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Dedicated Epithelial Recipient Cells Determine Pigmentation Patterns

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SUMMARY

Mammals generate external coloration via dedicated pigment-producing cells but arrange pigment into patterns through mechanisms largely unknown. Here, using mice as models, we show that patterns ultimately emanate from dedicated pigment-receiving cells. These pigment recipients are epithelial cells that recruit melanocytes to their position in the skin and induce the transfer of melanin. We identify Foxn1 (a transcription factor) as an activator of this "pigment recipient phenotype" and Fgf2 (a growth factor and Foxn1 target) as a signal released by recipients. When Foxn1—and thus dedicated recipients—are redistributed in the skin, new patterns of pigmentation develop, suggesting a mechanism for the evolution of coloration. We conclude that recipients provide a cutaneous template or blueprint that instructs melanocytes where to place pigment. As Foxn1 and Fgf2 also modulate epithelial growth and differentiation, the Foxn1 pathway should serve as a nexus coordinating cell division, differentiation, and pigmentation.

INTRODUCTION

It is well known that external coloration affects the survival and reproduction of animals, as pigmentation can provide camouflage, prevent damage from light, influence body temperature, and facilitate social interactions, such as the acquisition of mates. Consistent with these diverse functions, coloration itself can be strikingly diverse, as populations vary in both the types of pigment produced and the arrangement of pigments into patterns. For centuries, this diversity has attracted human interest, and in recent decades, much has been learned about the production of pigment. Nonetheless, much remains unknown about the patterning of pigmentation, in particular, how patterns become organized and new patterns emerge over time.

Mammals develop most of their coloration through a system comprised of two types of cells (reviewed in Slominski et al., 2004), referred to here as pigment donors and recipients. The pigment donors are melanocytes, which synthesize melanin in distinct organelles called melanosomes. The pigment recipients are epithelial cells, which acquire and hold most cutaneous melanin. As the system forms, each melanocyte extends dendrites and contacts multiple epithelial cells, creating a "pigmentary unit." Melanosomes are then transported along the dendrites and into the epithelial cells, which may internalize the melanosomes via phagocytosis. As donors connect directly to recipients, melanin is placed in precise locations, which often differ among or within species. In humans, pigment is targeted to the epidermis and a subset of hairs. In mice, the hair coat receives most of the melanin produced, as the coat-covered epidermis loses the melanocyte lineage in early life and thus remains unpigmented. Once inside epithelial cells, melanin plays site-specific physiological roles (e.g., UV protection in the case of human epidermis). Thus, in mammalian skin, pigmentary tasks are sharply divided, as one cell type creates pigment while another puts it to use.

With their melanosomes and dendrites, melanocytes are highly specialized cells and clearly built for the production and distribution of pigment. Accordingly, it may be asked whether epithelial cells are built for melanization, that is, whether pigment recipients are specialized counterparts to pigment donors. As the skin develops its particular pattern of coloration, some epithelial cells become melanized while others do not. At the morphological level, there is no basis for this difference, as epithelial cells produce no observable structures that facilitate or prevent pigmentation. At the functional level, there is little information about pigment recipients and no trait known to distinguish these cells from the unmelanized population. Thus, it has never been determined whether epithelial cells, like melanocytes, are specially dedicated to pigmentary functions. Moreover, little is known about the role of epithelial cells in the development of pigmentary interactions.

In particular, it has never been shown whether epithelial cells are primarily "reactive," acquiring pigment if a melanocyte offers it, or "proactive," recruiting melanocytes and inducing the transfer of pigment. Thus, the question arises as to how the skin forms pigmentary units, and by extension, what determines where pigment is placed.

Foxn1 (*Whn*, *Hfh11*) is a murine gene essential for the proper development of several epithelial tissues, including tissues with melanocyte populations. Its product has the properties of a transcriptional activator, as it contains a sequence-specific DNA-binding domain (Nehls et al., 1994; Schlake et al., 1997) and a negatively charged transactivation domain (Brissette et al., 1996; Schlake et al., 1997; Schüddekopf et al., 1996). In rodents, the loss of Foxn1 function results in the nude phenotype (Nehls et al., 1996, 1994), which is characterized by the lack of visible hair (Flanagan, 1966), defects in the epidermis (Köpf-Maier et al., 1990; Lee et al., 1999), impairments in mammary gland development (Militzer and Schwalenstocker, 1996; R. Baxter and J.L.B., unpublished), aberrant differentiation of nails (Mecklenburg et al., 2004), and absence of a thymus (Pantelouris, 1968). Human *FOXN1* is 86% identical to its murine counterpart (Schorpp et al., 1997), and a nonsense mutation in *FOXN1* is associated with T cell immunodeficiency, congenital alopecia, and nail dystrophy (Frank et al., 1999). Thus, the loss of FOXN1 activity leads to a disease that closely resembles the nude phenotype, demonstrating the functional conservation of the Foxn1 orthologs.

In this study, we identify novel functions for Foxn1 and epithelial cells in the development of coloration. At specific cutaneous sites, epithelial cells use Foxn1 to recruit melanocytes and induce their own pigmentation. Foxn1 thus defines a distinct cell population that ultimately controls the targeting of pigment in the skin.

RESULTS AND DISCUSSION

Foxn1 in Murine Skin

In mice, *Foxn1* expression appears restricted to epithelial cells and has been detected in various organs, such as the skin, thymus, mammary gland, and eye (Lee et al., 1999; Nehls et al., 1996). In the epidermis, Foxn1 is induced as cells lose the ability to divide and initiate terminal differentiation (Lee et al., 1999; Prowse et al., 1999). Hair follicles exhibit a comparable *Foxn1* expression pattern, as *Foxn1* becomes active during cellular transitions from proliferation to differentiation (Lee et al., 1999). Within epithelial cells, the Foxn1 protein localizes to the nucleus (Prowse et al., 1999), consistent with the actions of a transcription factor. As the epidermis matures, *Foxn1* activity mirrors the tissue's dynamics; the number of *Foxn1*-expressing cells peaks while the epidermis develops, then falls after birth as the tissue thins, fewer cells divide or differentiate, and melanoblasts disappear (Lee et al., 1999). A similar correlation exists between the transcription of *Foxn1* and the dynamics of the hair cycle (Lee et al., 1999; Meier et al., 1999). *Foxn1* expression peaks during anagen, when a hair is produced, then falls during catagen and telogen, when the follicle shortens, becomes quiescent, and loses its differentiated melanocytes.

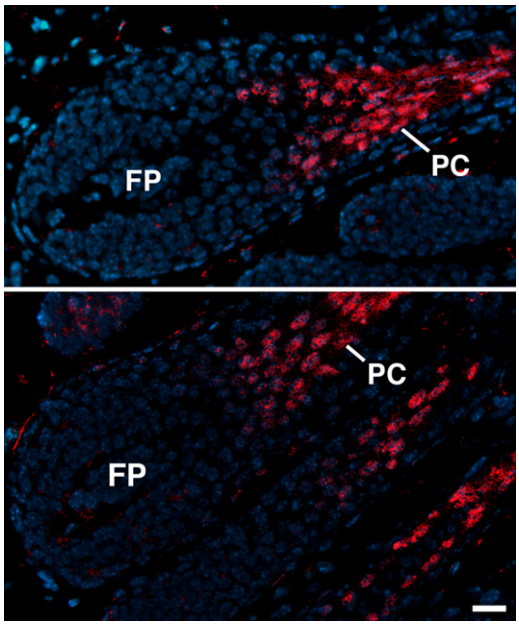

Figure 1. *Foxn1* Is Expressed in Melanocyte Target Cells of the Hair Follicle

Wild-type murine hair follicles are shown at postpartum day 9 (P9). Foxn1 was stained by immunofluorescence (red). DNA was stained with Hoechst dye 33258 (blue). PC, precursor cells of the hair cortex; FP, follicular papilla. The scale bar represents 20 μm.

Figure 1 shows immunofluorescent staining for Foxn1 as a hair grows from a mature follicle. Foxn1 was detected primarily in the differentiating precursors of the hair cortex, which generate the main structural support for the hair shaft and receive pigment from melanocytes. Foxn1 is normally present therefore in epithelial cells acquiring a keratinized and melanized phenotype. This localization of Foxn1 is consistent with the findings of previous studies, which detected the expression of the *Foxn1* promoter (Lee et al., 1999), the *Foxn1* mRNA (Lee et al., 1999; Meier et al., 1999), and an amplified *Foxn1* locus (Cunliffe et al., 2002) in the precortex.

Pigmentation and a Gain of Foxn1 Function

To elucidate the role of Foxn1 in epithelial morphogenesis, we generated transgenic mice that produce full-length Foxn1 from the promoter for keratin 5 (KRT5; Figure 2A) (Ohtsuki et al., 1992). This promoter is active in cutaneous epithelial cells capable of proliferation and lacking differentiated features. Thus, the transgene should misexpress *Foxn1* in epithelial progenitor populations, which like melanocytes typically border the dermis and attach to the basement membrane.

In general, the *Krt5-Foxn1* transgenics were healthy and fertile, though on rare occasions, a runted pup with flaky skin and sparse hair emerged from a backcross. While