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Notch signaling in bulge stem cells is not required for selection of hair follicle fate

Shadmehr Demehri and Raphael Kopan*

Notch signaling plays an important role in hair follicle maintenance, and it has been suggested that Notch is also required for follicular fate selection by adult hair follicle stem cells in the bulge. Here we demonstrate that, on the contrary, Notch signaling in bi-potential bulge stem cells or their uncommitted descendents acts to suppress the epidermal fate choice, thus ensuring follicular fate selection. To examine the role of Notch signaling in adult hair follicle stem cells, we used a *Krt1-15-CrePR1* transgenic mouse line to delete Rbpj or all Notch proteins specifically in the bulge stem cells. We conclusively determined that in the absence of Notch signaling, bulge stem cell descendents retain their capacity to execute the follicular differentiation program but fail to maintain it owing to their genetic deficiency. The defect in terminal differentiation caused the diversion of Notch-deficient hair follicles to epidermal cysts, and the presence of wild-type cells could not prevent this conversion. Importantly, our analysis revealed that a functional Notch signaling pathway was required to block bulge stem cells from migrating into, and assuming the fate of, interfollicular epidermis. Taken together, our findings yield detailed insight into the function of Notch signaling in hair follicle stem cells and reveal the mechanism of the replacement of Notch-deficient adult hair follicles by epidermal cysts.

KEY WORDS: Notch, Bulge, Fate selection, Stem cells, Mouse

INTRODUCTION

The mammalian hair follicle continuously cycles through three distinct phases: (1) anagen of variable length (regenerative/proliferative phase); (2) catagen, a short destructive phase during which the lower part of the hair follicle containing the proliferating and keratogenous zones is removed; and (3) telogen (resting phase) (Muller-Rover et al., 2001). The quiescent hair follicle stem cells reside in the bulge located in the permanent part of the hair follicle near the arrector pili muscle attachment site (Blanpain and Fuchs, 2006; Cotsarelis et al., 1990). During each hair cycle, a few bulge stem cells respond to signals from dermal papillae and give rise to new progenitor cells that reside in the hair matrix. This highly proliferative structure surrounds the dermal papilla at the base of the bulb and generates the new anagen hair shaft (Hardy, 1992; Millar, 2002). Matrix cells divide and differentiate into the outer root sheet (ORS), inner root sheet (IRS), cuticle, cortex and medulla of the hair (Legue and Nicolas, 2005). An elaborate network of signaling pathways regulates hair follicle morphogenesis and maintenance (Fuchs and Horsley, 2008; Millar, 2002). The Notch signaling pathway contributes to the maintenance of the follicular structure but not to cell fate selection during hair follicle morphogenesis (Pan et al., 2004). In addition, Notch signaling ensures an optimal proliferative environment in the matrix during first anagen by suppressing Tgfβ and activating Kit ligand (Lee et al., 2007).

Notch regulates keratinocyte proliferation, commitment and differentiation decisions in intact skin and in culture (Blanpain et al., 2006; Lee et al., 2007; Pan et al., 2004; Rangarajan et al., 2001). In response to ligand binding, Notch receptors undergo sequential proteolysis by two enzymes (ADAM metalloprotease followed by γ-secretase) to release the active Notch intracellular domain fragment (NICD), which translocates into the nucleus, binds to Rbpj and nucleates the recruitment of a transcription-activating complex (Lubman et al., 2004). This overall scheme is termed 'canonical' Notch signaling. Part of Notch function in epidermal keratinocytes is mediated by a poorly defined, Rbpj-independent signal (Demehri et al., 2008; Rangarajan et al., 2001).

Although Notch receptors do not function during hair germ cell induction or cell fate acquisition within the embryonic hair follicle, they are required to complete terminal differentiation in IRS cells. In the anagen hair follicle bulb, three Notch receptors are expressed in partially overlapping domains (Pan et al., 2004). Each follicle is derived from two to four multi-potent bulge stem cells (Jaks et al., 2008; Kopan et al., 2002), which give rise to oligo-lineage hair follicular progenitors (Legue and Nicolas, 2005) located adjacent to the dermal papilla in the matrix. Notch proteins are not expressed in these oligo-lineage progenitors (Kopan and Weintraub, 1993; Pan et al., 2004; Powell et al., 1998). Notch1 is expressed and activated in mitotically active IRS and cortex precursors (Cai et al., 2009; Pan et al., 2004), whereas Notch2 and Notch3 are expressed in their postmitotic descendents, respectively (Pan et al., 2004). In the absence of Notch signaling, a hair shaft still forms and contains properly positioned cells expressing markers for each fate (Pan et al., 2004). However, because IRS cells fail to properly adhere to each other, follicular architecture cannot be maintained, leading to the transformation of these aberrant hair follicles into epidermal cysts during the first anagen by overproliferating ORS cells (Pan et al., 2004).

Partial reduction in Notch signaling has also been associated with the conversion of hair follicles to epidermal cysts in adults (see Fig. S1 in the supplementary material) (Vauclair et al., 2005; Yamamoto et al., 2003). However, it is unclear whether the switch from a hair follicle to an epidermal unit during the hair cycle in adult Notch-deficient animals reflects (1) epidermal fate selection by Notch-deficient hair follicle stem cells in the bulge or (2) terminal differentiation defects caused by loss of Notch proteins in committed hair follicle progenitors that lead to aberrant hair shaft formation and to conversion of the hair follicle into an epidermal cyst. Previous studies have supported the former possibility by demonstrating that mosaic loss of Notch signaling by *Rbpj* removal in hair follicles

Department of Developmental Biology, Division of Dermatology, Washington University School of Medicine, Box 8103, 660 South Euclid Avenue, St Louis, MO 63110, USA.

*Author for correspondence (e-mail: kopan@wustl.edu)

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results in reduced follicular regeneration during postnatal life owing to a fate switch at the level of the Rbpj-deficient bulge stem cells (Yamamoto et al., 2003). In addition, fate mapping of the cells that have experienced Notch1 activation in mice marks cells in the bulge (Vooijs et al., 2007), consistent with a role for Notch1 in this structure.

To differentiate between these possibilities, we used the inducible stem-cell-specific *Krt1-15-CrePR1* transgenic line (Ito et al., 2005) to remove Notch pathway components in hair follicle stem cells. We determined that Notch signaling is not required for stem cells to select and execute the follicular program; instead, it plays an inhibitory role in preventing bulge stem cells from differentiating into epidermal cells. Therefore, our findings describe the role of Notch signaling in adult hair follicle stem cells, where it acts to ensure a follicular choice in bi-potential stem cells or in their uncommitted descendents.

MATERIALS AND METHODS

Mice

We generated mouse strains lacking Notch signaling pathway components in skin keratinocytes using the *Msx2-Cre* transgene (Pan et al., 2004). For the hair follicle stem cell studies, *Krt1-15-CrePR1; Rbpj$^{flox/flox}$; Rosa26R* (K15-RBP-jCKO), *Krt1-15-CrePR1; Rbpj$^{flox/+}$; Rosa26R* (control), *Krt1-15-CrePR1; Notch1$^{flox/flox}$; Notch2$^{flox/flox}$; Notch3$^{-/-}$; Rosa26R* (K15-N1N2N3CKO), *Krt1-15-CrePR1; Notch1$^{flox/+}$; Notch2$^{flox/+}$; Notch3$^{-/-}$; Rosa26R* (control), and *Krt1-15-CrePR1; Notch1$^{flox/flox}$; Rosa26R* (K15-N1CKO) were used (Ito et al., 2005). The CrePR1 fusion protein may be leaky in the absence of RU486 (Zhou et al., 2002); however, examination of K15-RBP-jCKO or K15-N1N2N3CKO animals prior to administration of RU486 revealed no evidence of gene deletion until after P120. Therefore, we induced gene deletion at P50, well before this leaky expression, in order to avoid any confounding effect. All mice were kept in accordance with Washington University animal care regulations.

Lineage analysis

To induce Cre-mediated floxed gene deletion in the *Krt1-15-CrePR1* system, mice were shaved and treated topically with 1% RU486 (Sigma, St Louis, MO, USA) dissolved in 70% ethanol for 5 consecutive days, starting at the beginning of the second telogen (~P50). A subgroup of animals was depilated mechanically using the Hair Remover Wax Strip Kit (Del Laboratories, Uniondale, NY, USA). At the indicated time points, dorsal skin was collected and analyzed.

Histology and immunohistochemistry

For Hematoxylin and Eosin (H&E) staining, skin samples from various mutant and wild-type animals were fixed in 4% paraformaldehyde in PBS, and paraffin-embedded tissues were sectioned at 5 μm. Staining for β-galactosidase activity (X-Gal staining) was performed on whole-mount skin samples as described (Kopan et al., 2002). Skin sections that were only X-Gal stained were counterstained with Nuclear Fast Red (Vector Laboratories, Burlingame, CA, USA). X-Gal-stained paraffin-embedded skin samples were used for immunohistochemical analysis. Anti-hair follicle keratin (AE13) antibody (a kind gift from Dr Tung-Tien Sun, New York University), anti-Rbpj antibody (clone T6709, Institute of Immunology, Tokyo, Japan), anti-keratin 10 antibody (Covance Research Products, Princeton, NJ, USA), anti-keratin 6 antibody (Abcam, Cambridge, MA, USA), anti-loricrin antibody (Covance Research Products), and anti-pan-leukocytic marker (CD45) (BD Biosciences Pharmingen, San Diego, CA, USA) were used. After treatment with biotinylated secondary antibody, HRP-conjugated streptavidin (Vectastain ABC Kit) and the DAB Substrate Kit (Pierce, Rockford, IL, USA) were applied to visualize the signal. No counterstaining was performed in immunohistochemical staining. Immunofluorescence staining was conducted on paraffin-embedded skin samples using a combination of the anti-keratin 10 and anti-loricrin antibodies. Fluorochrome-conjugated secondary antibodies were used to visualize the signals and DAPI nuclear stain was used as the counterstain.

RESULTS AND DISCUSSION

Notch signaling is not required for follicular fate determination of bulge stem cells

To directly examine the possibility that Notch is required for follicular fate selection during hair regeneration, we used *Krt1-15-CrePR1* transgenic mice (Ito et al., 2005) to remove canonical Notch signaling specifically in bulge stem cells in adulthood and determine whether bulge stem cells lacking Notch signaling could produce daughters that choose the hair follicle fate. *Rbpj* (Yamamoto et al., 2003) deletion was induced by topical application of RU486 onto the skin of postnatal day 50 (P50) *Krt1-15-CrePR1; Rbpj$^{flox/flox}$; Rosa26R* (K15-RBP-jCKO) mice at the beginning of the second telogen. Fourteen days later, bulge cells were activated by depilation, and 14 days after that we harvested the skin to examine the extent of labeling within the regenerating hair follicles (Fig. 1A). If Notch signaling were required to select/execute the follicular fate, Rbpj-deficient *lacZ*-positive cells would not be able to enter the follicular program. Contrary to this prediction, X-Gal and antibody stainings conclusively showed that blue Rbpj-deficient stem cells contributed descendents to hair matrix progenitors as well as to hair follicle keratin (AE13)-expressing cortex and cuticle cells (Fig. 1B-D). To rule out the possibility that depilation overcame the Notch deficiency, we repeated the experiment by topically treating skin with RU486 and waited for the next spontaneous anagen before harvesting the skin (see Fig. S2A in the supplementary material). As seen with follicles regenerating after depilation, hair follicles spontaneously entering anagen contained blue Rbpj-deficient cells (see Fig. S2C in the supplementary material). Therefore, canonical Notch signaling is not required for hair follicle fate selection by descendents of bulge stem cells.

The analysis of a complete allelic series of mice lacking Notch signaling components in their hair follicles revealed a tight inverse correlation between Notch dosage in follicular keratinocytes and the level of hair follicle distortion (Fig. 2A,B). Importantly, we noticed that *Msx2-Cre/+; Rbpj$^{flox/flox}$* hair follicles displayed a milder disruption at P9 than that seen with total loss of Notch receptors or γ-secretase, which led to epidermal keratin cyst formation during the first anagen (Fig. 2A,B) (Pan et al., 2004). Similar to hair follicles retaining some Notch activity (*Msx2-Cre/+; Notch1$^{flox/flox}$; Notch2$^{flox/+}$; Notch3$^{+/-}$*, or N1N2hN3hCKO) that formed epidermal cysts only in the second anagen (Fig. 2C and see Fig. S1 in the supplementary material), *Msx2-Cre/+; Rbpj$^{flox/flox}$* follicles did not form epidermal cysts in the first anagen and retained a recognizable follicular morphology at P9 (Fig. 2). As shown previously (Demehri et al., 2008; Rangarajan et al., 2001), Rbpj-independent Notch signals contribute to hair follicle maintenance (see Fig. S3 in the supplementary material). Thus, to address the concern that the demonstrated ability of Rbpj-deficient bulge stem cells to choose a follicular fate was preserved by Rbpj-independent Notch signal(s), we generated mice lacking all Notch proteins in bulge stem cells (*Krt1-15-CrePR1; Notch1$^{flox/flox}$; Notch2$^{flox/flox}$; Notch3$^{-/-}$; Rosa26R*, or K15-N1N2N3CKO). Following the Cre induction/hair depilation protocol (Fig. 1A), we showed that Notch-deficient stem cell descendents were also fully capable of contributing daughters to hair follicle structures (Fig. 1E). This finding demonstrates that neither arm of Notch signaling is required for stem cells to choose the follicular fate.

Although Notch-deficient bulge stem cells (blue) migrated down and formed anagen hair follicle progenitors and differentiated hair keratinocytes, the defect in maintaining terminal differentiation resulted nonetheless in the formation of

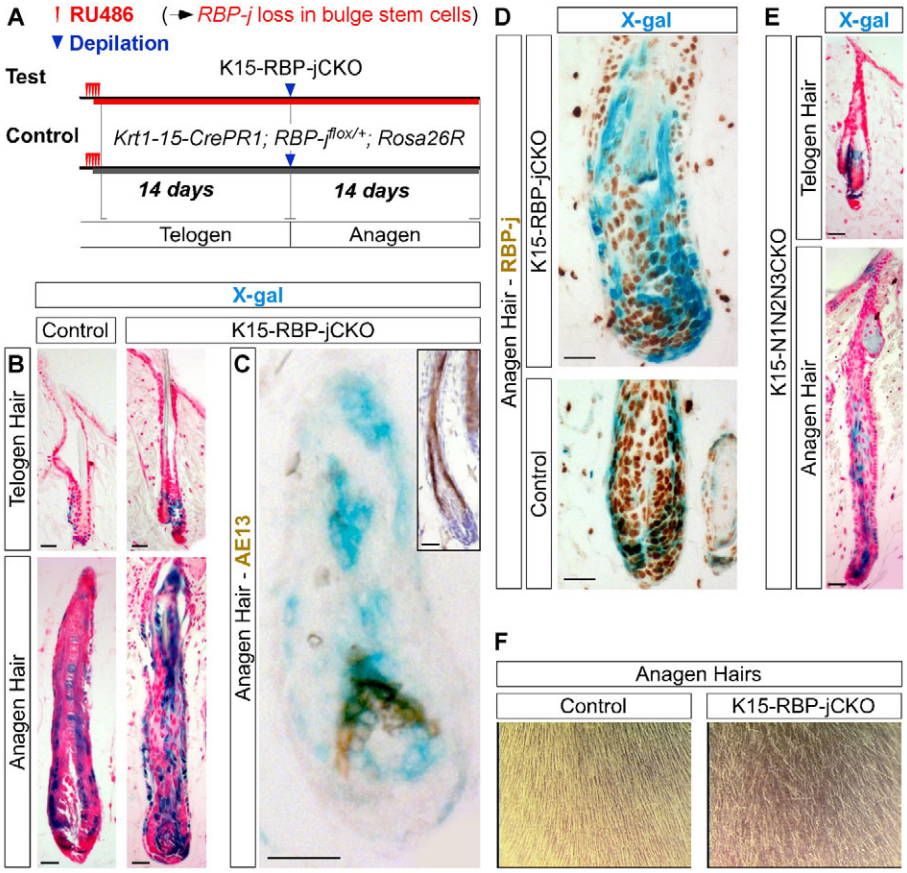

Fig. 1. Hair follicle stem cells lacking total Notch signaling are capable of choosing a follicular fate. (**A**) Diagram of the timeline/cohorts used to delete *Rbpj* (or Notch genes) in adult hair follicle stem cells, to induce the hair cycle, and to examine the behavior of anagen hair follicles 14 days after hair cycle induction. Note that the topical application of RU486 started when the mice were at ~P50 and the following analyses are performed at the end of the experimental period (i.e. 28 days after the last RU486 treatment). (**B**) X-Gal staining shows *Rbpj*-deleted bulge cells (blue) in *Krt1-15-CrePR1; Rbpj*$^{flox/flox}$*; Rosa26R* (K15-RBP-jCKO) and control hair follicles that are not depilated and hence are still in the telogen phase of the hair cycle. Depilated hairs, however, have entered anagen. Blue *Rbpj*-deleted cells in K15-RBP-jCKO hair follicles have migrated down to the bulb and are detectable in all layers of the anagen hair follicle. (**C**) AE13 (cortex and cuticle marker) staining confirms that blue *Rbpj*-deleted keratinocytes in K15-RBP-jCKO undergo the follicular differentiation program. The inset shows the normal pattern of AE13 staining in an anagen hair follicle (counterstained with Hematoxylin). (**D**) Immunohistochemistry for Rbpj demonstrates that blue cells in K15-RBP-jCKO anagen hair follicles are depleted of Rbpj protein. (**E**) Bulge stem cells deleted for all Notch receptors can differentiate into hair follicle keratinocytes and are detectable in anagen hair by X-Gal staining on *Krt1-15-CrePR1; Notch1*$^{flox/flox}$*; Notch2*$^{flox/flox}$*; Notch3*$^{-/-}$*; Rosa26R* (K15-N1N2N3CKO) skin after Cre/hair induction as outlined in A. (**F**) Back skin of K15-RBP-jCKO and control mice highlighting the aberrant hair shafts produced by keratinocytes derived from *Rbpj*-deficient stem cells. The genotype of control mice is shown in A. Scale bars: 25 μm.

aberrant hair shafts (Fig. 1B,E,F, Fig. 2 and see Fig. S2C in the supplementary material). To study the long-term consequences of Notch loss in bulge stem cells, we deleted *Rbpj* (K15-RBP-jCKO) or Notch receptors (K15-N1N2N3CKO) by topical application of RU486 onto the skin during the second telogen, and monitored the hair phenotype over time by histological analyses (Fig. 3A). In the absence of Notch signaling, hair follicles eventually transformed into keratin 10- and loricrin-positive epidermal cysts (Fig. 3B,C) following the destruction of anagen hair follicles in K15-RBP-jCKO and K15-N1N2N3CKO skin. Such cysts were predominantly located deep in the dermis (Fig. 3B). Hair follicles are polyclonal, being derived from two to four stem cells (Jaks et al., 2008; Kopan et al., 2002), and not all bulge cells undergo Notch gene/*Rbpj* deletion with our topical application paradigm. Thus, most follicles are chimeric, containing descendents of Notch/Rbpj-deficient stem cells as well as wild-type descendents of stem cells that are Notch signaling competent. Importantly, a significant number of keratin cyst-forming cells contained intact Notch signaling, demonstrating that the 'field effect' of aberrant hair shafts formed by Notch/Rbpj-deficient keratinocytes was sufficient to set them on the path to become epidermal cells (Fig. 3B). Accordingly, inactivation of Notch signaling in most bulges resulted in complete alopecia within 10 weeks (Fig. 3D), despite the presence of many stem cells that did not experience Cre activation. Importantly, K15-N1N2N3CKO hair loss was much more severe than that of RU486-treated *Krt1-15-CrePR1; Notch1*$^{flox/flox}$*; Rosa26R* (K15-N1CKO) mice (Fig. 3D and see Fig. S4 in the supplementary material). Considering that *Notch3* is a null allele and that *Notch1/3*-deficient hair follicles are indistinguishable from *Notch1*-deficient hair follicles (Pan et al., 2004), the severity of the K15-N1N2N3CKO hair phenotype confirms that deletion of *Notch2* and *Notch1* occurred in these *Notch3*-deficient bulge cells. In conclusion, replacement of Notch-deficient hair follicles by epidermal cysts is a secondary by-product of terminal differentiation defects that cannot be rescued by normal cells, and does not reflect a defective hair follicle fate-selection process. These findings show that the function of Notch signaling during hair regeneration is similar to its role during hair morphogenesis.

Removal of the Notch signaling pathway exposes the bi-potentiality of bulge stem cells

To examine the developmental potential of Notch signaling-deficient bulge stem cells, we mapped the cell lineages derived from these cells in K15-RBP-jCKO skin following the induction of Cre-mediated gene deletion (Fig. 4A). Importantly, a significant number of bulge-derived Rbpj-deficient blue cells migrated upward and generated keratin 10^{+}, keratin 6^{-} interfollicular epidermal cells in the isthmus and epidermis (Fig. 4B-D). Of the K15-RBP-jCKO hair follicles counted on X-Gal-stained 5 μm skin cross-sections, 39% (78 of 200) contained blue Rbpj-deficient cells above the bulge region (see Fig. S5 in the supplementary material). Similar

evidence for epidermal fate selection by K15-N1N2N3CKO bulge stem cells was also observed after RU486 treatment (data not shown). The migration of Notch/Rbpj-deficient bulge cells into the epidermis preceded the secondary effects of aberrant hair shaft formation: bulge-derived Notch/Rbpj-deficient cells were already present in the interfollicular epidermis as hair follicles spontaneously entered anagen (Fig. 4). At this point, epidermal thickness, hair follicle structure and dermal cellularity were normal. In addition, staining the skin sections for CD45 (Ptprc – Mouse Genome Informatics), a pan-leukocyte marker, confirmed that there

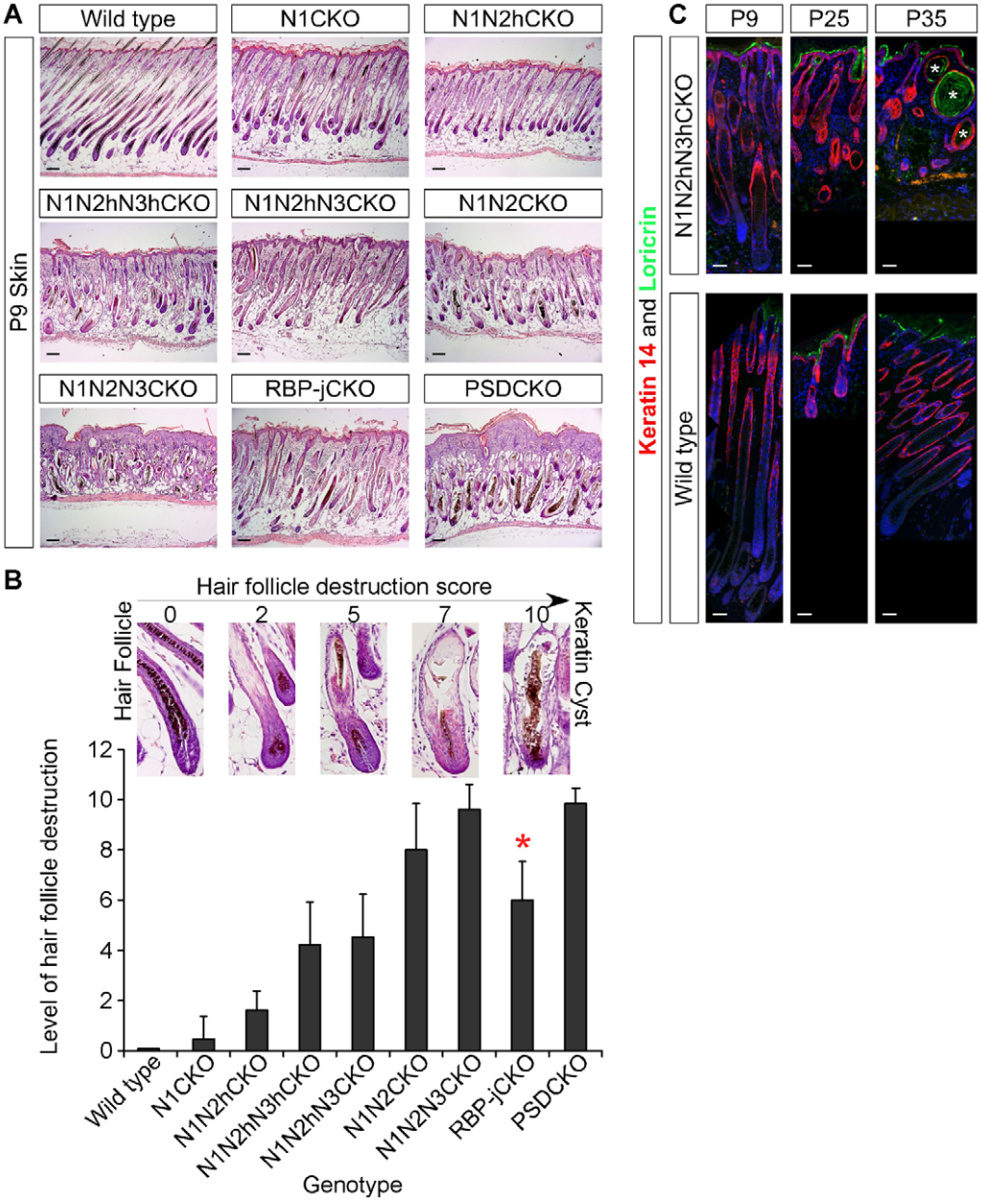

Fig. 2. Stepwise deletion of Notch pathway components in hair follicle keratinocytes results in progressive hair follicle deterioration culminating in transformation into epidermal keratin cysts. (**A**) H&E-stained skin sections from a comprehensive allelic series of Notch-deficient mice at P9 demonstrate progressive hair follicle destruction, which is most evident in mice lacking total Notch signaling (N1N2N3CKO and PSDCKO), which show densely packed keratin cysts beneath the epidermis. Note that Rbpj-deficient hair follicles have a milder phenotype than those lacking total Notch signaling. (**B**) A scoring system devised to permit a quantitative measure of hair follicle destruction (e.g. 0, intact hair follicle; 10, keratin cyst) confirms the inverse correlation between Notch dosage and the level of hair follicle deterioration. The destruction level of RBP-jCKO hair follicles (asterisk) is similar to that of N1N2hN3CKO. Hair follicles in three randomly selected 100× microscope fields are scored and the average score ± s.d. is presented in the bar chart. (**C**) Immunofluorescence staining for keratin 14 and loricrin (an epidermis-specific marker) shows the transformation of *Msx2-Cre/+; Notch1*$^{flox/flox}$*; Notch2*$^{flox/+}$*; Notch3*$^{+/-}$ (N1N2hN3hCKO) hair follicles to fully formed epidermal keratin cysts (asterisks) in the second anagen (P35). *Msx2-Cre/+; Notch1*$^{flox/flox}$ (N1CKO), *Msx2-Cre/+; Notch1*$^{flox/flox}$*; Notch2*$^{flox/+}$ (N1N2hCKO), *Msx2-Cre/+; Notch1*$^{flox/flox}$*; Notch2*$^{flox/+}$*; Notch3*$^{-/-}$ (N1N2hN3CKO), *Msx2-Cre/+; Notch1*$^{flox/flox}$*; Notch2*$^{flox/flox}$ (N1N2CKO), *Msx2-Cre/+; Notch1*$^{flox/flox}$*; Notch2*$^{flox/flox}$*; Notch3*$^{-/-}$ (N1N2N3CKO), *Msx2-Cre/+; Rbpj*$^{flox/flox}$ (RBP-jCKO), and *Msx2-Cre/+; PS1*$^{flox/flox}$*; PS2*$^{-/-}$ (PSDCKO). Scale bars: 100 μm in A; 50 μm in C.

was no inflammation present at this early stage when epidermal migration of Notch/Rbpj-deficient bulge cells was well underway (Fig. 4E). Furthermore, the occasional presence of bulge-derived Rbpj-deficient cells in the isthmus of otherwise normal-looking hair follicles (i.e. club hairs of telogen hair follicles; see Fig. S2B and Fig. S6 in the supplementary material) demonstrated that the upward migration of Notch/Rbpj-deficient bulge cells started before the hair follicles entered anagen. Considering that under similar circumstances, wild-type bulge stem cells do not choose an epidermal fate (Fig. 4 and see Fig. S5 in the supplementary

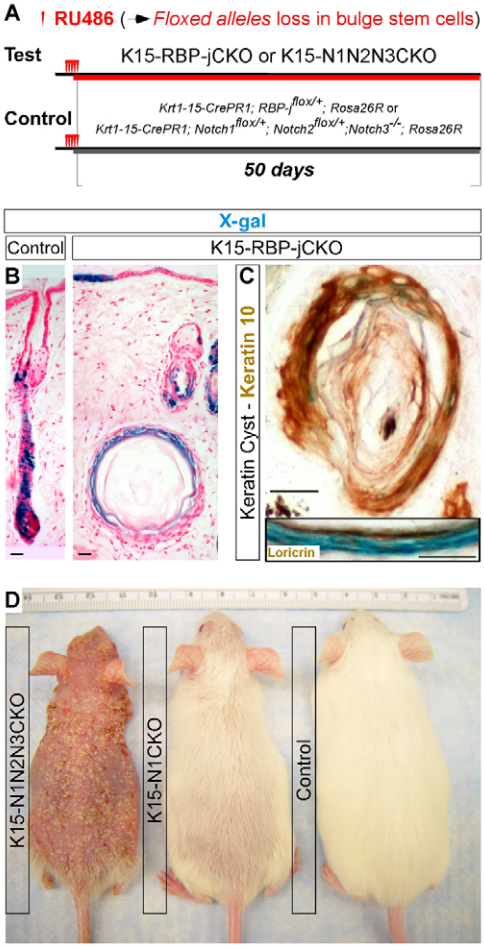

Fig. 3. Hair follicles that are derived from Notch/Rbpj-deficient stem cells transform into epidermal keratin cysts. (**A**) Diagram outlining the experimental approach used to delete *Rbpj* or Notch genes in adult hair follicle stem cells during the second telogen (~P50) and to examine the status of hair follicles in the subsequent anagen phase. Note that there is no depilation step. (**B**) X-Gal-stained skin sections collected 50 days after the last RU486 treatment demonstrate the replacement of hair follicles by keratin cysts in K15-RBP-jCKO skin. Note that *Rbpj*-expressing (pink) cells also contribute to this epidermal structure. The same transformation happens with K15-N1N2N3CKO hair follicles (data not shown). The genotype of control mice is shown in A. (**C**) Expression of keratin 10 (suprabasal epidermal marker) and loricrin (granular layer marker within a *lacZ*-positive cyst; inset) confirms that K15-RBP-jCKO keratinocytes forming the keratin cysts have undergone the epidermal differentiation program. (**D**) Ten weeks after RU486 treatment, K15-N1N2N3CKO mice show severe alopecia with numerous keratin cysts visible on the back skin. Keratin cyst formation in K15-N1N2N3CKO and/or barrier defects associated with the loss of Notch proteins in bulge-derived epidermal keratinocytes lead to an intense dermal inflammation in these mice (see Fig. S4 in the supplementary material). RU486-treated *Krt1-15-CrePR1; Notch1*$^{flox/flox}$*; Rosa26R* (K15-N1CKO) mice have a mild hair loss phenotype compared with similarly treated age-matched controls (*Krt1-15-CrePR1; Notch1*$^{flox/+}$*; Notch2*$^{flox/+}$*; Notch3*$^{-/-}$*; Rosa26R*). Scale bar: 25 μm.

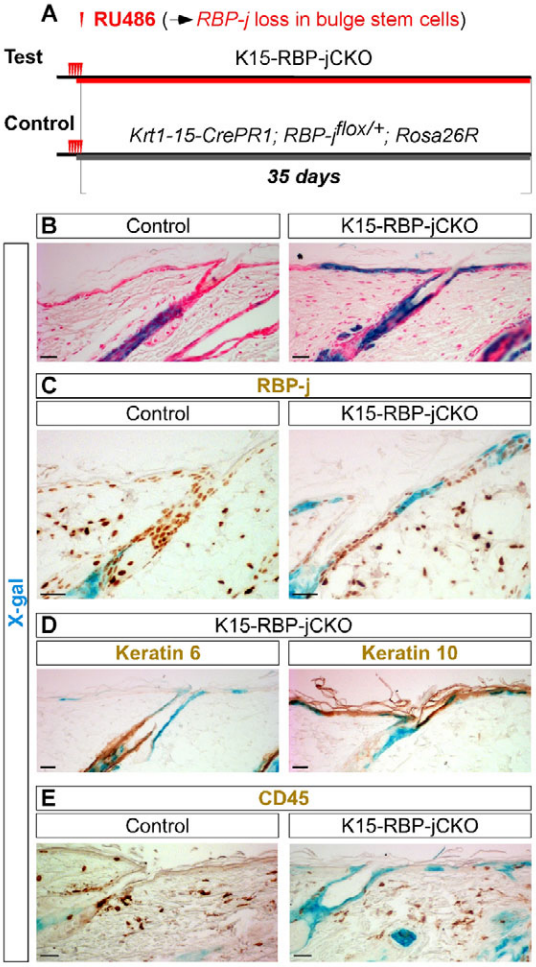

Fig. 4. Hair follicle stem cells lacking Notch signaling contribute to epidermal homeostasis under normal conditions. (**A**) Diagram of the approach used to delete *Rbpj* in adult hair follicle stem cells during the second telogen (~P50) and to determine their fate 35 days later without depilation. (**B**) X-Gal staining of the skin shows that blue cells migrate up from the bulge to form epidermis in K15-RBP-jCKO mice. Note that no blue clones emanating from hair follicles are found in the RU486-treated control animals (counterstain is Nuclear Fast Red). (**C**) Rbpj antibody staining confirms that most (>90%) blue cells in K15-RBP-jCKO are Rbpj-deficient. The few Rbpj-positive blue cells in K15-RBP-jCKO epidermis either responded to a signal produced by Rbpj-deficient cells or followed deleted cells in their migration towards the epidermis (a group effect). (**D**) Immunohistochemical staining for keratin 6 (ORS marker) and keratin 10 (epidermal marker) demonstrate that blue Rbpj-deficient bulge stem cells have differentiated into epidermal keratinocytes. (**E**) The leukocyte content of K15-RBP-jCKO dermis is comparable to that of control dermis 35 days after Cre induction. The CD45 pan-leukocyte marker is used to demonstrate the absence of inflammation in K15-RBP-jCKO skin at the location where Rbpj-deficient bulge cells have migrated into epidermis. In C-E, no counterstaining was performed; the genotype of control mice is shown in A. Scale bars: 25 μm.

material) (Ito et al., 2005; Levy et al., 2005), these observations demonstrate that Notch signaling acts to prevent hair follicle stem cells or their uncommitted descendents from randomly adopting the epidermal fate. How wounds reverse this block (Ito et al., 2005) remains an interesting question to be investigated.

Conclusions

In summary, and in contrast to Wnt signaling (Huelsken et al., 2001), neither arm of Notch signaling is required for follicular fate selection by bulge stem cells. Instead, Rbpj-dependent Notch signals restrict bulge cells (or their uncommitted, migratory descendents) to the follicular fate. In addition to cells selecting the follicular fate, a substantial fraction of Notch/Rbpj-deficient stem cells produce descendents that spontaneously choose the epidermal fate and migrate upwards, joining the interfollicular epidermis and producing epidermal cells deficient in terminal differentiation. The hair follicles formed by Notch-deficient stem cells properly associate with dermal papillae, produce a bulb expressing hair keratins, but fail to maintain the identity of IRS cells and medulla (Pan et al., 2004). Thus, both during hair follicle development and regeneration, Notch contributes to terminal differentiation. The progressive transformation of hair follicles to epidermal cysts caused by a stepwise reduction in Notch dosage is the direct result of hair shaft disintegration due to increasingly defective terminal differentiation.

This report identifies Notch signaling as a novel regulator of bulge stem cell fate selection, acting to constrain this bi-potential cell to the hair follicle fate. The ability of Notch/Rbpj-deficient stem cells to enter both hair follicle and epidermal fates under the normal homeostatic state with similar probability indicates a stochastic fate-selection process. This is in contrast to the classical role for Notch in fate selection as seen in the fly neuroectoderm, where a default fate (sensory organ precursor fate) is selected by all Notch-deficient cells owing to the presence of a dominant factor (Achaete-Scute proteins) (Simpson, 1997). The identities of molecules governing this stochastic fate-selection process in mouse bulge stem cells, and the details of how Notch constrains the fate choice, remain to be determined.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/136/6/891/DC1

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