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New activators and inhibitors in the hair cycle clock: targeting stem cells' state of competence

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Summary

The timing mechanism of the hair cycle remains poorly understood. However, it has become increasingly clear that the telogen-to-anagen transition is controlled jointly by at least the bone morphogenic protein (BMP), WNT, fibroblast growth factor (FGF), and transforming growth factor (TGF)-β signaling pathways. New research shows that Fgf18 signaling in hair follicle stem cells synergizes BMP-mediated refractivity, whereas Tgf-β2 signaling counterbalances it. Loss of Fgf18 signaling markedly accelerates anagen initiation, whereas loss of Tgf-β2 signaling significantly delays it, supporting key roles for these pathways in hair cycle timekeeping.

Hair cycle clock

FGFR3/4 inhibitors and TGF-β mimetics can be exploited for their hair growth–inducing effects. One of the most intriguing questions in hair biology is what controls the hair cycle, a repetitive process of hair follicle regeneration. The hair cycle is typically divided into resting, growing, and involution phases, referred to as telogen, anagen, and catagen, respectively. The duration of anagen determines the length of the hair shaft produced by the follicle, whereas the duration of telogen determines how soon a new hair shaft is made. Telogen-to-anagen and anagen-to-catagen transition events must be highly regulated, as they contribute to the achievement of a hair coat of optimal length and density, which is essential for the survival and adaptation of many mammals. The mechanism regulating the anagen-to-catagen transition targets proliferating matrix cells and induces them to undergo coordinated apoptosis, likely via signaling switches from within the matrix and/or in adjacent dermal papillae. The telogen-to- anagen transition mechanism targets progenitor populations of the bulge and hair germ, causing them to exit quiescence. This latter process has received much attention in recent years, shedding light on the general aspects of stem cell niche biology in other tissues and organs.

"Social networking" between hair follicles

Until recently, the hair follicle was considered a relatively closed system and was treated as such in the context of many experimental models. This concept worked fairly well for many purposes, partly because laboratory mice do not have obvious seasonal hair growth and partly because many experiments were designed around the first and second postnatal hair cycles, when hair growth stages can be reliably associated with the age of the animal.

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Studying the hair cycle in adult mice was thought to be difficult because telogen-to-anagen transition events could not be predicted, and, when they occurred, would result in patches of hair growth that appeared to be random.

Recent inquiries into the adult hair cycle have revealed several interesting facts. The quiescence of hair follicle progenitor cells is maintained not only by the immediate niche microenvironment but also by the larger dermal macroenvironment. The microenvironment consists of the dermal papilla, the dermal sheath, and keratin 6–positive bulge cells, whereas the macroenvironment includes dermal fibroblasts, cutaneous adipocytes (Plikus *et al.*, 2008), preadipocytes (Festa *et al.*, 2011), and probably other extrafollicular components such as intradermal blood vessels, the nerve plexus, and immune cells (Figure 1). Neighboring hair follicles also communicate with one another during the telogen-to-anagen transition, exchanging growth-inducing signals (Plikus *et al.*, 2011). Curiously, signaling pathways previously implicated in autonomous anagen initiation—bone morphogenic protein (BMP) (Botchkarev *et al.*, 2001) and WNT (Lowry *et al.*, 2005; Enshell-Seijffers *et al.*, 2010)—are reused to mediate follicle-to-follicle and macroenvironment- to-follicle communications.

As a population, pelage hair follicles strive to regenerate as seldom as possible. Telogen follicles can retain old hair shafts for long periods of time, eliminating the need for continuous regeneration to maintain hair coat integrity. Working against that goal are anagen-spreading waves, carrying high levels of activating signals to telogen follicles. To balance between resisting anagen-spreading waves for a minimal period of time and efficiently responding to them thereafter, a protection mechanism has developed. It renders hair follicles refractory to anagen-activating signals during early telogen and sensitive to the same signals during late telogen, yet dormant in their absence.

Timing refractory telogen

At the heart of the telogen refractivity mechanism is a signaling threshold switch operating in the dermal macroenvironment (Figure 1). In mice, the refractory-to-competent transition occurs after about 1 month in telogen and is accompanied by a precipitous drop in the expression of Bmp2/4 ligands (Plikus *et al.*, 2008) and Dkk1/Sfrp4 WNT antagonists (Plikus *et al.*, 2011) in the dermis and cutaneous adipocytes. This macroenvironmental switch is aided by the intrafollicular switch operating, at a minimum, across the BMP, WNT, and fibroblast growth factor (Fgf)7-Fgfr2-IIIb signaling pathways (Greco *et al.*, 2009). Once these switches have occurred, competent telogen hair follicles are on the verge of an anagen transition. With inhibitory BMP signaling largely eliminated and the number of WNT antagonists dwindled, a transient rise in WNT ligands may surpass the signaling threshold and induce the onset of anagen. Curiously, despite this delicate signaling equilibrium during competent telogen, spontaneous WNT activation events are quite rare. In fact, they are so rare in mice that competent telogen can last for several months (Plikus *et al.*, 2008, 2011).

The refractory-to-competent signaling threshold switch works to a large extent at the level of ligands and antagonists. For example, competent telogen follicles can be turned refractory again simply by adding more Bmp4, and normal refractory telogen can be cut short by

overexpressing the soluble BMP antagonist noggin (Plikus *et al.*, 2008). Changes in the response patterns by bulge and hair germ progenitors via changes in receptor and transcription factor profiles constitute an additional component of the mechanisms underlying this switch (Greco *et al.*, 2009; Geyfman *et al.*, 2011; Oshimori and Fuchs, 2012). It is important to note that, although the role of macroenvironmental signaling in maintaining refractory telogen is clear, the mechanism that regulates gene expression changes in the dermis and cutaneous adipocytes after a predefined period of time is not known.

Discovering new “gears” in the hair cycle clock

Given the complexity of hair follicles, it is reasonable to expect that the hair cycle clock might display redundant features, such as overlapping regulation of the same event by several signaling pathways. Such redundancy would enable hair follicles to more reliably sort out true signals from signaling noise and to adapt more efficiently their regeneration dynamics to changing environmental conditions. Indeed, in this issue, Kimura-Ueki *et al.* (2012) provide compelling evidence for the key role of Fgf18 signaling in maintaining telogen refractivity. The authors show that in telogen follicles Fgf18 is preferentially expressed by bulge cells and to a lesser extent by hair germ cells and dermal papilla cells. Upon epithelial Fgf18 deletion, the telogen phase shortens from 1 month to just 1 week. These mutant mice display a fast hair cycling phenotype, closely reminiscent of that in *K14-Noggin* mice, in which noggin overexpression reduces BMP-mediated telogen refractivity (Plikus *et al.*, 2008). This suggests that Fgf18 and BMPs act in parallel as redundant regulators of refractory telogen. The fact that disruption of each of these signaling pathways is equally successful in eliminating refractive properties hints at signaling interdependence, the details of which are not fully understood.

The inhibitory effect of Fgf18 on hair follicle regeneration was independently suggested in earlier studies. Blanpain *et al.* (2004), Greco *et al.* (2009), and Hsu *et al.* (2011) showed that Fgf18 is indeed enriched in keratin 6–positive bulge cells and in the dermal papillae of telogen hair follicles, and that it exerts an antiproliferative effect on keratinocytes in vitro. Hsu *et al.* (2011) also showed that specific ablation of Fgf18high keratin 6–positive bulge cells results in precocious anagen initiation, the phenotype that can be rescued by exogenous Fgf18 administration. The report by Kimura-Ueki *et al.* (2012) provides important in vivo evidence that Fgf18 regulates the hair cycle clock, and it lays groundwork for further inquiries into the mechanism of telogen refractivity.

A recent study by Oshimori and Fuchs (2012) uncovered the essential role of transforming growth factor (Tgf)-β2 signaling in counterbalancing BMP mediated telogen refractivity. In hair follicles, Tgf-β2 is secreted by dermal papillae during the competent telogen phase, which is accompanied by transient activation of phospho-Smad2/3 signaling in hair germ progenitors. This paracrine Tgf-β2 signaling is central to the normal anagen initiation mechanism because epithelial-specific ablation of the TGF-β pathway greatly extends telogen duration whereas administration of recombinant Tgf-β2 results in precocious anagen initiation. Interestingly, Oshimori and Fuchs (2012) showed that the activating role of Tgf-β2 in the hair cycle is mediated by its direct antagonistic effect on BMP signaling in hair

germ progenitors. Tmeff1, the direct transcriptional target of the TGFβ pathway, dampens canonical BMP phospho-Smad1/5/8 signaling in hair germ cells. Small hairpin RNA–mediated deletion of Tmeff1 is sufficient to replicate the delayed anagen initiation phenotype of TGF-β signaling–deficient mice. In this respect, it is tempting to speculate that TGF-β signaling is also at the base of the well-known phenomenon of wounding induced hair regeneration. Tgf-βs are among the key mediators of the wound healing process, released by multiple cell types in the wound macroenvironment, including macrophages, mast cells, platelets, and fibroblasts. It is plausible that the transient rise in these macroenvironmental Tgf-βs simulates the effect of intrafollicular Tgf-β2 on hair germ cells and induces anagen initiation at the wound edge.

New tools in the hair researcher toolbox

Experimental assessment of telogen refractivity is challenging. Standard experimental techniques (such as histological hair cycle analysis at early postnatal time points or after hair plucking in adult animals) can be inadequate. Telogen phenotypes often do not become apparent until after the third hair cycle, and plucking- induced hair regeneration likely involves additional mechanisms that are not present during spontaneous physiological regeneration. There are, however, experimental techniques that can be adopted by almost any laboratory. Longterm hair growth pattern analysis is highly informative in evaluating the adult hair cycle. Skin pigmentation patterns resulting from anagen-coupled melanogenesis can be photographically recorded from a single animal over a prolonged period of time (Plikus et al., 2008). Temporal analysis of such patterns can reveal even subtle telogen timing defects. For example, consistent shortening of telogen to less than 1 month and simplification of hair growth patterns indicate defects in refractory telogen. Competent telogen length is normally highly variable, ranging from 1 day to many months, whereas decreased variability of this phase and increased asymmetry of hair growth patterns suggest enhanced spontaneous anagen initiation. Kimura-Ueki *et al.* (2012) adopt this technique elegantly and show that a refractory telogen defect in epithelial Fgf18-null mice manifests itself clearly in highly dynamic and symmetric wave-like hair growth patterns (see Figure 3 of their article).

Additional experimental techniques include the quantitative hair plucking assay (i.e., timing regenerative response following hair plucking from a group of just 50 or 200 telogen follicles), expression analysis on longitudinal skin strips spanning several adjacent hair growth domains, transgenic skin transplantation, and intradermal protein administration assays (Plikus *et al.*, 2008). Caution must be exercised, however, in designing and interpreting the results of such experiments. Anagen can be induced artificially during highly responsive, competent telogen as a result of manipulations such as unintentional micro-wounding upon animal handling. In fact, Kimura-Ueki *et al.* (2012) were not able to validate the anagen-inducing effect of Fgf18 protein reported earlier by the same group (Kawano *et al.*, 2005). Upon further careful experimentation, Kimura-Ueki *et al.* (2012) conclude that the hair growth– promoting effect of Fgf18, which contradicts its inhibitory role in the hair cycle, was likely indirect, resulting from experimental manipulations.

New targets in hair loss treatment

The lack of coupling between human scalp hair follicles contributes to hair growth pathology. Because anagen in the scalp lasts for several years in humans, the majority of normal scalp hair follicles are growing at any given time, even if they rely only on rare intrinsic anagen initiation. In androgenic alopecia, the anagen phase shortens substantially, whereas intrinsic anagen initiation does not become more efficient. With the telogen-to-anagen length ratio now altered in favor of telogen, there is a much larger proportion of telogen hair follicles at any given time. Thus, the human scalp skin appears bald because scalp follicles do not retain old hair shafts efficiently as compared with mouse pelage follicles. An attractive method for anti–hair-loss therapy would be reactivating the coupling between scalp hair follicles (reviewed in Plikus *et al.*, 2011) so that rare spontaneous activation events can spread, increasing the overall number of follicles in anagen. An alternative method would be counteracting intrafollicular telogen refractivity, and in this respect Fgf18 and TGF-β signaling emerge as potential targets. For example, further inquiries into the Fgf18-Fgfr3/4 pathway are encouraged by the fact that Fgf18 is indeed elevated in the epithelial progenitor cells of human hair follicles (Garza *et al.*, 2011). In addition, soluble Fgfr3/4 extracellular domain fragments promote hair growth in mice upon local and systemic delivery (Brennan *et al.*, 2011a,b).

In summary, Kimura-Ueki *et al.* (2012) and Oshimori and Fuchs (2012) add Fgf18 and Tgf-β2 firmly to the list of crucial hair cycle clock regulators. Future studies should aim to establish details of how the BMP, WNT, FGF, TGF-β, and other signaling pathways jointly regulate refractory telogen, competent telogen, and telogen-to-anagen initiation events within each hair follicle stem cell niche and throughout the skin (Figure 1).

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Clinical Implications

- Efficacy of hair growth–promoting agents can be reliably measured by studying changes in hair growth patterns in mice
- FGFR3/4 inhibitors and TGF-β mimetics can be exploited for their hair growth–inducing effects

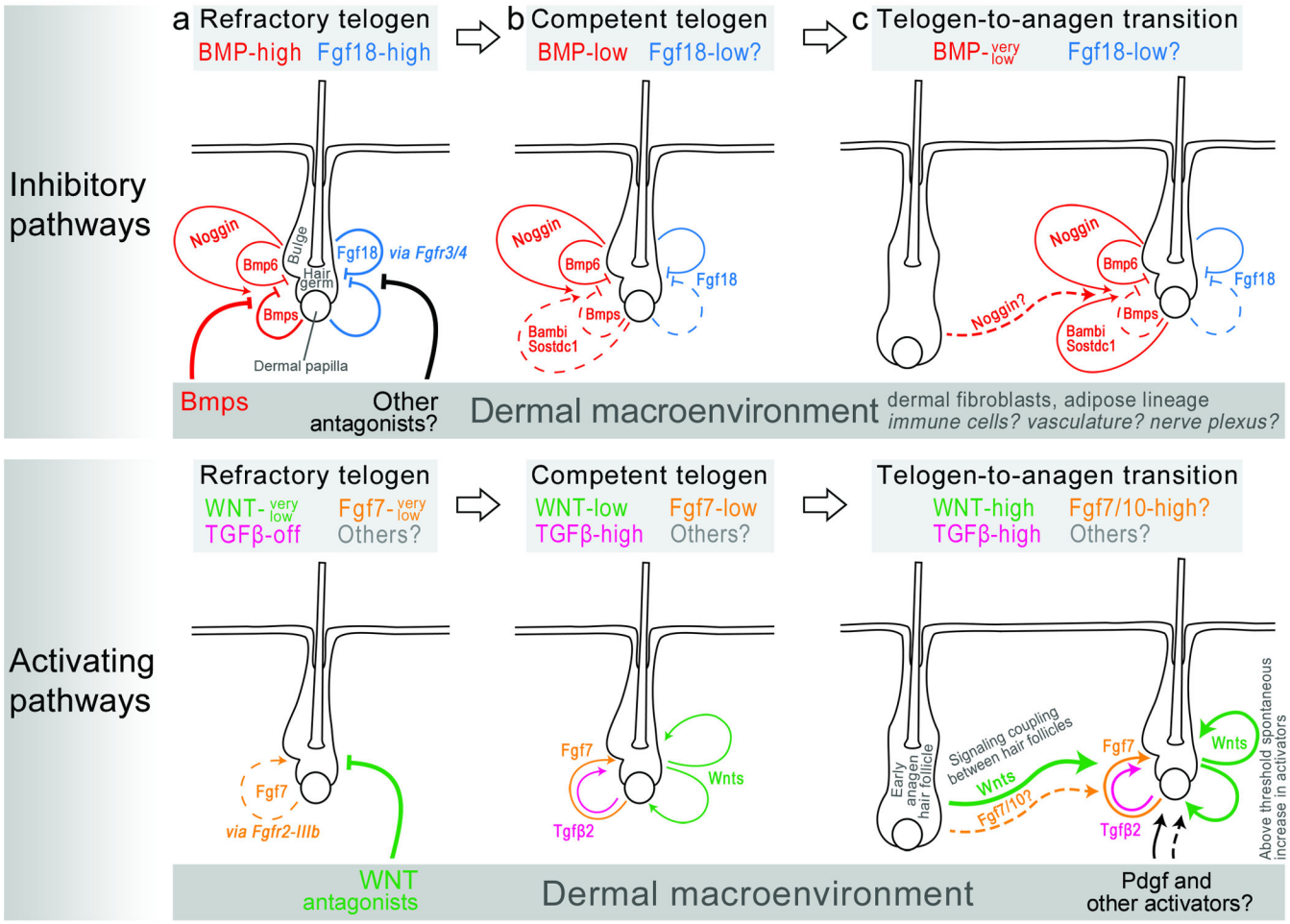

Figure 1. Signaling changes in the hair follicle during telogen and upon anagen initiation

Hair follicles are exposed to a different signaling environment during competent telogen than during refractory telogen. (a) In the refractory phase, a high level of inhibitory bone morphogenic protein (BMP) signaling is attributable to the multiple Bmp ligands produced by the hair follicles themselves (Botchkarev *et al.*, 2001; Blanpain *et al.*, 2004, Greco *et al.*, 2009) and by the surrounding dermal macroenvironment (Plikus *et al.*, 2008). Activating WNT signaling is low, partly because of the WNT antagonists present in the dermal macroenvironment (Plikus *et al.*, 2011). Inhibitory fibroblast growth factor (Fgf)18 signaling (mediated by Fgfr3/4 receptors) is high (Blanpain *et al.*, 2004; Greco *et al.*, 2009; Hsu *et al.*, 2011; Kimura-Ueki *et al.*, 2012), whereas activating Fgf7 signaling (mediated by the Fgfr2-IIIb receptor) is very low (Greco *et al.*, 2009). (b) Upon transition into the competent telogen phase, overall BMP signaling decreases and WNT signaling increases, partly because of the signaling threshold switch operating in the dermal macroenvironment (Plikus *et al.*, 2011) and partly as a result of increased production of Wnts, BMP antagonists, and transforming growth factor (Tgf)-β2 by hair follicles themselves (Greco *et al.*, 2009; Oshimori and Fuchs, 2012). During competent telogen, dermal papillae begin to produce more of the activating Fgf7 and less of the inhibitory Fgf18 (Greco *et al.*, 2009). It is uncertain whether Fgf18 production by bulge cells changes from refractory to competent

telogen. (c) Upon telogen-to-anagen transition, hair follicles experience transient activation of canonical WNT signaling, first in dermal papillae (Enshell-Seijffers *et al.*, 2010; Plikus *et al.*, 2011) and then in epithelial progenitor cells (Greco *et al.*, 2009). The WNT activation event is partly fueled by Wnt ligands secreted by neighboring anagen hair follicles. This so-called signaling coupling between neighboring telogen and anagen hair follicles likely occurs across multiple signaling pathways; the exact details of this process remain unknown. It is likely that the dermal macroenvironment provides activating signals during anagen initiation as well. Recently, dermal preadipocyte-derived platelet-derived growth factor (PDGF) emerged as one such activator (Festa *et al.*, 2011).