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A Hairy End to a Chilling Event

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In the skin, sympathetic nerves, arrector pili muscles, and hair follicles form a tri-lineage unit to cause piloerection or goosebumps. In this issue of *Cell*, Schwartz et al. report that, beyond goosebumps, muscle-anchored nerves form "synapse-like" connections with hair follicle stem cells to promote hair regeneration in response to cold.

The skin, the largest organ in the body, provides a sensory and protective interface with the external environment. In most mammals, the skin also undertakes a motor and behavioral function in the form of "piloerection," where skin hair becomes erect to enable thermoregulation and provide a social warning in response to aggression. Piloerection is controlled by sympathetic nerves, which innervate arrector pili muscle (APM) that undergo contraction to raise hair follicles during a "fight or flight response" to external stressors such as cold or danger (Fujiwara et al., 2011). The anatomical organization of sympathetic nerves, arrector pili muscle, and hair follicles is conserved across mammals including humans, where piloerection manifests as "goosebumps," although the thermoregulatory function is lost. The conserved arrangement also implies functions that extend beyond goosebumps, and this is of particular relevance when considering that hair follicles represent one of the few self-regenerating tissues in adult mammals (Gonzales and Fuchs, 2017). In this issue of *Cell*, Schwartz et al. (2020) report that beyond the acute response of piloerection, sympathetic nerves trigger activation of hair follicle stem cells (HFSCs) to produce a new hair coat in mice under prolonged cold exposure. In defining morphological, cellular, and functional interactions between nerves, arrector pili muscles, and HFSCs in exquisite detail (Figure 1), the study provides new insight into regulation of a stem cell niche by environmental cues.

Adult hair follicles undergo cycles of regeneration (anagen), degeneration (catagen), and rest (telogen), driven by HFSCs that reside in niches known as the "bulge" and "hair germ" (Gonzales and Fuchs, 2017). The hair follicle niche is one of the most intensively studied in stem cell biology because of the stereotypical and synchronized processes underlying hair growth (Gonzales and Fuchs, 2017). Sympathetic nerves are part of the niche because they innervate arrector pili muscle, bundles of smooth muscle cells, attached to the bulge region where HFSCs reside. Sympathetic nerve-derived adrenergic signaling stimulates hair growth (Botchkarev et al., 1999; Fan et al., 2018), and loss of innervation has been linked to hair defects (Botchkarev et al., 1999). However, the niche cell types that are direct targets of innervation and the molecular and cellular mechanisms underlying nerve-hair follicle interactions remain undefined.

To address functional effects of sympathetic innervation, Schwartz et. al. (2020) ablated nerves by injecting a neurotoxin or by transgenic expression of diphtheria toxin in mice and observed a delay in HFSC activation and anagen entry. Sympathetic nerves signal directly to HFSCs through β2-adrenergic receptors (Adrb2) that respond to the neurotransmitter, norepinephrine. Loss of this receptor specifically in HFSCs recapitulated the effects of sympathectomy in delaying hair growth in mice. Conversely, topical delivery of adrenergic receptor agonists accelerated anagen entry. Transcriptome analyses of HFSCs lacking Adrb2 showed upregulation of transcripts that promote quiescence, suggesting that neuronal signaling is required to prime HFSCs to enter the growth phase.

Using immunofluorescence-based imaging, serial section electron microscopy, and 3D-reconstructions, Schwartz et. al. (2020) observed an elaborate network of sympathetic fibers that bundled around arrector pili muscles and even extended beyond to directly contact HFSCs

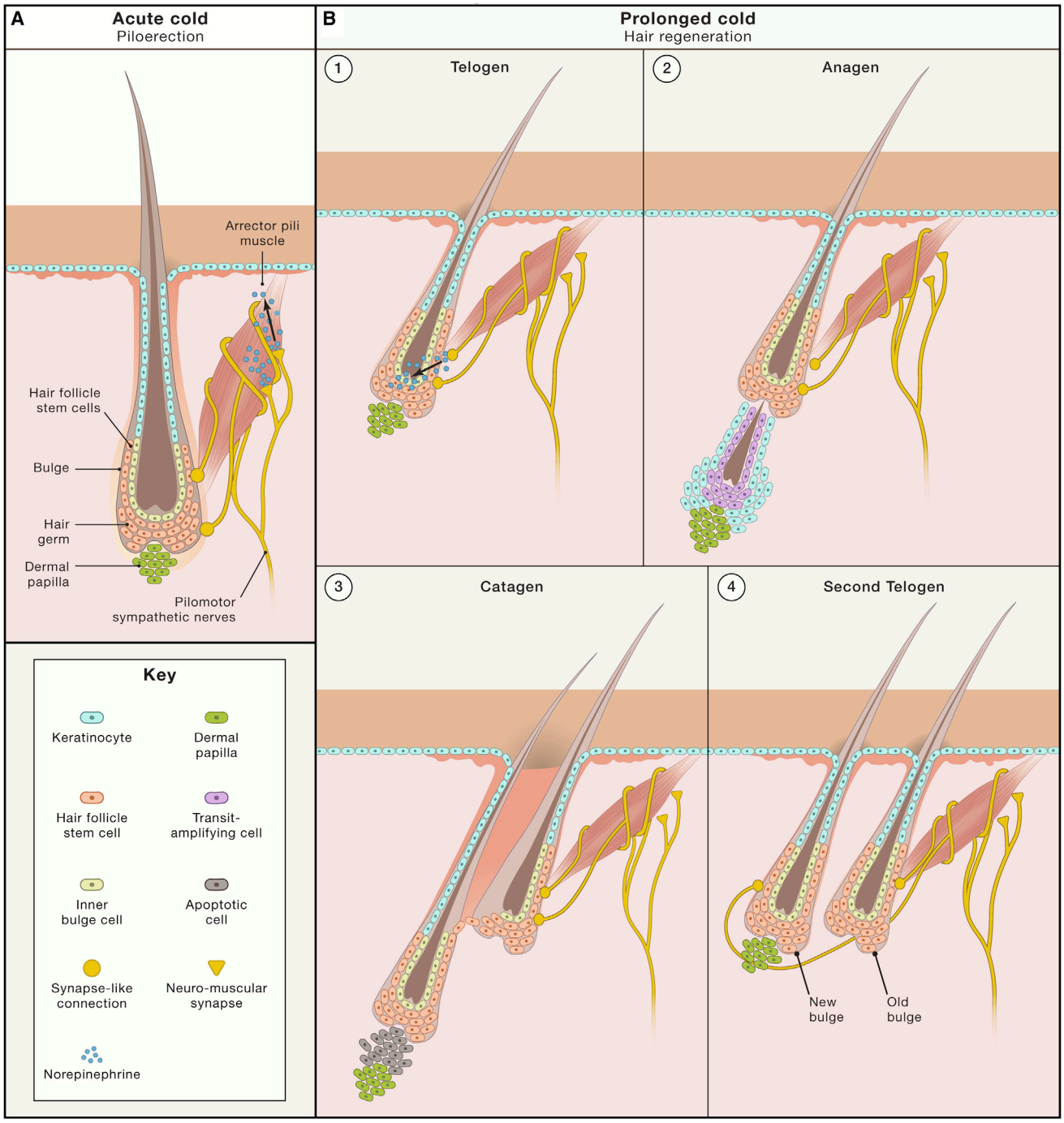

Figure 1. Sympathetic Nerves Promote Piloerection and Hair Regeneration in Response to Cold

(A) Hair follicles, arrector pili muscles, and sympathetic nerves form a tri-lineage unit, which promotes piloerection or goosebumps as an acute response to cold. Arrector pili muscles attach to the bulge region of hair follicles where hair follicle stem cells (HFSCs) reside. Arrector pili muscles are enwrapped by sympathetic pilomotor nerves, which release the neurotransmitter norepinephrine (NE) to trigger muscle contraction and cause hairs to become erect in response to cold. (B) Adult hair follicles spontaneously regenerate through cycles of growth (anagen), degeneration (catagen), and rest (telogen). Sympathetic pilomotor nerves, innervating arrector pili muscle, extend further to establish direct connections with bulge hair follicle stem cells (HFSCs) through "synapse-like" structures. Under prolonged cold exposure, sympathetic nerve-derived NE signaling activates quiescent HFSCs in the rest phase of the hair cycle (telogen) to accelerate their entry into the growth phase (anagen). HFSCs proliferate to produce transit-amplifying cells, which then undergo massive proliferation and differentiation to drive the growth of new hair. Proliferative capacity of transit-amplifying cells subsequently wears out, and hair follicles degenerate during catagen. Hair follicles are restored to their resting size as they enter the next telogen.

through synapse-like structures. Ultrastructural analyses revealed nerve terminal specializations at sites facing HFSCs including varicosities or swellings where neurotransmitter-loaded vesicles accumulate and the absence of endoneurium or glial sheaths that would interfere with neurotransmission. These findings provide rare and high-resolution insight into nerve-epithelium connectivity and suggest anatomical specializations that could allow subtle increases in neurotransmitter levels to trigger HFSC activity in response to physiological stimuli such as cold exposure.

Intriguingly, the intimate connection between sympathetic nerves and HFSCs do not rely on HFSCs themselves but rather on arrector pili muscles, the third cell type in the tri-lineage unit. The authors generated mice in which arrector pili muscle were selectively removed either by transgenic or viral expression of diphtheria toxin and observed loss of innervation to HFSCs and a delay in anagen entry. Lineage tracing of arrector pili muscle revealed that they undergo little turnover during several rounds of hair cycles, suggesting they act as stable anchors for nerves despite substantial remodeling of surrounding tissues.

The conserved anatomical arrangement of hair follicles, arrector pili muscle, and sympathetic nerves is best known for its role in causing piloerection or goosebumps. Do mice get goosebumps? It turns out, like humans, they do exhibit classical goosebumps in response to cold. However, these mice also entered anagen faster. Together, these results support that goosebumps are likely a first line of defense to provide rapid thermoregulation for animals in response to cold. However, under persistent cold exposure, sympathetic activity further triggers HFSC activation to produce new hair, a response that may represent an evolutionary mechanism to couple hair growth to environmental changes.

The discoveries of Schwartz et. al. (2020) present key advances in defining reciprocal morphological, functional, and developmental interactions between HFSCs, arrector pili muscle, and innervating sympathetic fibers that couple stem cell activity and tissue regeneration with environmental changes. Sympathetic nerves innervate almost all organs and tissues and are ideally positioned to modulate diverse stem cell pools throughout the body in response to physiological or environmental cues. For example, in skin, stress-induced sympathetic hyperactivity induces proliferation of melanocyte stem cells resulting in their depletion from the niche and leading to hair graying (Zhang et al., 2020). In bone marrow, chronic stress-induced sympathetic signaling regulates proliferation of hematopoietic stem and progenitor cells (HSPCs) that give rise to blood and immune lineages and causes inflammation (Heidt et al., 2014). Further, the sympathetic nervous system relays photic cues from the suprachiasmatic nucleus, the central pacemaker in the brain, for circadian regulation of mobilizing HSPCs into the circulatory system (Katayama et al., 2006). The findings of Schwartz et. al. (2020) raise key questions about the cellular targets of sympathetic nerves in other stem cell niches: whether direct synapse-like connectivity exists with other stem cell populations and whether there are additional nerve-derived signals that influence stem cell behaviors.

Recently, arrector pili muscles were shown to be specifically innervated by a sympathetic neuron subtype expressing the neurotrophic receptors GFRα2 and Ret, based on retrograde tracing and single-cell RNA sequencing (Furlan et al., 2016). This raises the question of how these pilomotor sympathetic axons navigate beyond arrector pili muscles to connect with HFSCs through synapse-like structures. In this regard, arrector pili muscles may be considered as intermediate pathfinding targets, whereas HFSCs are the final targets for these axons. Further, how this neuronal sub-type is specialized to relay environmental signals to stem cell targets remains to be determined. Finally, despite decades of studies on wide-spread physiological effects of sympathetic neurons, little is known about the neuronal heterogeneity at cellular or molecular levels that underlie functional diversity. It will be of interest in future studies to determine if there are dedicated sympathetic neuron sub-types and circuitry that underlie specific stem cell behaviors in response to diverse environmental stimuli. Beyond the potential of targeting sympathetic nerves in clinically managing hair loss, the work of Schwartz and colleagues opens the door for a fundamental understanding of how the nervous system controls stem cell niches in regenerating tissues.

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