

Hair Shaft Formation and Mitochondrial Bioenergetics: Combining Biology, Chemistry, and Physics

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Synopsis

Research into biological manipulation of hair “quality” has ebbed and waned but today is in a resurgence. Hair appearance is regulated by multiple intervention opportunities—adding more hairs; increasing hair “amount” by modulating shaft diameter or shape; or, in principle, by altering shaft physical properties by changing its synthesis. It is likely that improved benefits may be achieved by combining multiple areas—minimizing follicle loss and miniaturization, maximizing shaft production, and treating the existing shaft. A previously overlooked opportunity is follicle metabolism: building “better” hairs. Hair production is energy intensive, and it is known that follicle metabolism influences shaft diameter. Multiphoton microscopy enables metabolic investigation of live, growing, human, hair follicles. This allows definition of multiple “zones” with vastly different metabolism: proliferation—where keratinocytes proliferate and migrate into specialized layers; production—proliferation ceases, and synthesis and patterning begin; construction and elongation—the structural framework is seeded and cells extend to create the nascent fiber; and maturation—gradual hardening and transformation into mature shaft. Recent investigations into the transition from construction to maturation reinforce this as a key developmental threshold, where shaft production transforms from a biologically driven into a biochemically driven process. We now name this “Orwin’s transition.”

INTRODUCTION

Although in general mammalian hair has many useful functions, in humans, terminal scalp hair remains of unknown evolutionary utility. It is almost certainly less about physical functions such as temperature regulation and protection and has become primarily important aesthetically and sexually, very likely an essential part of mate selection. In both men and women, scalp hair is a key perceptual indicator of age, health, and beauty (1–4). Inadequate hair condition and loss of scalp hair clearly has adverse psychological

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impact, resulting in significant anxiety and distress (1,5). This is the basis for the estimated \$100 billion dollar plus per year demand for treatments that effectively promise to improve hair growth and appearance (6).

Despite the controversy and lack of clear biological data on why humans have terminal scalp hair, we certainly dedicate a vast amount of energy into its production. Production of hair depends on adequate regulation of the follicle growth cycle (7) and maintenance of a well-functioning factory synthesizing hair shaft. If one considers that human scalp hair grows at approximately 0.3 mm/day/follicle and one assumes a total of 100,000 terminal scalp hairs, we produce approximately 2 m of hair shaft per day (8,9). It is difficult to define the exact energy required to produce this much hair, but if we consider the human scalp hair as being relatively similar to wool, some data exist. There are many studies that examine sheep diet in respect to wool properties (10,11). A direct application of sheep diet data to humans might not be appropriate because sheep are herbivores, relying on bacterial fermentation in digestion; however, the energy required to produce a set weight of hair fiber is probably similar. In sheep, about 630 kJ of metabolizable energy is required to produce 1 g of clean dry wool (containing 24 kJ gross energy) and 0.27 g wool grease (sheep sebum, containing 11 kJ gross energy). This may seem like a lot of energy; it is equivalent to about 10 min of hard exercise with both arms and legs (12) or 400 shots from a Smith and Wesson Model 29 revolver (13) of "Dirty Harry" fame. It is also a lot of hair, with a single ~4-cm human hair weighing in at 0.62 mg. Hence, 1 g of human hair represents about 1,500 hairs of 4 cm in length.

Although a rough estimate, this demonstrates the energy intensity of human anagen follicles. For example, human scalp hair grows on average 10 mm/month with an estimate of 17–170 vertical cell divisions per cell type (e.g., cortex, cuticle, and Huxley's layer) per day (14). Although this is almost certainly imprecise (having been derived from various data sources), it highlights the point that a growing hair implies a population of cells at their most active, continually dividing and rapidly differentiating. Mammalian mitochondria have for many years been a well-researched target for drug therapy, particularly focusing on inhibiting tumor growth *via* inhibition of mitochondrial metabolism and as a generator of toxic reactive oxygen species after radiation exposure. Thus, there is a wealth of information available to investigate targeting of mitochondrial metabolism in hair loss prevention, and the new techniques reviewed herein should enable more detailed assessment.

An optimal head of hair requires adequate follicle density to produce enough visible hair shafts, and each shaft must be functional for coverage and style (2,3). In addition, each hair must be robust enough with strength and durability to sustain years of both deliberate and unintentional physical and environmental damage. Unfortunately, research into the biology of hair and the physical/chemical properties lie in separate realms: follicle regulation and the biological basis of hair production lies in biology and medicine; physical/cosmetic properties of the mature hair shaft fall under physics and physical chemistry; whereas construction and assembly of the hair shaft are considered oxidative or biochemistry. Hence, subjects (or patients) have suffered because communication between research centers and coordinated treatments from each of the singular aforementioned domains has lagged. In order for the best possible outcomes for hair loss, the scientific community must implement collaborative programs containing biology, chemistry, and physics.

BIOLOGY: THE HAIR FOLLICLE AND THE PROLIFERATION STAGE

Hair follicles are complex skin-associated organs within the dermis/epidermis (Figure 1), where each “pilosebaceous” or “follicular” unit comprises the hair synthesis machinery, the sebaceous (oil) gland, the apocrine (sweat) gland, and an arrector pili muscle (15,16). Each follicle consists of both epithelial and mesenchymal components: the outer root sheath (ORS), inner root sheath (IRS), and germinative matrix (GM) are epithelial in derivation, whereas the dermal papilla (DP) and encapsulating connective tissue surrounding the follicle known as the dermal sheath (DS) are mesenchymally derived (17,18). Importantly for full understanding of follicle function, the dermal components are vascularized and enervated.

On human scalp, most of the cells within the GM eventually form the cortex of the hair shaft. Although these structures are derived from the same source, the division of the scientific bodies researching hair production often has differing lexicons and confusing nomenclatures. As this is an attempt to bring together these disparate fields, societies, and institutions, we will attempt to define each group’s terminology. Throughout this review, where we see confusion or duplication, we will revert to the nomenclature defined in Figure 1.

The follicle bulb creates hair shaft and other support layers similar to how the epidermis creates the stratum corneum: basal cells progressively differentiate, die, and cornify into the dead outer protective layer (20). In the follicle bulb, a basal epidermal layer attached to a basement membrane surrounding the DP rapidly divides and produces a GM. It is believed that each cell lining along the DP membrane is fully fated to a specific lineage to create a specific hair follicle layer. Maintenance of a healthy follicle is fundamentally

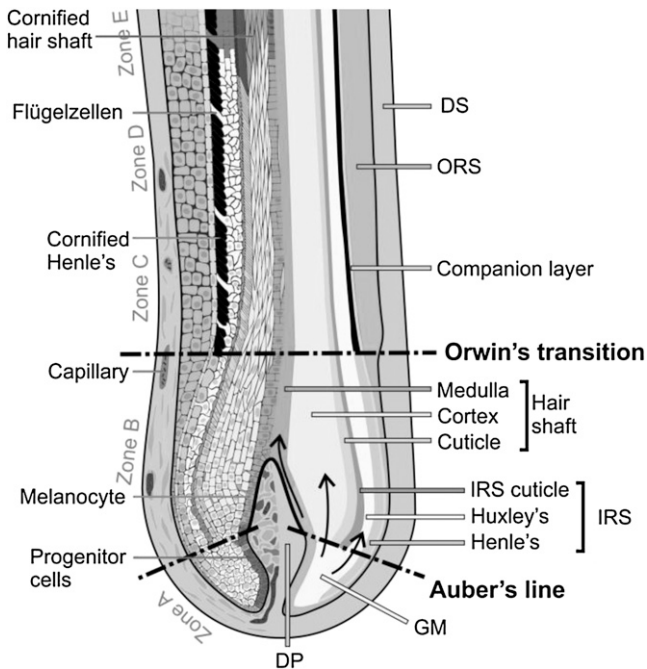


Figure 1. Nomenclature of the key structures of the human hair follicle bulb. Reprinted by permission from Springer Nature: Introduction to hair development by D. Harland, Copyright 2018 (19).

dependent on epithelial–mesenchymal interactions across this complex basement membrane and how each follicular compartment communicates with the others (21). The DP is quiescent, essentially never containing mitotic activity and having relatively low metabolic activity but likely controls the number, size, and fate of all the GM cells. The DP thus determines the fiber size, content, and hence final cosmetic properties (17,22). As GM cells cease division and migrate above the Auber's line, differentiation begins: they pack closely, compress and align along the soon to be fiber axis (23,24). In some hairs, other GM cells migrate to a centrally located medulla, whereas in all hairs, the cortex is surrounded by cells that will become the hair shaft cuticle.

The outer follicular layers that provide structure and support during shaft growth also originate in the GM and migrate and differentiate similarly to the shaft cell lines. The IRS differentiates into three cell lines named medially to laterally: the IRS cuticle, Huxley's layer, and Henle's layer. Adjacent to Henle's layer is a distinct layer of flattened, elongated cells termed the "companion layer." The companion layer is closely associated with both Henle's and Huxley's layers through numerous direct connections, leading to speculation that the companion layer nourishes the IRS and may even guide its differentiation and eventual destruction in the follicle lumen (25–27). The aforementioned IRS layers surround and move with the hair shaft – progressing outward to the skin surface and then disintegrate into the follicle infundibulum (where the follicle reaches the skin surface). The companion layer is hence most likely the "shear plane" between the outwardly moving hair shaft and its associated structures, and the stationary, ORS.

The ORS resides outside the companion layer and consists of characteristic cuboidal cells that thicken from one to multiple layers as the follicle extends distally. At the proximal end, the ORS is contiguous with GM cells lining the lower half of the DP. At the distal end, the ORS is contiguous with the interfollicular epidermis. Interestingly, these cells are competent to regenerate a stratified, fully differentiated interfollicular epithelium *in vitro* (28). ORS cells express keratins, keratin-associated proteins (KAPs), adhesion molecules, and signaling molecules uniquely distinguishing them from interfollicular epidermis (17,29,30). The human stem cell compartment is located near the attachment of the arrector pili muscle. In humans, it is important to note that the "stem cell" cluster of the ORS does not form a visible "bulge" as it does in murine follicles. Finally, the entire epidermal follicle apparatus is encased in a tough, mesoderm-derived connective tissue which forms a robust DS, from which it is separated by a thick basement membrane.

CHANGES IN BIOLOGY LEAD TO A DIFFERENT PRODUCT

There are numerous examples of biology-mediated alterations in hair follicles that result in direct, if difficult to explain, alterations in hair shaft properties. For example, monilethrix (OMIM #158000) was described in 1879 (31) as a genetic hair disease where the hair shaft has a beaded appearance (32). The between-bead regions are breakage prone and lead to fragile hair and, hence, alopecia. Monilethrix has unknown and complex genetics and clinical overlap with other hair disorders such as localized autosomal recessive hypotrichosis (LAH). The most of the data suggest that the disease may be caused by mutations in hair cortex keratin or KAP genes and/or the cell–cell contact–associated gene desmoglein 4 (32–37). The obvious hereditary nature of monilethrix, the obvious hair phenotype, and the lack of a clear cause emphasizes the complex nature of hair shaft biology.

Many of the early hair development events we described previously and which occur in the lower bulb are firmly based in the field of cell biology. However, many of the traits, especially those that are functional only in the dead hair, must undergo final transformative processes within an environment in which conventional cell biology processes may hinder that function. For example, the final organization of the disulfide network within macrofibrils occurs in a densely packed molecular environment in which enzymes and chaperone molecules cannot easily function. These stages of development fall within the field of protein chemistry. Explaining the full development of normal hair, or the etiology of monilethrix, requires an interdisciplinary effort, with final hair functional phenotype falling into the area of physical material science.

CHEMISTRY: CELL LINE-SPECIFIC MODES OF MATURATION

A feature of all cell lines in the developing fiber, IRS, and companion layer is that the cytoplasm is packed with free-floating ribosomes (38,39). Protein expression directly into the cytoplasm without modification or packaging is important to the interactions that lead to structure development. Production and assembly of structural proteins must also continue within cortical and other cell lines even at late stages of fiber development, distal of the bulb, where the cytoplasmic space is becoming crowded, the chemical environment becomes increasingly oxidative, and the organelles are being actively degraded (40). As each cell line moves up the bulb distal to its origin at the edge of the DP basement membrane, it progresses down a specific path of differentiation, keratinization, cornification, and death.

Four distinct patterns of maturation occur, and within these, each cell line has its own specific timing and features.

Type I maturation includes the ORS, companion, Henle's and Huxley's layers, and the IRS cuticle, and all share sudden, demarcated cornification (41) occurring within one cell length (42–44), similar to generation of cornified cell envelopes in skin (45).

Type II maturation occurs in the cuticle and is a prolonged multistage keratinization, which is far from understood but involves integration of nonfilament-forming keratin and KAPs into amorphous layers, with introduction of enzymatically induced isopeptide bonds and nonenzymatic disulfide bonds (46).

Type III maturation takes place in the medulla. The medullary core of some hairs cornifies relatively suddenly, and the process also involves transglutaminase, nonfibrillar keratin, and trichohyalin. However, the mechanism differs from that in the root sheath (14,47).

Type IV cortical maturation is gradual, nonenzymatic, involves large-scale protein conformational changes, and cumulates in a relatively sudden cornification event, which, along with the medulla and cuticle, appears to be a dewatering process in which the fiber shrinks about 25% in diameter (48–50).

ORWIN'S TRANSITION MARKS A KEY DEVELOPMENTAL STAGE AT THE TOP OF THE BULB

In the lower follicle bulb, keratinocyte division stops abruptly before the cells reach the apex of the DP, and this threshold, where differentiation replaces proliferation, is

known as the Auber's critical level (51,52), or the Auber's line, after its originator (48). Biologically driven differentiation processes dominate the remainder of the bulb, including significant cell reshaping (24) and expression of the first waves of keratins and KAPs (30).

The top of the bulb is another significant developmental transition. By the top of the bulb, IRS and fiber cell nuclei have lost transcriptional function and cell shaping is complete. Around this point, high-energy metabolic events including a dramatic plume of reactive oxygen species called the "ring of fire" is observed (53) and Henle's layer cornifies *via* a rapid enzymatic process (42,54). In the restricted tube formed by the cornified Henle's cells, with the fiber shape finalized and mitochondria degrading (40), it is likely that the physical and chemical environment begins to transform into one that may be considered cytotoxic and within which maturation is driven largely by chemical processes (55).

We define "Orwin's transition" as the crossover from where development is primarily driven by biology in a slightly reducing environment to one driven primarily by protein chemistry and thermodynamics in a chemically oxidizing environment. Orwin's transition crosses the follicle at the point at which Henle's layer hardens, and in the scheme developed by Orwin, this is the border of zones B and C (19,39) (Figure 1).

Distal of Orwin's transition, macrofibrils continue to grow, but there are distinct signs of gradual keratinization in terms of protein changes (56), increasing stiffness (57), affinity to electron microscopy stains (56) and filament alignment, and conformational change (57,58). Also distal of Orwin's transition, the cytoplasmic environment is hypothesized to become chemically oxidizing, possibly because of the release of superoxide from degrading mitochondria (40,53). The oxidizing environment encourages disulfide bond formation and reduction in IF diameter to about 7 nm (59–62). To date, final cornification is a process that is still not well understood (Figure 2).

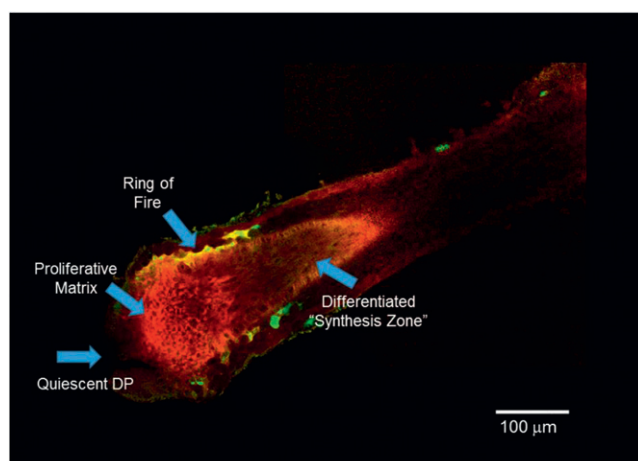


Figure 2. Compartmentation of hair follicle metabolism. Red, active mitochondria *via* tetramethylrhodamine (TMRM); green, ROS *via* 2',7'-dichlorofluorescein diacetate (DCF). The figure shows red proliferative matrix, weak mitochondrial activity during differentiation, and the ROS generation of the "ring of fire" in the upper bulb of a viable, whole human hair follicle.

BIOENERGETICS: MITOCHONDRIA AS A TARGET FOR HAIR BENEFITS

Overall, hair follicle metabolism shows high compartmentalization. The DP is relatively quiescent, with little mitochondrial metabolism and low ROS generation. The base of the bulb indicates an area of high mitochondrial membrane potential but low ROS generation, indicative of the proliferative nature of the bulb base. Moving upward past Auber's line (Figure 3), the cells continue to have high mitochondrial membrane potential but now increasing ROS production and presence of acidic secretory vesicles (53). The "ring of fire" indicates the highest local region of ROS production and is likely associated with initial keratinization of the IRS. Once passing the "Orwin's transition," the mitochondrial membrane potential is lost and there only remains the generation of ROS associated with keratinization and cross-linking. Leverage of real-time imaging of living tissues, in particular growing isolated human hair follicles will provide new insights into hair follicle morphology and bioenergetics and increase our fundamental knowledge of hair growth and metabolism. Better understanding of the mechanisms underlying hair growth may lead to better strategies to treat alopecia and hirsutism, enhance wool production, and develop new cosmetic products.

PHYSICS: THE DEAD HAIR PRODUCT/FUNCTIONAL PHENOTYPE

At this point, biology and chemistry have created the basic hair shaft structure. The current state of cosmetic hair science employs physics and physical chemistry to measure the properties of the hair shaft and relate them to consumer perception and benefit. Understanding how the physical measures work and how they quantify properties gives insight into how potential biological changes may contribute to hair physical properties.

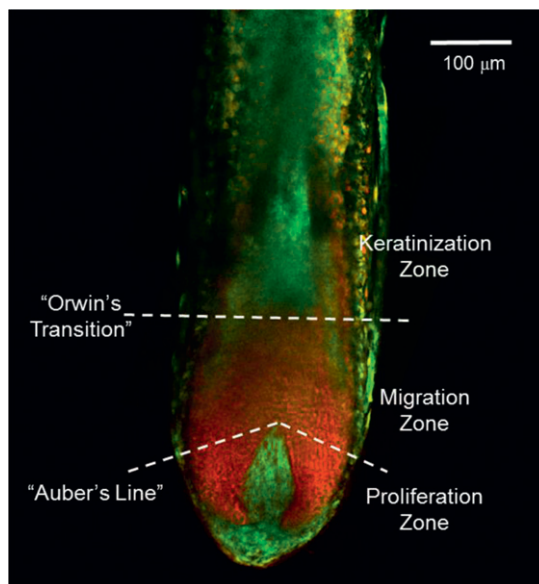


Figure 3. Location of key compartments of follicle metabolism, biological, and chemical activity in a living human hair follicle.

Mature hair is dead hair, composed of the dead remnants of cells that have been sacrificially transformed into the cuticle, cortex, and medulla (63). The function of hair, defined by their structure, protein chemistry, and mechanical properties, is codified in life to generate a phenotype that only becomes functional after death. How human hair phenotype is determined by specific genetic controls remains mostly unknown. Most genetic disorders that may provide interesting clues come from human heritable diseases, such as monilethrix mentioned previously, or similar scenarios in animal models. We propose that macroscale studies of hair phenotype should form an essential complement to biologically driven hair research (64).

SUMMARY AND CONCLUSIONS

Generation of human hair is a complex process involving biology to create biomass and generate biosynthetic building blocks and chemistry to use these building blocks to create the final hair shaft. These activities are compartmentalized within the hair follicle, a complex structure with many cell types coordinated into a single production unit. Measurement of the end product, the hair shaft, is mainly based on physics. To best serve patients, hair loss subjects, and consumers in need of better hair, the scientific communities in the diverse disciplines of biology, chemistry, and physics need to work together. When merged, a joint program has defined follicle energy production and mitochondrial energy production as high potential intervention points for designing new products.

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