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## *Review*

# The Notch pathway: hair graying and pigment cell homeostasis

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**Summary.** The Notch signaling pathway is an essential cell-cell interaction mechanism, which regulates processes such as cell proliferation, cell fate decisions, differentiation or stem cell maintenance. Pigmentation in mammals is provided by melanocytes, which are derived from the neural crest, and by the retinal pigment epithelium (RPE), which is part of the optic cup and hence orginates from neuroectoderm. The importance of functional Notch signaling in melanocytes has been unveiled recently. Here, the pathway is essential for the maintenance of proper hair pigmentation. Deletion of Notch1 and Notch2 or RBP-Jκ in the melanocyte lineage resulted in a gene dosage-dependent precocious hair graying, due to the elimination of melanoblasts and melanocyte stem cells. Expression data support the idea that Notch signaling might equally be involved in development of the RPE. Furthermore, recent analyses indicate a possible role of Notch signaling in the development of melanoma. In this review, we address the essential role of Notch signaling in the regeneration of the melanocyte population during hair follicle cycles, and discuss data supporting the implication of this signaling pathway in RPE development and melanoma.

**Key words:** Melanocytes, Notch, RPE, Knockout, Melanoma, Graying

### Introduction

In animals, pigment cells are essential for coloration, as seen in feathers, fur, skin and eyes. Their main function is to produce melanin, a major contributor to skin color besides carotenoids and hemoglobin. In mammals, a minor population of pigment cells originates from the optic cup of the developing forebrain and forms the retinal pigment epithelium (RPE), a cell monolayer lying between the choroid and the photoreceptor cells (reviewed in Martinez-Morales et al., 2004). In the mouse, eye development begins at around embryonic day (E)7.5, with an optic cup and a presumptive RPE clearly detectable between E9.5 and E10.5 (reviewed in Bharti et al., 2006). In contrast, melanocytes differentiate from pluripotent neural crest cells at about E8.5 in mice, migrate along the dorsolateral pathway and subsequently proliferate through the dermis horizontally to the ventral region (Yoshida et al., 1996; reviewed in Yoshida et al., 2001). By E14.5, melanocytes exit from the dermis and invade into the epidermis to finally be located in the skin and the hair follicles. Additionally, some melanocytes remain dermal, as seen in ear skin, and more rarely in interfollicular dermis, or are found in the eye (choroid, iris, ciliary body), the brain (leptomeninges) and the inner ear (cochlea) (Mayer, 1973).

During morphogenesis, melanocyte precursors migrate into developing hair follicles and segregate into two populations: differentiated melanocytes that localize in the bulb where they actively produce and transport pigment into the keratinocytes that form the hair shaft (Botchkareva et al., 2003), and melanocyte stem cells that colonize the bulge region at the bottom of the permanent portion of the hair follicle, just below the sebaceous gland (Fig. 1) (Nishimura et al., 2002; reviewed in Reya and Clevers, 2005). Hair follicles are in a continuous cycle, alternating periods of growth (anagen), regression (catagen), and rest (telogen), in which the hair is released allowing a new cycle to begin. During the anagen phase, melanocytes arising from the bulge migrate along the ORS (outer root sheath) to colonize the hair matrix, thus regenerating the pool of differentiated melanocytes that deliver melanin to keratinocytes while new hair is being synthesized (Fig.

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1). In regions without hairs, melanoblasts stay immature on the basement membrane of the epidermis until they are stimulated by keratinocytes to differentiate into mature pigment cells. These surrounding keratinocytes have been proposed to regulate melanoblast homeostasis through cell-cell interactions; however, the contributing factors remain largely unknown (reviewed in Haass et al., 2005).

Over the past years, an increasing number of genetic loci (more than 120) were identified as coat color loci in the mouse, affecting pigmentation of hair, skin, and/or eyes (reviewed in Bennett and Lamoreux, 2003). In more than 60 loci, the corresponding genes have been identified. The proteins encoded by such genes affect diverse processes, such as the development of melanocytes, the processing and transport of melanosomal components and melanosomes, or the synthesis of melanin and its transfer from melanocytes to surrounding keratinocytes. In melanin biosynthesis, the transcription factor Mitf (Microphthalmia) plays a pivotal role in regulating pigment cell-specific genes containing E-boxes in their promoter region (reviewed in Steingrimsson et al., 2004; Levy et al., 2006). Amongst these genes are *Tyr* (Tyrosinase), *Tyrp1* (Tyrosinase related protein 1) and *Dct* (Dopachrome tautomerase), which catalyze the multi-step transformation of tyrosine into melanin pigments, the eumelanin and the pheomelanin (reviewed in Murisier and Beermann, 2006). Functionally, these processes occur in melanosomes, lysosome-related organelles dedicated to the synthesis and storage of the pigment. These trafficking organelles move from the perinuclear region to the dendrites of melanocytes where they are transferred to surrounding keratinocytes, which then distribute them in pigmented areas (reviewed in Hearing, 2005). In response to UV irradiation, the melanosomes are transported from the keratinocyte periphery to be positioned above the nuclei as a “sunshield”.

## Hair graying and follicular melanocytes

Hair graying is a natural process which occurs with aging in humans. This change in hair color, from natural color to gray and then to white, is caused by the gradual decrease of pigmentation that occurs when melanin ceases to be produced in the hair root, for example due to a loss of melanocytes, and, in consequence, new hairs have few or no pigments (Commo et al., 2004). Among the large variety of genetic loci in the mouse which are implicated in hair color, some encode for genes involved in the maintenance of melanocyte stem cells. The constitutive or conditional absence of these genes in the melanocyte lineage leads to precocious hair graying. This is due to the lack of newly differentiated melanocytes that can invade the hair bulb during the new hair cycle (reviewed in Steingrimsson et al., 2005). For example, the Bcl2 and Mitf genes have been suggested to be involved in the process of graying. Bcl2$^{-/-}$ mice are normally pigmented at birth, but turn gray at the second hair follicle cycle due to a perinatal loss of epidermal and bulge melanoblasts, which is caused by selective apoptosis of melanocyte stem cells at their entry into the dormant state (Nishimura et al., 2005; Mak et al., 2006). In contrast, in Mitf$^{vit/vit}$ mutants, the gradual decrease of melanocyte stem cells is caused by premature differentiation at early-mid anagen of the third hair cycle, and thus preceded by the appearance of unexpected pigment-producing cells in the bulge region (Nishimura et al., 2005). Interestingly, such aberrant pigmentation in the bulge seems to precede age-related depletion of the melanocytic population (Nishimura et al., 2005). The occurrence of hair graying is an interesting phenomenon and is attractive to experimental testing, since effects are easily visible. Once regulatory pathways and genetic interactions are identified, it might

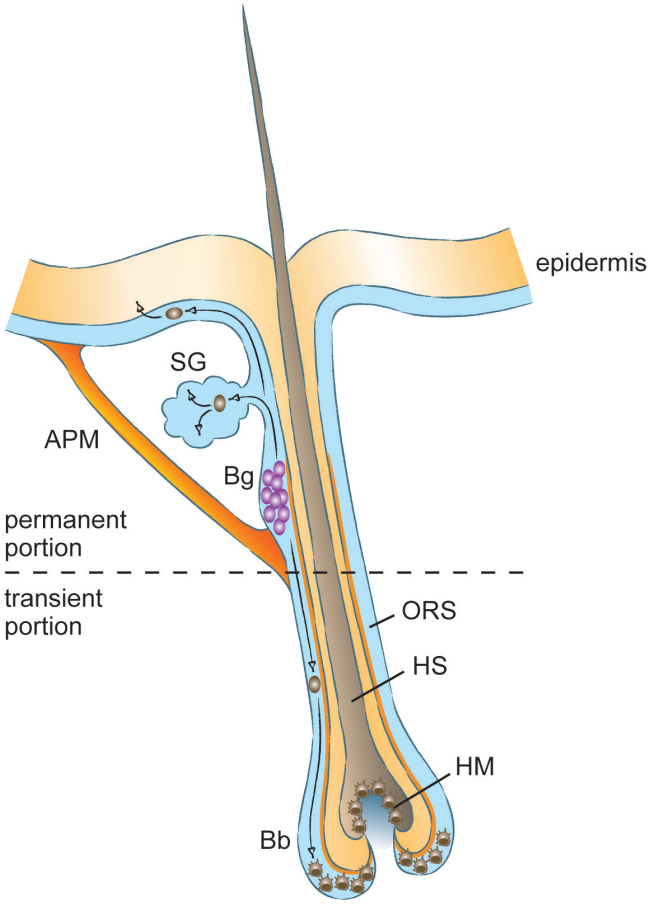


**Fig. 1.** Hair follicle structure. Hair follicle stem cells (purple), that consist of epithelial and melanocyte stem cells, are located in the lower permanent portion of the hair follicle, known as the bulge region. During hair growth, the melanocyte stem cells migrate from the bulge along the outer root sheath to colonize the hair matrix in the bulb. Mature melanocytes then transfer the melanin to the keratinocytes that will form the pigmented hair shaft. ORS, outer root sheath; Bg, bulge; Bb, bulb; HS, hair shaft; HM, hair matrix; SG, sebaceous gland; APM, arrector pilli muscle (adapted from Reya and Clevers, 2005).

also be amenable to treatment. Obviously, this is of great interest for the cosmetic industry, which is eager to develop products that are able to prevent graying in human and thus to contribute to "eternal youth".

### The Notch signaling pathway

The Notch signaling pathway is a cell-cell interaction mechanism that has been highly conserved throughout the animal kingdom (reviewed in Greenwald, 1998). All receptors and ligands are single-pass transmembrane proteins with large extracellular domains that consist primarily of epidermal growth factor (EGF)-like repeats (Fig. 2). The number of ligands and receptors differs between different species. For instance, signaling in *Drosophila* is triggered by one Notch receptor and two ligands (Delta and Serrate) while two receptors (LIN-12 and GLP-1) signal through four different ligands (APX-1, LAG-2, ARG-1 and DSL-1) in *Caenorhabditis elegans*. In mammals, four Notch receptors (Notch1-4) and five ligands (Jagged-1 and 2, and Delta-like [Dll] 1, 3 and 4) have been described (reviewed in Radtke et al., 2005; Bray, 2006). The interaction between ligand and Notch receptor results in two successive proteolytic cleavages. The first cleavage occurs extracellularly and is initiated by a metalloprotease of the ADAM family (TACE, tumor necrosis factor-α-converting enzyme). This allows the second cleavage to take place at the transmembrane domain, mediated by a protein complex with γ-secretase activity (presenilin, nicastrin, APH1, and PEN2) (reviewed in Fortini, 2002). The released intracellular domain of Notch (NotchIC) then translocates to the nucleus where it binds to CSL transcription factors (CBF1 in human, Suppressor of Hairless in *Drosophila* and LAG in *C. elegans*, also known as RBP-Jκ in mouse) and thereby activates transcription of Notch target genes (Fig. 2).

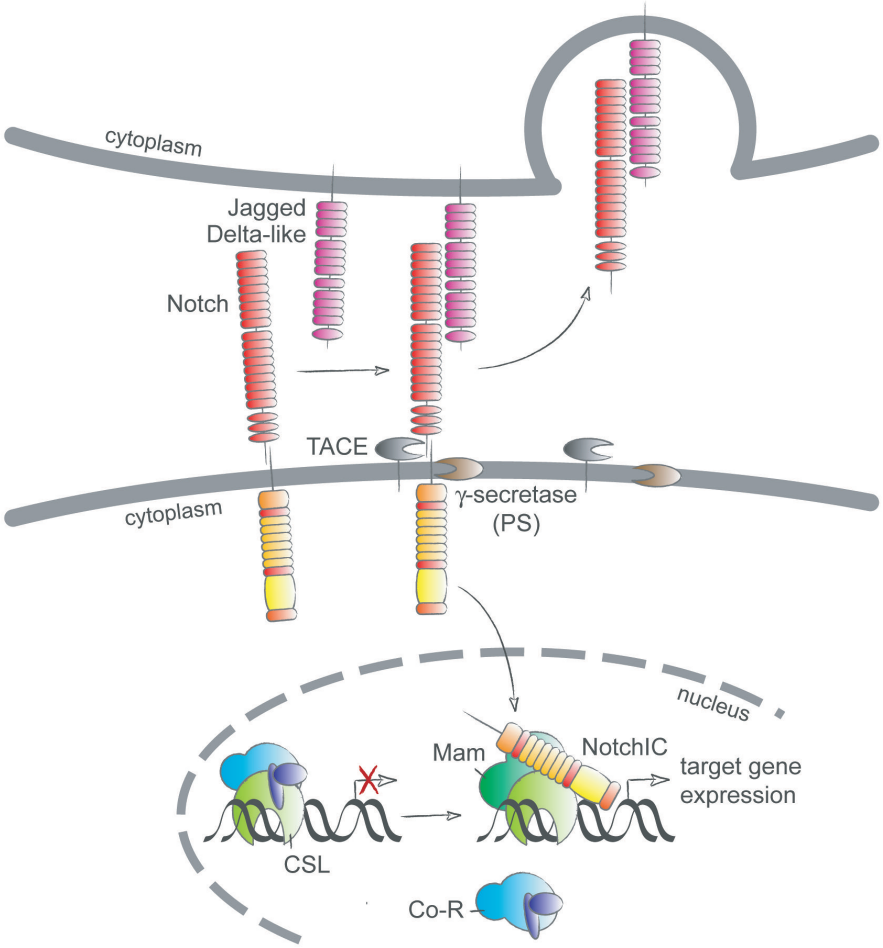


**Fig. 2.** The Notch signaling pathway. Interactions between the Notch receptors (red/yellow) and their ligands, such as Delta-like or Jagged (purple), result in a succession of two proteolytic cleavages. The TACE (TNF-α-converting enzyme) metalloprotease (gray) catalyzes the first cleavage, generating the substrate for the second cleavage by the γ-secretase activity of presenilin (PS; brown). The resulting free intracellular subunit of Notch (NotchIC; yellow) then translocates to the nucleus and associates with the DNA-binding CSL transcription factor (light green). While co-repressors (blue) are displaced, co-activators such as Mastermind (Mam; green) are recruited to the CSL-NotchIC complex to activate transcription of target genes. Subsequently to the cleavages, the extracellular domain of Notch is "trans-endocytosed" by the ligand-expressing cell.

The Notch signaling pathway has been demonstrated to play critical roles in many biological processes, such as cell proliferation, differentiation, apoptosis, or stem cell maintenance (reviewed in Artavanis-Tsakonas et al., 1999; Radtke et al., 2005; Wilson and Radtke, 2006). During embryonic and postnatal development, Notch signals are involved in cell differentiation by either influencing binary cell fate decisions of progenitor cells or inducing terminal differentiation of a particular cell lineage. Examples include the nervous system, the hematopoietic system, or the pancreas (reviewed in Hansson et al., 2004). In particular, Notch signaling has been shown to inhibit neurogenesis while promoting glial cell fate (reviewed in Lundkvist and Lendahl, 2001; Gaiano and Fishell, 2002; Ge et al., 2002; Lai, 2004). Finally, Notch signaling might be of importance in maintenance and self-renewal of stem cells (reviewed in Radtke et al., 2005; Wilson and Radtke, 2006). It can thus be concluded that diverse and sometimes opposing roles of the Notch pathway exist and are functional in a context-dependent manner.

In some instances, the different Notch receptors display redundant functions. Notch3 homozygous mutant embryos fail to develop a severe phenotype. Hence, it was suggested that the Notch3 deficiency might be compensated largely by expression of either Notch1 and/or Notch2 in many Notch3-expressing tissues (Krebs et al., 2003; Kitamoto et al., 2005). In angiogenic vascular remodeling, embryos homozygous for mutations of both *Notch1* and *Notch4* genes often displayed a slightly more severe phenotype than Notch1 homozygous mutant embryos (Krebs et al., 2000). Moreover, *Notch1* and *Notch2* had mostly overlapping functions in the pigmentary system, with the combined deletion of both genes in the melanocyte lineage leading to a more severe phenotype (Schouwey et al., 2007).

This is in contrast with other systems where Notch1 and Notch2, although expressed in the same cells, have non-redundant functions. For example, in the skin, conditional deletion of Notch1 in the embryonic ectoderm (*Msx2::Cre/°; Notch1$^{flox/flox}$*) results in a mosaic pattern of hair growth, whereas Msx2::Cre/°; Notch2$^{flox/flox}$ mice are indistinguishable from wild-type mice (Pan et al., 2004). Other examples include the hematopoietic system, in which Notch1 plays a critical role in intrathymic T-cell development, whereas Notch2 signaling is essential for development of marginal zone B-cells (reviewed in Robey and Bluestone, 2004; Wu, 2006).

### The Notch signaling pathway in melanocytes

Until a few years ago, and in contrast to other organisms or organ systems, nothing was known on Notch signaling in the mammalian pigmentary system. It was only recently that the involvement of Notch signaling in hair and skin pigmentation as well as in melanoma began to be addressed.

In mouse vibrissa follicles - not distinguishing keratinocytes from melanocytes -, expression of *Notch1* and *Notch2* as well as of several ligands was found from embryonic to adult stages (Powell et al., 1998; Favier et al., 2000). During follicle morphogenesis, *Notch1* is expressed in the epithelial cells of the hair plug, in the dermal condensation, as well as in the interfollicular epidermis. At later stages, the activated protein is absent in the inner root sheath (IRS) and the dermal papilla, but is detected in the suprabasal and basal layers of the outer root sheath (ORS) and epidermis that include Dct-positive melanoblasts (Lin and Kopan, 2003; Moriyama et al., 2006). Similarly to Notch1 activation, *Jagged2* is expressed in the basal layer of the epidermis. *Notch2* and *Delta1* are expressed complementarily during embryogenesis, with the ligand being found in the dermal condensation, and the receptor in the interfollicular dermis. After birth however, no specific expression pattern was detected for Notch2, whereas Notch1 is expressed during anagen in epithelial cells of the ORS and the hair matrix, with the exception of cells above the dermal papilla. In contrast, all cells of the hair matrix and those located above the papilla are positive for Notch1 in catagen (Favier et al., 2000).

It should be noted that most of these data refer to the hair follicle *in toto* and do not specify an expression in melanocytes. Due to the relative difficulty in isolating or detecting melanocytes in situ, expression data are rare. Immunofluorescence staining revealed the presence of Notch1 in Dct-positive melanoblasts (Moriyama et al., 2006). In FACS-purified melanoblasts from the E16.5 epidermis, Hes1 was identified as the major Notch target gene, whereas Hes5 and Hey1 expression seems to be of less importance (Moriyama et al., 2006). In addition, Notch and Hes1 expression was recently demonstrated to be the highest in FACS-purified melanocyte stem cells, as compared with embryonic melanocytes as well as melanocytes of the hair matrix of adult follicles (reviewed in Nishikawa and Osawa, 2007). Separate ex vivo analyses equally showed that members of the Notch signaling pathway are expressed in normal mouse and human melanocytes (Hoek et al., 2004; reviewed in Haass and Herlyn, 2005). Transgenic and knockout mice were instrumental in further analyzing the Notch signaling pathway in the pigmentary system. Since Notch3$^{-/-}$ and Notch4$^{-/-}$ mutant mice were described as viable and without any coat color phenotype (Krebs et al., 2000, 2003; reviewed in Louvi and Artavanis-Tsakonas, 2006), it was concluded that these two receptors are of reduced or no importance for melanocytes and dispensable for pigmentation. In contrast, constitutive deletion of RBP-Jκ, Notch1 and Notch2 results in embryonic lethality (reviewed in Louvi and Artavanis-Tsakonas, 2006). Therefore, to address the disruption of Notch signaling in melanocytes, mice carrying floxed alleles of Notch1, Notch2 and RBP-Jκ (Radtke et al., 1999; Tanigaki et al., 2002; Besseyrias et al., 2007) were mated to Tyr::Cre/° mice, which express the Cre recombinase specifically in the melanocyte lineage from E10.5 (Delmas et al., 2003).

Due to a dramatic reduction of melanoblasts and follicular melanocytes, the first coat of mice with conditional deletion of RBP-Jκ was a mixture of pigmented and unpigmented hairs. Subsequently, melanocytes completely disappeared from the hair follicles, thus rendering almost all the hairs unpigmented (Fig. 3 H) (Moriyama et al., 2006). In addition, treatment of skin with DAPT, a γ-secretase inhibitor that blocks Notch signaling, induced apoptosis in melanoblasts and thus led to development of unpigmented hairs. This DAPT-induced hair graying was rescued by the expression of the Notch target Hes1 in melanocytes of Dct::Hes1 transgenic mice, thus arguing for the essential role of Notch signaling in the maintenance of melanocyte stem cells in the bulge (Moriyama et al., 2006). However, this effect of RBP-Jκ ablation did not address the contribution of the individual Notch receptors. Conditional deletion of Notch1 and Notch2

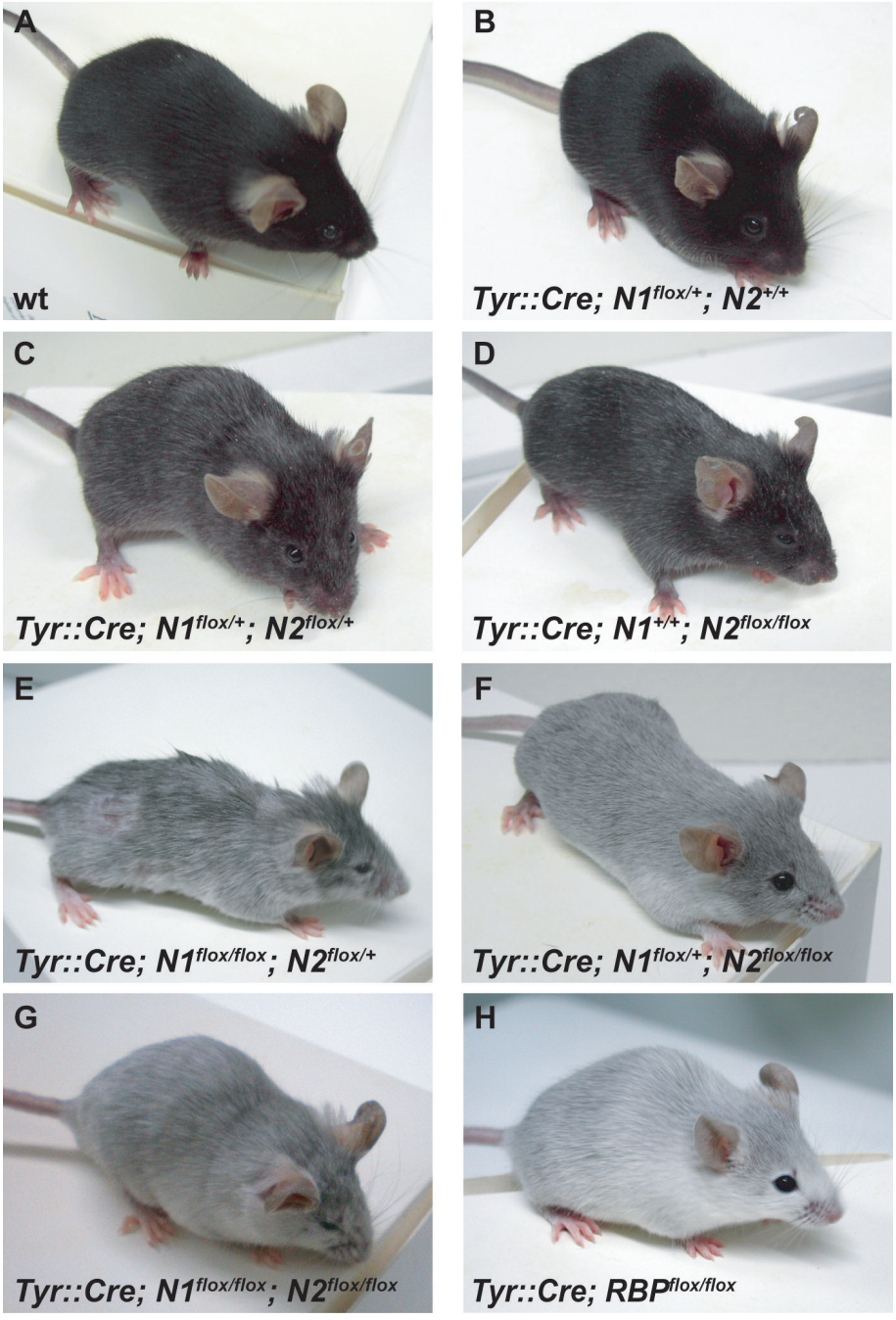


**Fig. 3.** Notch1 and Notch2 signaling through RBP-Jκ is essential for the maintenance of hair pigmentation with an effect of gene dosage. Melanocyte-specific deletion of a single Notch allele is not sufficient to induce a coat color phenotype (**B**, *Tyr::Cre/°; Notch1$^{flox/+}$; Notch2$^{+/+}$*) and mice are pigmented as controls not expressing the *Tyr::Cre* transgene (**A**). In contrast, mice with conditional deletion of two Notch alleles (**C**, *Tyr::Cre/°; Notch1$^{flox/+}$; Notch2$^{flox/+}$*) display a scattered hair graying that is more pronounced in *Tyr::Cre/°; Notch1$^{+/+}$; Notch2$^{flox/flox}$* mice (**D**) than in *Tyr::Cre/°; Notch1$^{flox/flox}$; Notch2$^{+/+}$* mice (not shown, gray hairs not yet visible at 8 weeks). A more intense coat color dilution results from the recombination of three Notch alleles in melanocytes, with a completely gray coat observed in *Tyr::Cre/°; Notch1$^{flox/flox}$; Notch2$^{flox/+}$* (**E**) and *Tyr::Cre/°; Notch1$^{flox/+}$; Notch2$^{flox/flox}$* (**F**) mice. Finally, disruption of Notch signaling by removing RBP-Jκ in *Tyr::Cre/°; RBP-Jκ$^{flox/flox}$* mice (**H**) leads to a coat color phenotype similar to mice with inactivation of all four Notch alleles (**G**, *Tyr::Cre/°; Notch1$^{flox/flox}$; Notch2$^{flox/flox}$*). All mice were 8 week-old and kept on a mixed genetic background with > 75% contribution of C57BL/6. They were all pigmented (*Tyr$^{+}$*), nonagouti (*a*) and black (*Tyrp1$^{+}$*).

revealed that both are required for proper hair pigmentation with an effect of gene dosage (Schouwey et al., 2007). Three intact alleles of *Notch1* and Notch2 were necessary to prevent precocious hair graying (Fig. 3). Even though Notch2 seemed to be more relevant for the maintenance of melanocyte stem cells and hair pigmentation, the combined deletion of both *Notch1* and *Notch2* led to a more severe phenotype than a single mutation, suggesting that both genes have mostly overlapping functions in the pigmentary system.

In contrast to follicular melanocytes, the pigmentation produced by neural crest-derived pigment cells located in the dermis and the choroid layer was not affected by the *Tyr::Cre/°*-mediated disruption of Notch signaling (Schouwey et al., 2007). Although Notch1 and Notch2 signaling through RBP-Jκ is required for survival of melanoblasts and melanocyte stem cells (Fig. 4), it could be postulated that these populations of non-follicular melanocytes are not regenerated by stem cells and thus not dependent on Notch signaling.

In contrast to stem cells, neither survival nor functionality of differentiated melanocytes seemed to be affected by the disruption of the Notch signaling pathway in follicular and non-follicular melanocytes (Fig. 4) (Schouwey et al., 2007). Indeed, in wild-type mice, the Notch signaling pathway was shown to be inactive in the differentiated melanocytes at the hair matrix, while transcription of Hes1 as well as activation of Notch1 were detected in melanoblasts at the lower permanent portion (LPP) of hair follicles (Moriyama et al., 2006).

The Notch signaling pathway is implicated, amongst others, in cell proliferation, migration and differentiation (reviewed in Radtke and Raj, 2003; Radtke et al., 2005; Chiba, 2006). In the mouse, melanocytes proliferate and migrate through the dermis from E8.5 and then invade into the epidermis between E12.5 and E14.5 (Yoshida et al., 1996; reviewed in Yoshida et al., 2001). Thus, most of this occurs before the Notch signaling pathway is disrupted by expression of Cre recombinase from Tyr::Cre/° mice. Additional experiments are needed to address whether Notch signaling is required at earlier stages of development, for differentiation, proliferation and/or migration of melanoblasts.

## RPE and Notch signaling

The retinal pigment epithelium (RPE) is a highly polarized and specialized pigmented cell monolayer that is placed at the interface between the photoreceptors of the neural retina and the choroid layer in the vertebrate eye. During embryogenesis, RPE cells that are generated directly from the optic neuroepithelium participate in the development of various structures of the eye (reviewed in Chow and Lang, 2001; Martinez-Morales et al., 2004; Bharti et al., 2006). In the adult, they provide nutritional support to retinal visual cells, form a blood/retinal barrier, are involved in retinoid metabolism, control water and ion flow between the neural retina and the choroid, protect against oxidative damage, and phagocyte the outer segments of the photoreceptors, thereby ensuring their renewal. As well as the inner neural retina, the outer retinal pigment epithelium is largely developed by the early postnatal period, with the number of RPE cells increasing about 4-fold between E15 and postnatal day (P)15 (Bodenstein and Sidman, 1987). However, the number of mitotic cells decreases considerably from E13 to P15 and the adult mammalian eye is deprived of retinal stem cells. Thus neither the neural retina nor the RPE show evidence for adult regeneration (Tropepe et al., 2000).

Notch signaling has been proposed to be involved in the patterning of the eye as well as in cell fate determination and differentiation (Bao and Cepko, 1997). During rat eye development (E12.5, E15.5), *Notch1* and *Delta* are expressed within undifferentiated progenitor cells of the neural retina. In contrast, *Notch2* was only found in the non-neuronal derivatives of the optic cup, including the RPE, the optic stalk, and the ciliary margin (Bao and Cepko, 1997). Moreover, one of the downstream target genes of RBP-Jκ-dependent Notch signaling, *Hes1*, is expressed in the RPE and seems to affect at least early RPE development (Lee et al., 2005).

In addition, Numb, a known inhibitor of the Notch signaling pathway, is preferentially distributed to the apical daughter cell during the asymmetric vertical division of rat retinal neuroepithelial cells (Cayouette et al., 2001). The proportion of these vertically dividing cells, which is maximal (20%) at P0, depends on the underlying RPE, and changes during development, thus suggesting an involvement of Numb and Notch signaling in the development of the neural retina and the RPE. The putative role of Notch signaling in the RPE could be addressed in future using mice carrying floxed alleles of RBP-Jκ, *Notch1* and *Notch2*, and transgenic for a Cre recombinase expressed in the RPE (Mori et al., 2002).

## Putative role of Notch signaling in melanoma development

In addition to embryonic and adult development, it has been suggested that the Notch signaling pathway is also involved in tumorigenesis, as aberrant Notch signaling is frequently observed in several cancers. Depending on the cell type and context, Notch can either promote cell proliferation and cancer growth, or act as a tumor suppressor (reviewed in Radtke and Raj, 2003; Wilson and Radtke, 2006). The oncogenic property of Notch was first described in T-cell acute lymphoblastic leukemias (T-ALL) (Ellisen et al., 1991) and activating mutations of Notch1 are actually found in more than 50% of all cases (Weng et al., 2004). Moreover, abnormal signaling through Notch receptors was implicated in small cell lung cancer (Sriuranpong et al., 2001), neuroblastoma (Gestblom et al., 1999; Grynfeld et al., 2000), and cervical (Zagouras et al., 1995; Talora et al., 2002) and prostate (Shou et al., 2001) carcinomas. In keratinocytes however, Notch signaling acts as a tumor suppressor and induces cell growth arrest and

differentiation of squamous epithelia (Lowell et al., 2000; Rangarajan et al., 2001). Consequently, deletion of Notch1 in mouse epidermis results in hyperproliferation of the basal epidermal layer and renders mice susceptible to skin carcinoma (Nicolas et al., 2003).

Melanoma is a malignant tumor of melanocytes, which causes the majority of skin cancer-related deaths (48'000/year). Its incidence is increasing, with an estimated 132'000 new cases of melanoma diagnosed worldwide each year (World Health Organization, http://www.who.int/topics/melanoma/en/). Melanoma development and progression is a step-wise process

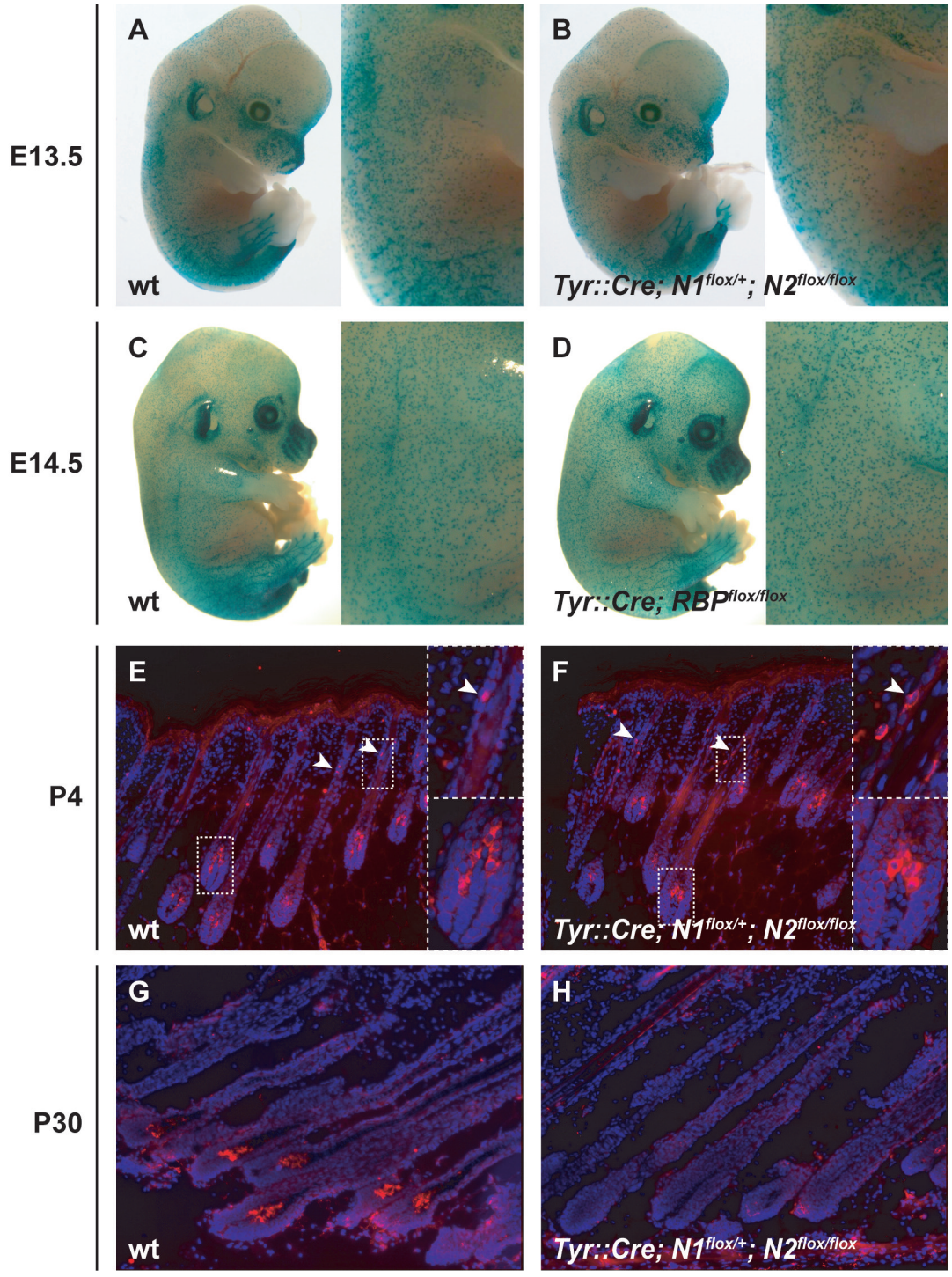


**Fig. 4.** Melanocyte-specific deletion of Notch1 and Notch2 or RBP-Jκ does not lead to early congenital defects but to the elimination of melanocytes in mutant hair follicles after birth. At embryonic stages, the density and the migration of melanoblasts visualized by *Dct::LacZ* staining (MacKenzie et al., 1997; Schouwey et al., 2007) are similar in control **(A)** and *Tyr::Cre/°; Notch1$^{flox/+}$; Notch2$^{flox/flox}$* (**B**) embryos at E13.5, as well as in control (**C**) and *Tyr::Cre/°; RBP-Jκ$^{flox/flox}$* (**D**) embryos at E14.5. Immediately after birth, at P4, melanocytes are still present, when comparing dorsal skin of control (**E**) and *Tyr::Cre/°; Notch1$^{flox/+}$; Notch2$^{flox/flox}$* (**F**) genotype. Immunohistochemistry using anti-Dct (PEP8) antibody revealed *Dct*-expressing melanocytes in the bulb and also detected some cells in the bulge region of control and mutant hair follicles (**E**, **F**, arrow heads). After the first regeneration of hair follicles, dorsal skin analyzed by immunohistochemistry at one month (P30) showed the obvious absence of Dct-expressing melanocytes in hair follicles of *Tyr::Cre/°; Notch1$^{flox/+}$; Notch2$^{flox/flox}$* mice (**H**) while some positive cells were clearly detected in hair bulbs of control mice (**G**).

(reviewed in Chin, 2003). While common acquired and congenital nevi have structurally normal melanocytes, dysplastic nevi with structural and architectural atypia may evolve to a radial growth phase (RGP), where primary melanomas have not yet acquired metastatic competence. In the next step, the vertical growth phase (VGP), tumorigenic primary melanomas are competent for metastasis, and finally they metastasize and cause secondary tumors (Balint et al., 2005; reviewed in Haass and Herlyn, 2005). Amongst others, melanoma cells are characterized by their independence from growth factors and their escape from control by keratinocytes as well as growth inhibitory factors.

Abnormalities in different signaling pathways in melanocytes have been proposed to be responsible for melanoma induction and progression (reviewed in Chin et al., 2006). These include B-Raf (Davies et al., 2002) and N-Ras (Ball et al., 1994) which are amongst the most common mutated genes found in human melanoma (B-Raf, >50%; N-Ras, around 20%) (Forbes et al., 2006; reviewed in Schubbert et al., 2007), as well as p16INK4A (Cannon-Albright et al., 1992), p53/Apaf-1 (Soengas et al., 2001), PTEN/Akt (Robertson et al., 1999), cyclin D1/cyclin-dependent kinase 4 (Wolfel et al., 1995; Sauter et al., 2002), Wnt5a (Weeraratna et al., 2002), and Grm-1 (Pollock et al., 2003).

Recently, experimental evidence supported the notion that activated Notch signaling might be an essential event in the development of melanoma. Initial experiments demonstrated that Notch receptors and target genes are upregulated in melanoma cell lines compared to normal melanocytes (Hoek et al., 2004; reviewed in Nickoloff et al., 2005). Using immunohistochemistry and RT-PCR analyses, it was shown that *Notch1, Notch2* and the ligands *Jagged-1, Jagged-2* and *Delta-like* 1 were overexpressed in dysplastic nevi and melanomas, compared to common melanocytic nevi and melanocytes (Balint et al., 2005; Massi et al., 2006). Moreover, primary melanoma cells were described to have acquired an enhanced metastatic ability when the Notch1 pathway was constitutively activated, an effect that was suggested to be mediated by ß-catenin (Balint et al., 2005).

*In vitro* and *in vivo* analyses reinforced this oncogenic role of Notch1 in melanoma survival, growth and progression, and showed that this effect is mediated through the MAPK and PI3K-Akt pathways (Liu et al., 2006). Moreover, signaling through Notch1 was demonstrated to promote the expansion of vertical growth phase (VPG) melanoma cells, to increase N-cadherin expression and to enhance tumor cell adhesion. Another study reported that the Notch target Hes1 downregulates the expression of microtubule-associated protein 2 (MAP2) in primary melanoma cell lines (Bhat et al., 2006). MAP2 is often activated in human cutaneous melanoma, and its expression inversely correlates with the aggressiveness of the tumor (Soltani et al., 2005). Moreover, inhibition of Notch signaling upregulated MAP2 promoter activity, with higher sensitivity in primary than in metastatic melanoma cells (Bhat et al., 2006).

Thus, activation or suppression of the Notch signaling pathway affected primary melanoma cell growth both *in vitro* and *in vivo* but had only little effect on metastatic melanoma cells. This suggests an importance of Notch signaling at early stages of melanocytic tumor development and thus places Notch as a putative therapeutic target in melanoma patients. One approach would be to block the generation of the activated NotchIC using γ-secretase inhibitors. Such inhibitors have been used in clinical trials to block the cleavage of the amyloid precursor protein (APP) that releases the amyloid ß-peptide, the precursor of amyloid plaques, in Alzheimer's diseases (reviewed in Shih and Wang, 2007). The antitumor effect of such compounds remains to be confirmed and has to be fine-tuned to balance the damaging consequences that they could have on the wide-range physiological processes that require Notch signaling.

## Concluding remarks

The Notch signaling pathway is required for development and maintenance of the melanocyte lineage, and thus is essential for hair pigmentation. The effect is due to the disappearance of melanocyte stem cells in the bulge, but it remains open whether this is due to apoptosis. This issue might be resolved by identification of the target genes of RBP-Jκ in the melanocytes, amongst which *Hes1* might be a strong candidate. Pigment cells in mammals not only exist as melanocytes, derived from the neural crest, but equally as cells of the retinal pigment epithelium (RPE), which originates from neuroectoderm. Both lineages share characteristics of pigment cells, such as melanogenic enzymes, but are subject to distinct regulation during development. Members of the Notch pathway are expressed in the RPE, and future experiments will show how the putative role of Notch signaling in RPE can be compared to melanocytes. Finally, there is increasing evidence that Notch acts as an oncogene in the development of melanomas, with several receptors, ligands as well as target genes upregulated at early stages of melanocyte transformation and tumor progression. Open questions concerning the targets of Notch signaling in melanocytes, melanoma and RPE cells, as well as the genetic context, timing and requirement for Notch signaling in melanoma remain to be addressed to understand the contribution of this classical signaling pathway in pigment cell homeostasis.

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