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# Bridging the "Dead Hair"—"Live Follicle" Divide in Applied Hair Research

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**Synopsis**

For many decades, applied hair research has been hampered by an unproductive intellectual and conceptual divide between researchers who are primarily interested in the hair shaft (HS), its structural properties, visual appearance and cosmetic manipulation, and those investigators who are mainly interested in the fascinating miniorgan that cyclically regenerates the HS, the hair follicle (HF). This article attempts to bridge this unproductive divide between the "dead hair" and "live follicle" worlds by summarizing both current key concepts and major open questions on how the HF, namely, the anagen hair bulb and its precortical hair matrix keratinocytes, generate the HS, focusing on selected key signaling pathways. We discuss current theories of hair shape formation and avenues toward impacting on human HS structure. The article closes by delineating which instructive preclinical research assays are needed to ultimately close the experimental gap between HS and HF researchers in a manner that benefits consumers.

The hair follicle (HF) is a unique miniorgan whose main function is to produce a pigmented hair shaft (HS) (1–3). The number of HFs in the human body, about 5 million, is determined during embryogenesis when HFs develop as the result of a bidirectional cross-talk between cutaneous ectoderm and mesenchyme (4–6). During fetal life, lanugo HFs generate soft but long fine filaments, which are present during the prenatal period and are usually shed *in utero* or during the first weeks of life. These first hairs are then replaced by vellus HFs that develop into terminal HFs only in particular body areas. Vellus HFs form short, unmedullated, non- or light-pigmented hair and cover most areas of the body surface. Terminal HFs, which give rise to long, thick, and pigmented hair, are developed in the scalp or after puberty in androgen-dependent body areas, such as the pubic area, underarm, and male beard (2,7).

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The structure of mature HFs is composed of ectoderm- or mesoderm-derived tissue compartments that are arranged in the shape of an inverted-wine glass with many layers like in an onion. The distal compartment of the HF, close to the skin surface, is the infundibulum, which continues into the isthmus, delimited by the insertion of the sebaceous gland and arrector pili muscle, and the suprabulbar and bulbar areas (1,8,9). The isthmus area contains the so-called "stem cell niche," the bulge, where the multipotent epithelial HF stem cells responsible for the constant renewal of the HFs during the hair cycle reside (10–13).

The hair bulb is called the "HS machinery" because it is the HF part where hair matrix (HM) keratinocytes, melanocytes, and highly specialized inductive fibroblasts in the dermal papilla interact with each other to make the hair fiber (1,8,9).

The HF during its growth phase (anagen) is composed of more than 20 different cell populations. The HF's epithelial compartments, from the outermost to the innermost layer, are the outer root sheath (ORS), the inner root sheath (IRS), and the hair fiber. The ORS represents the continuation of the epidermal basal layer. The IRS is further divided into four parts, the companion layer, Henle's layer, Huxley's layer, and IRS cuticle and reaches the insertion of the sebaceous gland duct. The HS is constituted by the cuticle, cortex and, only in some cases, the medulla. A substantial basement membrane surrounds the ORS, which in turn is enclosed by a mesenchyme-derived layer, the perifollicular connective tissue sheath (1,8,9,14,15).

The HF undergoes life-long process of cyclic transformations (1,8,16–18). The hair fiber is only produced during the HF growth phase, called anagen, which is proportional to the size of the dermal papilla, dictating the size, shape, and length of the hair (19). The growth phase is followed by an apoptosis-driven regression phase called catagen and by a relative stage of rest (telogen). Approximately 100,000 terminal HFs are found in the human scalp, which produce dead hair fibers that are typically long but can vary in shape, color, and physical behavior. The growth of the hair fibers in the human scalp is reported to be around 0.3–0.5 mm per day (20).

During anagen, the HS and IRS are produced by highly proliferating germinative HM keratinocytes generated by stem cells that originated from the bulge and migrated to the hair bulb during early anagen (10–13). These cells lose their proliferative activity after a critical anatomically defined level in the follicle structure (called as the Auber line), and start to differentiate into trichocytes of all HF epithelial cell layers with the exception of the ORS (9,14,21). The mechanisms that are involved in proliferation–differentiation switch of the HM keratinocytes remain largely unknown. The HS production is associated with the initiation of the hair-specific keratin gene expression program (13,14,22,23). The mystery of what occurs at the border between the germinative and precortical HM remains one of the major fascinating open questions in the field. It has recently been shown that Cyclin-dependent kinase (CDK) interacting protein/kinase inhibitory protein (CIP/KIP) family members regulating cell cycle progression/arrest, differentiation, and endoreplication are involved in this process (13). Other factors have also been shown to be implicated in the switch between HM keratinocytes proliferation and differentiation, such as the distance from the dermal papilla, which supply a gradient of growth factors (14), as well as miRNAs and other epigenetic regulators (24).

The HS is composed of dead (fully differentiated) keratinocytes (cortical cells) containing mainly proteins. Although the cuticle comprises 6–11 overlapping cell sheaths with an

exposed edge, cells in the cortex become elongated and the hair keratins are organized into macrofibrils (15,25,26). These are complex structures containing intermediate filaments embedded in a matrix composed of keratin-associated proteins (KAPs). The intermediate filaments are collections of heterodimers of hair keratin proteins. Macrofibril structures are stabilized by cross-links established by a range of disulphide bonds, ionic bonds and hydrogen bonds (25–27). The array of hair keratins and KAPs expressed in the HS and other HF compartments have been very well characterized (14,22,23,28–30). Although these clearly determine the structure and strength of the HS, researchers have failed to understand how the modulation of HM keratinocyte activities correlates with hair keratins and KAP's expression and influences HS properties. Some attempts have been made by "live follicle" researchers, who have shown that precortical hair keratins can be regulated by selected endogenous and exogenous factors *ex vivo*, using human HF organ culture (31,32). Unfortunately, methodological limitations prevented these researchers from translating their findings to changes in HS quality. In addition, decreased expression of selected hair keratins and KAPs is correlated with hair ageing (33), which is also characterized by thinning of the HS (34).

Ultimately, the changes occurring in the "live follicle" contribute to alterations in character of "dead hair" structure and quality; however, research within the two HF worlds remains separated. This is quite surprising considering that these two worlds share the hair care market and try to satisfy common consumers. The "live follicle" and "dead hair" researchers have clearly different scientific interests and different backgrounds; biologists are unraveling the secret life of the "live follicle," whereas physicists and chemists are involved in learning about the material properties of the "dead hair." Indeed, an intense literature search is required to reveal the aspects of the "live follicle" that affect the "dead hair" structure and quality.

Most of such studies come from the wool industry, for which obviously wool hair fiber quality is more important than hair fiber length and, therefore, anagen maintenance. For example, an elegant study by Bond et al. (35,36) showed that *ex vivo* treatment of wool adult anagen HFs with fibroblast growth factors 1 and 2, whose receptors are expressed in the living HFs, has an impact on protein synthesis for the "dead hair" but did not alter the cells of the "live" HM. Although the authors have not analyzed the hair fiber quality, they suggested that these changes likely affect tensile strength.

In humans, some information can be extrapolated from genetic hair conditions. It is clear that anything that interferes with the production of "dead hair" shaft keratins during keratinocyte differentiation in the "live follicle" results in decrease in HS thickness and integrity, as seen in monilethrix (5,23,37). Regulation of the production of trichohyalin (THH) or its solubility contributes to HS abnormalities. Also, genetic mutations of genes encoding for *THH* or enzymes involved in THH post-translational modification result in the condition called uncombable hair syndrome, manifested by a production of silvery-blond or yellowish-colored disorderly hairs, which are difficult to comb (38). Hair disorders characterized by abnormal HS curvature have been linked to changes in the expression of IRS keratins (3,5). Patients with deficiency in Adenosine triphosphate (ATP)7A (or Menkes' ATPase), which is involved in copper trafficking, feature hypopigmented brittle hairs, also called kinky hairs (39,40). Mutation in genes encoding for a protein involved in the cell cycle, namely, M phase–specific Serine/threonine-protein kinase (PLK1), causes trichothiodystrophy, a syndrome in which hairs are brittle because of deficiency in sulfur, a fundamental component for intermediate filament cross-linking in the HS (41). We have

recently demonstrated that patients showing a new form of ectodermal dysplasia carry the mutation in gene *TSPEAR* encoding the thrombospondin-type laminin G domain and EAR repeats protein. This mutation causes scalp hypotrichosis as a result of reduced number of HFs and abnormalities in HS cuticle cells, which appeared flattened. Interestingly, the silencing of *TSPEAR* in mouse HFs *ex vivo* promoted HM keratinocyte apoptosis and reduction in the hair bulb diameter (42). TSPEAR deficiency negatively impacts HF structure and HS quality by regulating the expression of Notch homolog 1, translocation-associated (Drosophila) (NOTCH)1 (42), a protein involved in HF development and regeneration (6,43).

Among "live follicle" researchers, it is very well known that insulin-like growth factor (IGF)-1 signaling is fundamental for hair cycle regulation and anagen maintenance (44–46). Dysregulation of the insulin pathway in the dermal papilla fibroblasts is associated with androgenetic alopecia (47), and the addition of insulin or IGF-1 to the culture medium is essential for delaying catagen during HF organ culture *ex vivo* (48–50). Surprisingly, only few studies reported about the role of the IGF-1 pathway on HS quality and structure. DNA polymorphism of IGF-binding protein (IGFBP)-3 causes changes in combed cashmere weight, cashmere fiber length, and guard hair length but not in cashmere fiber diameter (51). Interestingly, IGFBP5 is expressed in the HM, dermal papilla, and/or medulla in mice. However, only zigzag but not guard or awl HFs express IGFBP-5. Overexpression of IGFBP5 *in vivo* in the IRS and HS medulla results in hair bending and a thicker and longer HS (52). The fact that IGF-1 signaling is involved in hair bending was also confirmed in human HF *ex vivo* (53). Overexpression of IGF-1 in the IRS and medulla in mice prevented hair bending in zigzag HFs but promoted the development of longer and thicker hair (44). Therefore, it is conceivable that any compounds increasing the expression of IGF-1 in human HFs would not only stimulate anagen prolongation but also could lead to the production of thicker, straighter HS.

The Wingless/Integrated (WNT)/β-catenin, Sonic Hedgehog Homolog (SHH), transforming growth factor (TGF)β, and Bone Morphogenetic Protein (BMP) signaling pathways are required for hair development and regeneration (1,6). In addition, the regulation of proteins belonging to WNT or BMP families is also implicated in HS quality and structure. Transgenic mice overexpressing BMP antagonist noggin in the ORS (K5 promoter) show bigger HFs and the replacement of zig-zag and auchene hairs by awl-like hairs with increased diameter (54). Interestingly, this effect is related to decrease in the expression of Cdk inhibitor p27 (Kip1) and increased expression of selected cyclins (13) in the HM (54). Instead, overexpression of Wnt3 in the ORS (K14 promoter) causes the production of shorter and thinner hairs in mice (55). Similar phenotype was seen when the Wnt pathway was inhibited by overexpression of miR-214 in mouse keratinocytes (56). Also, the overexpression of only one selected growth factor in the HF ORS *in vivo*, such as vascular endothelial growth factor (VEGF), stimulates the production of thicker hairs, which is associated with the increase in hair bulb size and vascularization around HFs (57). Therefore, although it is evident that modulations in "live follicle" activities may highly contribute to HS quality, somehow, this aspect of HF research is often forgotten or limited to gross information such as hair diameter.

Very interesting conclusions can be drawn about the link between "live follicle" and "dead hair" based on the information extracted from several articles about the hair benefits of caffeine, the most widely used nutraceuticals used in cosmetic formulations. "Live follicle" researchers have known for some time that caffeine treatment of human HFs

*ex vivo* results in anagen prolongation associated with increased HM keratinocyte proliferation, reduced apoptosis, and TGFβ1 expression (58,59). In addition, an *in vivo* study showed increased hair weight and stiffness after regular employment of a caffeine-based shampoo (60). These *ex vivo* and *in vivo* observations are very well supported by the experience of consumers. However, because caffeine can also be absorbed by the HS itself (60), it is not clear whether hair fiber quality is improved because of the impact of caffeine on the "live follicle," "dead shaft," or both. Davis et al. (61) reported that a hair care formulation containing caffeine improved HS quality, increasing hair strength and rigidity by measuring these parameters on hair tresses. This renders the possibility that caffeine acts directly on hair fiber still more plausible.

This is exactly where researchers from the "live follicle" or the "dead hair" worlds cannot close the gap. Unfortunately, at present, we do not have any well-described preclinical assays which would allow modulation of activities of the "live follicle" followed by the measurement of parameters involved in "dead hair" fiber quality. However, taking advantage of new tools and techniques developed in both fields, such a preclinical assay could be established if experienced "live follicle" and "dead hair" researchers join forces. It is quite fulfilling to imagine a preclinical assay in which a "live follicle" could be treated while continuously producing the HS, allowing not only parameters of the "live follicle" to be investigated (e.g., anagen prolongation and keratins expression) but also HS properties (e.g., hair protein structures and hair mechanical properties) and signaling pathways involved in such modulation.

## CONFLICT OF INTERESTS

M.B. is an employee of Monasterium Laboratory (ML) GmbH, a company owned and founded by R.P., for which N.B., G.W., C.W., and R.P. serve as consultants. ML services include the testing of hair care cosmetic ingredients *in vitro*, *ex vivo*, and *in vivo*, and have recently, together with TRI Princeton, opened a call for sponsors financing the development of a new *ex vivo* preclinical assay for predicting the effects of follicle actives on hair quality. For additional information, please visit: www.monasteriumlab.com (NEWS) and www.triprinceton.org.

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