a self-renewing epidermis and its appendages. Mi-2β is highly expressed in the E10.5 ectoderm, when it begins to commit to an epidermal/appendage lineage. This suggests that the capacity of Mi-2β to modify chromatin might be actively required to reprogram the cell fate of these early progenitors. Nonetheless, depletion of Mi-2β in the E10.5 ectoderm does not interfere with the initial differentiation of the epidermis that begins a few days later. The differentiation of the successive layers of the epidermis occurs on schedule. Instead, Mi-2β depletion at this early stage in skin development appears to alter the properties of the emerging epidermal precursors allowing them an apparently reduced capacity for self-renewal and maintenance of the differentiated cell types of the epidermis. The progressive depletion of squamous layers observed in the ventral part of the skin, where Mi-2β is depleted early, is consistent with a defect in the ability of a basal epidermal precursor to regenerate itself (Fig. 7, phenotype 1). This effect is unlikely to be due to a defect in the general ability of basal keratinocytes to enter the cell cycle as normal numbers of proliferating cells are seen in the basal layer early in development. Reduced proliferation is only observed after depletion of the basal, suprabasal and granular layers has begun. Similarly, increased cell death does not account for depletion of the basal cells. Later deletion of Mi-2β (after E13.5) does not interfere with the development and maintenance of a multilayered epidermis. Once established, basal cells subsequently deleted for Mi-2β can sustain epidermal differentiation and expansion throughout fetal development to the postnatal stage.

In the parlance of stem cells and TA cells, these studies suggest that as ectodermal progenitors acquire an epidermal/appendage progenitor fate, they require Mi-2β to achieve the extended proliferative and self-renewal capacities of an epidermal stem cell. In its absence, they acquire the more restricted proliferative capacity and generative potential of a transit-amplifying cell (Fig. 7, phenotype 1).

Epigenetic regulation in the development of epidermal lineages

The specific blocks at follicular lineage specification, and the subsequent transition of a follicular progenitor to a matrix stem cell, suggest a crucial role for Mi-2β and its associates in restructuring a chromatin environment permissive for the gene expression changes required in the specified path of differentiation. The brahma (Brm) and brahma-related Brg1 (also known as *Smarca4* in mouse – Mouse Genome Informatics) ATP-dependent nucleosome remodelers act in the context of the SWI2/SNF2 complex and have also been deleted in developing epidermis (Indra et al., 2005). Ablation of *Brg1*, or of both *Brg1* and *Brm*, causes progressively more severe defects in the later terminal differentiation of the stratum corneum and in its barrier function. However, defects in earlier stages of epidermal differentiation or follicular development were not observed. Thus, the requirement for Mi-2β and possibly the NURD complex to mediate chromatin remodeling during early differentiation of the integument is distinct from the functions of the SWI/SNF complex.

Perhaps less clear is how Mi-2β activity might be required to instil the self-renewal capacity that is lost upon early deletion during epidermal development. The phenotype of early depletion of Mi-2β is, in some respects, similar to disruption of p63 activity (Mills et al., 1999; Yang et al., 1999). Distinct isoforms of p63 are thought to be required for the commitment to formation of stratified epidermis and subsequent maintenance of the proliferative potential of basal keratinocytes (Koster et al., 2004; Koster et al., 2005; Koster and Roop, 2004; McKeon, 2004; Suh et al., 2006). No gross deregulation of p63 expression is observed in Mi-2β-depleted skin (data not shown). However, genome-wide analysis of p63 binding has suggested that chromatin states might regulate p63 access to cognate sites, and it is possible that Mi-2β activity is permissive for p63 function (Yang et al., 2006). Whether mediated by p63 or other factors, a unifying hypothesis that provides a common mechanistic explanation for the effects of Mi-2β depletion on the plasticity and self-renewal capacity of keratinocytes, is that extended self-renewal capacity is actively conferred on a progenitor with more limited proliferative capacity. In this model, the self-renewal defects observed in the basal epidermis in ventral skin could be ascribed to a lack of plasticity in the epidermal progenitor, precluding the imposition of this aspect of epidermal stem cell character that normally occurs between E10 and E14 (Fig. 7, phenotype 1).

Defects in terminal differentiation

Although the most dramatic effects of Mi-2β depletion are observed at critical transitions in cell fate and potential, more modest defects in the execution of terminal differentiation programs are also detected. Depletion of Mi-2β in epidermal precursors does not interfere with their ability to give rise to a multilayered epidermis, as exemplified by the normal expression of basal and suprabasal layer markers such as K5, K1 and loricrin in the dorsal side of the mutant skin. However, abnormal expression of K6 was detected in the suprabasal layers of the mutant animals, but only in cells that lack Mi-2β. K6 induction occurs in response to defects in terminal differentiation and/or barrier function of the epidermis. Barrier function studies indicated a severe defect in the ventral side, but not in the dorsal side, of the skin where K6 induction is also observed. Although signals associated with a modestly affected barrier not revealed by the permeability assay might be responsible for K6 induction, they are only sufficient to activate K6 in Mi-2β-depleted cells. Whether this reflects a direct influence of Mi-2β on the keratin gene cluster, or an indirect consequence of its effects on the physiology of the cell, remains to be determined.

Conclusion

In summary, the progressive depletion of Mi-2β during the development of the epidermis in this experimental model has revealed critical transition points at which this chromatin remodeler is required for the normal development of the skin and its appendages. Significant changes in the developmental potential and regenerative capacity of the progenitor cells that give rise to the epidermis and its appendages depend on the activity of Mi-2β. Once these changes have been imposed, more modest deficits in the execution of developmental programs are observed in the absence of Mi-2β. Further investigation of the mechanisms by which Mi-2β exerts these effects in this tractable system will provide additional insight into the role that chromatin remodelers play in the specification of stem cell identity and potential.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/134/8/1571/DC1

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