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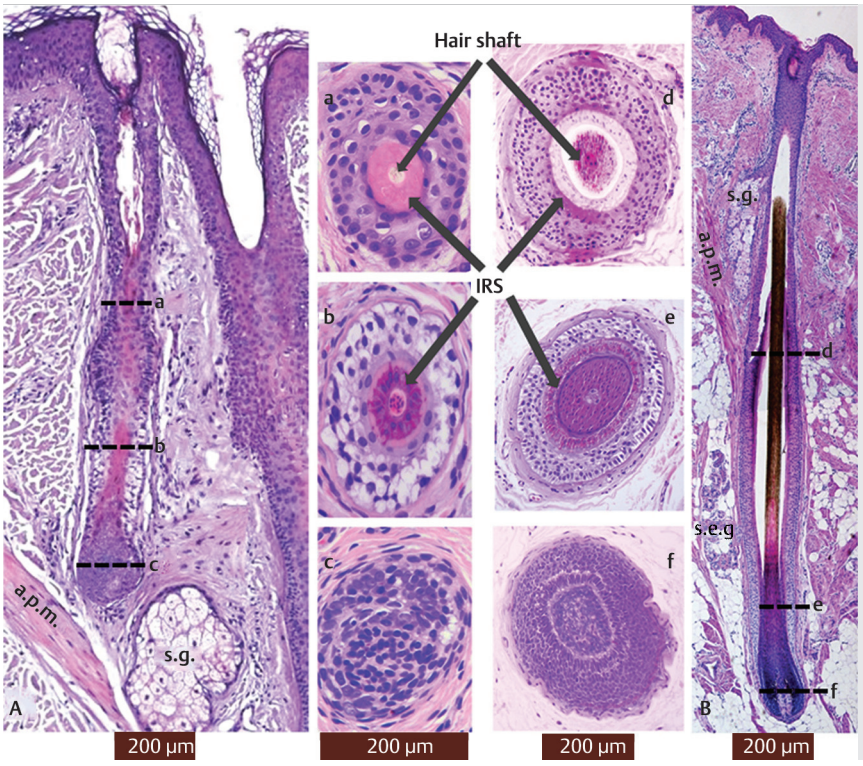

Fig. 3.1 Histological differences between vellus and terminal hair follicles. The image on the left **(A)** shows a vertical section of a vellus follicle. Note that vellus follicles do not have arrector pili muscle attachment and that the bulb is rooted in the dermis. Images **a**, **b**, and **c** are horizontal sections made at their respective level of depth. The image on the right **(B)** shows a vertical section of a terminal hair follicle. Images **d**, **e**, and **f** are horizontal sections of a terminal hair follicle. The purpose of these figures is to show the differences in hair shaft diameter between vellus and terminal follicles: by definition, vellus hair shafts are thinner than the inner root sheath (IRS). Note that terminal follicles are rooted in the subcutaneous fat. a.p.m., arrector pili muscle; s.e.g., sweat eccrine gland; s.g., sebaceous gland.

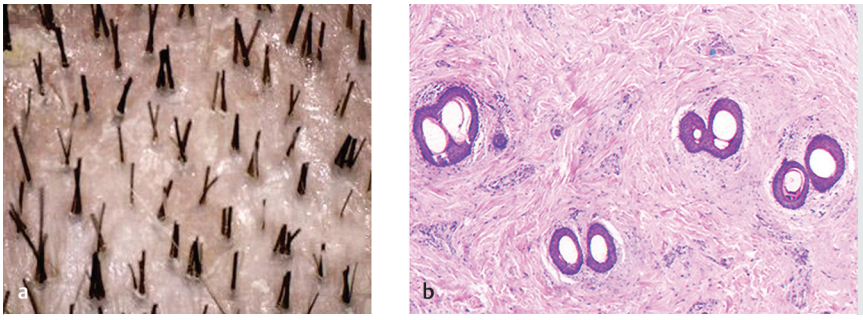

Fig. 3.2 Follicular units (FUs). **(a)** Close-up photo of the occipital scalp skin showing how hairs exit the surface, forming groups known as follicular units (FUs). **(b)** A horizontal histologic section of four 2-hair FUs. This section was made at the infundibulum level (above the sebaceous glands).

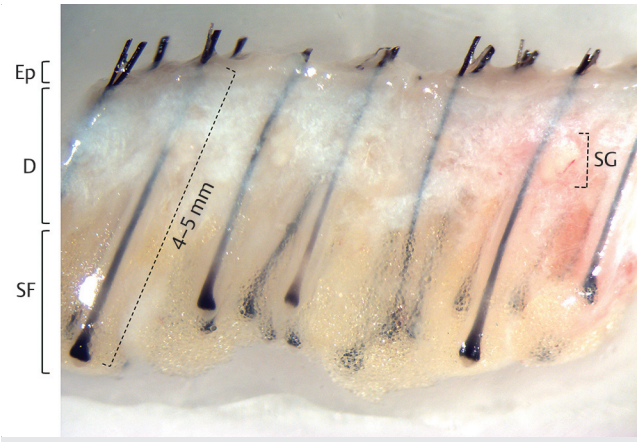

Fig. 3.3 Sliver dissected from a strip excision. This photo shows a sliver dissected under the stereomicroscope from a donor strip. It illustrates the different compartments of the scalp skin. D, dermis; Ep, epidermis; SF, subcutaneous fat. Note how deep the bulbs of the terminal follicles are located (normally between 4 and 5 mm). Also note the yellowish color of the sebaceous glands (SG), and the arrangement of the follicles in groupings. Other structures present in the skin including arrector pili muscles, sweat glands, small vessels, and nerves are invisible under the stereomicroscope.

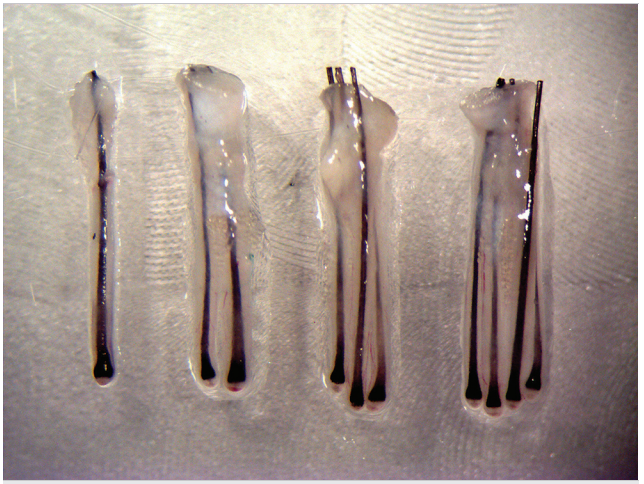

Fig. 3.4 Follicular units represent the main transplant graft. FUs of human scalp may contain one, two, three, or four terminal hairs (from left to right). These FUs were harvested with a 0.95-mm punch using the FUE technique.

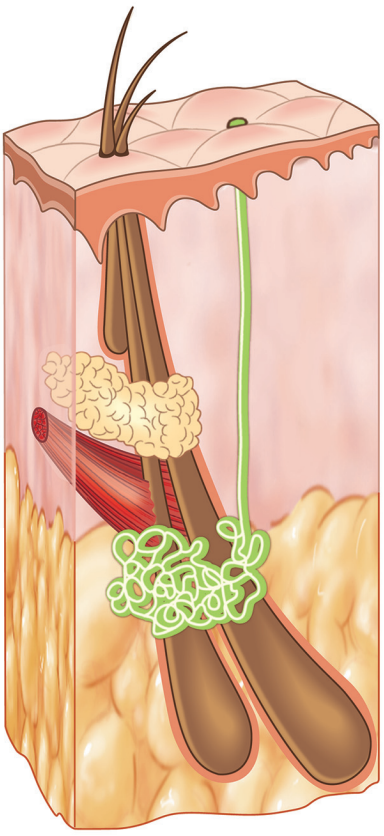

Fig. 3.5 Anatomy of the follicular unit. This is a drawing showing the spatial relationship between the different components of an FU transplant graft: hair follicles, sebaceous gland (*yellow*), eccrine gland (*green*), and arrector pili muscle (*red*).

model takes into consideration the FU as a unit structure, introducing the concept of one FU served by one AP muscular unit,[5] in which the AP muscles that originate from their respective follicles join together, forming a single muscular structure that extends upward to its superior attachment zone. We could imagine the AP muscles acting as a string that ties all the HFs of each FU together, like a ribbon on a bunch of flowers (▶ Fig. 3.5). Below that area, the inferior portion of the anagen follicles tends to splay out, which is the main reason why sharp FUE punches inserted too deep (usually deeper than 3 mm) cause excessive follicular transection.

The AP muscle is attached to the follicle in a portion of the outer root sheath known as the bulge zone. It has been shown that the bulge stem cells are responsible for guiding the attachment of the AP muscle by means of the deposit of a protein called nephronectin.[6] In hair transplantation, the AP muscles are obviously transected during donor harvesting but the muscle seems to be regenerated after implantation in the recipient area[7] maintaining its contractile capacity.

3.2.3 FUs Contain Eccrine Sweat Glands

The surgeon and the hair transplant technicians cannot see eccrine glands under the stereomicroscope because they are not visible unless stained with specific dyes. Nevertheless, the majority, if not all, of FUs contain one eccrine coil (secretory portion of the eccrine sweat glands) as can be observed in many vertical histologic sections at the level of the inferior portion of the follicle (below the AP muscle; ▶ Fig. 3.5 and ▶ Fig. 3.7).[8] The functional significance, if any, of this eccrine–HF anatomic association is currently unknown.

3.3 The Terminal Hair Follicle: The Hair Surgeon's Most Precious Tissue

The terminal HF is what produces the thick and long hair shaft. It would seem to be a simple structure, but the terminal HF is in fact quite complex and can be considered a miniorgan per se, composed of many different types of cells that interact together and with the surrounding microenvironment: epithelial cells, mesenchymal cells from the dermal papilla (DP) and dermal sheath, several pools of epithelial, melanocyte, and mesenchymal stem cells involved in HF self-regeneration and pigmentation, a rich innervation and vascularization network, and resident immunocytes (mast cells, macrophages, T cells, and Langerhans cells). Some of these different cell types contribute to hair shaft growth and some to other very important functions (dermal remodeling, re-epithelialization after wounding, cutaneous stem cell homeostasis, etc.), which are beyond the scope of this chapter.

3.3.1 Changes in the Anatomy of the Terminal HF According to the Hair Cycle

HFs follow a continuous cycle of growth (anagen phase), involution (catagen phase), and rest (telogen phase) until a new cycle develops. In humans, these events are asynchronous, which means that each follicle contained in an FU is at a point in its cycle, which is independent of its neighbors (▶ Fig. 3.6a). In normal circumstances, approximately 90% of human scalp follicles are in anagen, and the remaining 10% in either catagen or telogen.

The anagen terminal follicles are the most common and the easiest to identify under the stereomicroscope due to their well-defined inferior segment. On the scalp, they have an average length of 4 to 5 mm, although they can be as short as 3 mm in patients with thin hair and up to 6 mm in thick hair scalps. Depending on the duration of the anagen phase, HFs in different areas of the body produce hairs of different length. For example, scalp follicles stay in anagen for a long time (2–8 years), with the hair shaft length increasing each day at an average rate of 0.30 mm.

The catagen phase starts with the destruction by massive apoptosis of the inferior portion of the HF. This event leaves an epithelial strand and a significant reduction in HF size. The DP changes from cone-shape to a more condensed shape and moves upward, remaining attached to the epithelial strand like a remnant DP. The contraction of the dermal sheath due to their smooth muscle molecular machinery seems to be essential for the upward movement of the hair shaft and the dermal papilla niche during catagen. This process allows the dermal papilla to relocate and reach its stem cell-adjacent position before

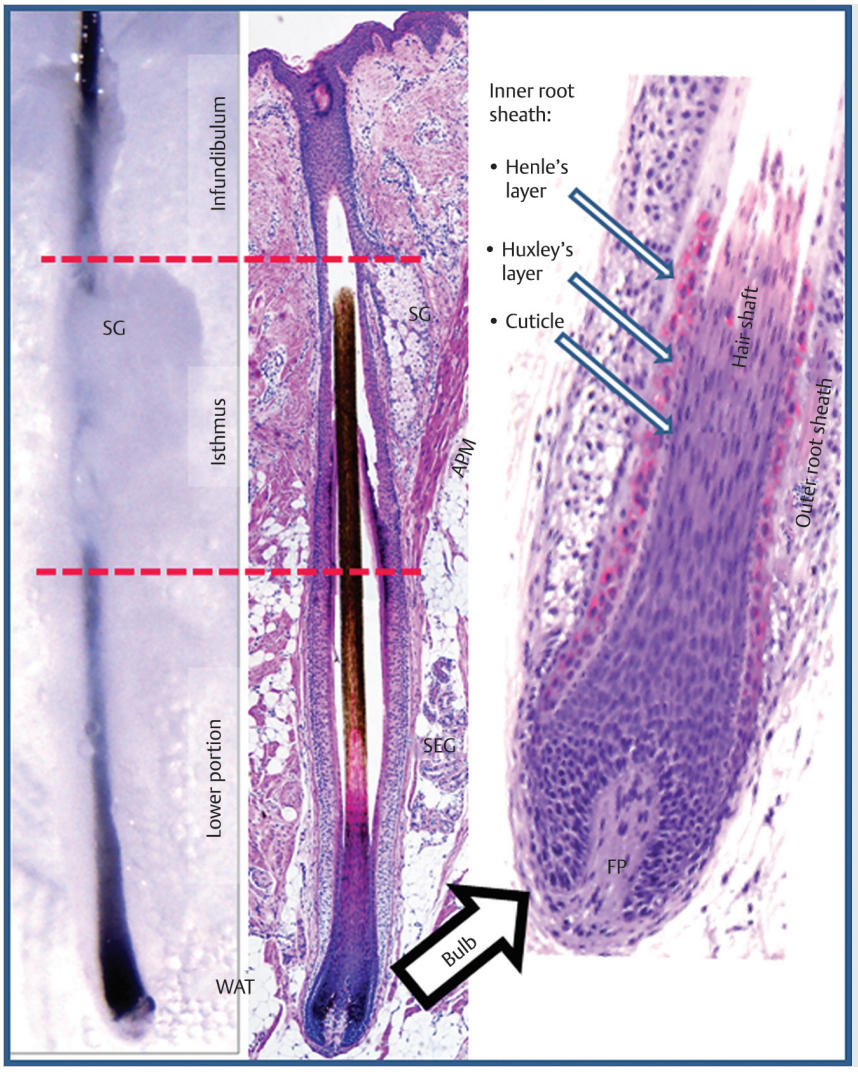

Fig. 3.7 Histology of a terminal hair follicle. Vertical section of an anagen terminal follicle stained with hematoxylin and eosin. The three compartments of the follicle (infundibulum, isthmus, and inferior portion) are delineated, as well as the different epithelial layers. Note the position of the secretory coiled portion of the eccrine gland (SEG), very close to the follicular epithelium and always below the arrector pili muscle (APM). FP, follicular (or dermal) papilla; SG, sebaceous gland; WAT, white adipose tissue.

3.4 The Location of the Stem Cell Niches for Hair Follicle Regeneration

The stem cells of the follicles reside in topographically well-defined locations. These specialized tissue compartments that host the stem cells and every other component necessary for their function, including neighboring cell populations, molecular signals, and other extracellular components, are commonly referred to as "niches." There are two niches in the follicle: the epithelial and the mesenchymal niche. These HF niches are critical for regulating the process of hair regeneration (from telogen to re-entry in anagen), and the hair surgeon should be aware of their precise location to avoid any damage.

The epithelial follicular niche is the best studied. It is located in a region of the follicle known as the bulge. The bulge zone can be easily recognized in hematoxylin and eosin vertical sections of murine and human fetus as a prominent protuberance of the ORS. In contrast, in adult human follicles, the bulge region is barely prominent. Initially, the bulge was only known for being the attachment zone of the AP muscle, until significant interest was aroused when it was identified as the area that contained the main pool of follicular epithelial stem cells. Anatomically, the bulge in anagen follicles extends between 1 and 2 mm below the skin surface and coincides with the location of the isthmus portion of the follicle,[12] while in telogen follicles the bulge represents the deepest epithelial portion of the follicle. Specific immunohistochemical markers such as CK15 and CD200, among others, can delineate bulge stem cells in human follicles (▶ Fig. 3.8).

Recent studies made in mice follicles have uncovered very interesting aspects of the hair stem cell dynamics. It seems that the bulge zone has several compartments of organization: a more activated and a more quiescent one, and the location of the stem cells within the niche is important in predicting their fate and contribution to hair growth. Specifically, cells located in the lower bulge generate ORS lineages, while those situated further down, in the hair germ, contribute to the IRS and hair shaft layers. Cells in the mid and upper bulge do not contribute directly to the regeneration of the follicle and remain quiescent. We now know that in telogen follicles the first stem cells that become activated and proliferate to enter a new regeneration cycle are cells of the hair germ.[13] The reason why hair germ

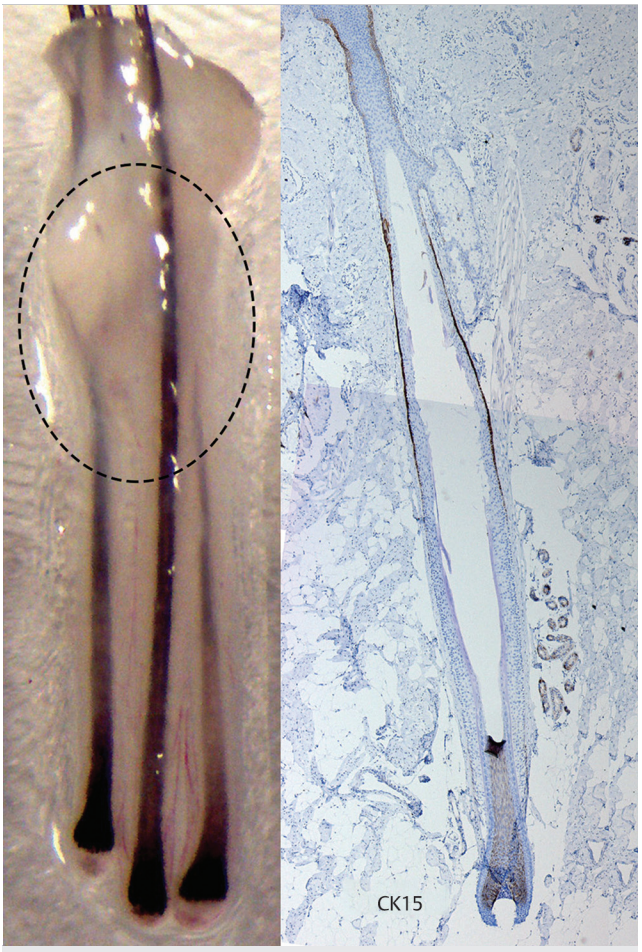

Fig. 3.8 Location of the bulge (epithelial stem cell zone). The figure on the left shows a typical FU transplant graft in which a *circle has been drawn* delineating the location of the bulge stem cell zone. This region, located approximately 1–2 mm below the skin, coincides with the region stained with the antibody anti-cytokeratin 15, which is a marker of human follicular epithelial stem cells (see positive brown staining of the outer root sheath cells in the bulge zone).

cells are the first to proliferate is thought to be due to their close proximity to the DP cells, which emanate activating signals for hair regeneration.

The mesenchymal niche is primarily composed of a group of fibroblast-like cells that form the DP and the lower portion of the dermal sheath that surrounds the bulb, which is known as the dermal cup. It is thought that in the dermal cup there is a population of self-renewing dermal stem cells, which, at the onset of each anagen growth stage, are mobilized to regenerate a new dermal sheath and supply new cells to the DP.[14] The DP cell number seems to be related to the follicle's capacity to initiate new hair growth. As DP cell numbers decline below a specific threshold, HFs are unable to initiate a new hair cycle, whereas follicles retaining a sufficient number of DP remain able to re-enter the growth phase.[15] Destruction of the DP in telogen follicles renders the HF incapable of initiating anagen growth.[13] The ability to isolate and culture DP cells in 3D cultures to maintain their inductive capacity for hair neoformation has been demonstrated by a variety of transplantation experiments; however, its translation into clinical practice in an efficient manner has not yet been possible.

Other important players in HF regeneration are molecules involved in signaling pathways as well as growth factors that regulate stem cell quiescence, proliferation, and differentiation. These molecules emanate from the DP or from the bulge or from neighboring cells. The most relevant ones in follicles include the Wnt pathway, BMP, TGF-beta, and FGF.

References

[1] Meng J, Wyss AR. Multituberculate and other mammal hair recovered from Palaeogene excreta. Nature. 1997; 385(6618):712–714

[2] Headington JT. Transverse microscopic anatomy of the human scalp. A basis for a morphometric approach to disorders of the hair follicle. Arch Dermatol. 1984; 120(4):449–456

[3] Jimenez F, Ruifernández JM. Distribution of human hair in follicular units: A mathematical model for estimating the donor size in follicular unit transplantation. Dermatol Surg. 1999; 25(4):294–298

[4] Lee IJ, Jung JH, Lee YR, Kim JC, Hwang ST. Guidelines on hair restoration for East Asian patients. Dermatol Surg. 2016; 42(7):883–892

[5] Poblet E, Ortega F, Jiménez F. The arrector pili muscle and the follicular unit of the scalp: a microscopic anatomy study. Dermatol Surg. 2002; 28(9):800–803

[6] Fujiwara H, Ferreira M, Donati G, et al. The basement membrane of hair follicle stem cells is a muscle cell niche. Cell. 2011; 144(4):577–589

[7] Sato A, Toyoshima KE, Toki H, et al. Single follicular unit transplantation reconstructs arrector pili muscle and nerve connections and restores functional hair follicle piloerection. J Dermatol. 2012; 39(8):682–687

[8] Poblet E, Jimenez F, Escario-Travesedo E, et al. Eccrine sweat glands associate with the human hair follicle within a defined compartment of dermal white adipose tissue. Br J Dermatol. 2018;178(5):1163–1172

[9] Heitman N, Sennett R, Mok KW, et al. Dermal sheath contraction powers stem cell niche relocation during hair cycle regression. Science. 2020;367 (6474):161–166

[10] Higgins CA, Westgate GE, Jahoda CAB. From telogen to exogen: mechanisms underlying formation and subsequent loss of the hair club fiber. J Invest Dermatol. 2009; 129(9):2100–2108

[11] Alexander CM, Kasza I, Yen CLE, et al. Dermal white adipose tissue: a new component of the thermogenic response. J Lipid Res. 2015; 56(11):2061–2069

[12] Jimenez F, Izeta A, Poblet E. Morphometric analysis of the human scalp hair follicle: practical implications for the hair transplant surgeon and hair regeneration studies. Dermatol Surg. 2011; 37(1):58–64

[13] Rompolas P, Greco V. Stem cell dynamics in the hair follicle niche. Semin Cell Dev Biol. 2014; 25–26:34–42

[14] Rahmani W, Abbasi S, Hagner A, et al. Hair follicle dermal stem cells regenerate the dermal sheath, repopulate the dermal papilla, and modulate hair type. Dev Cell. 2014; 31(5):543–558

[15] Chi W, Wu E, Morgan BA. Dermal papilla cell number specifies hair size, shape and cycling and its reduction causes follicular decline. Development. 2013; 140(8):1676–1683

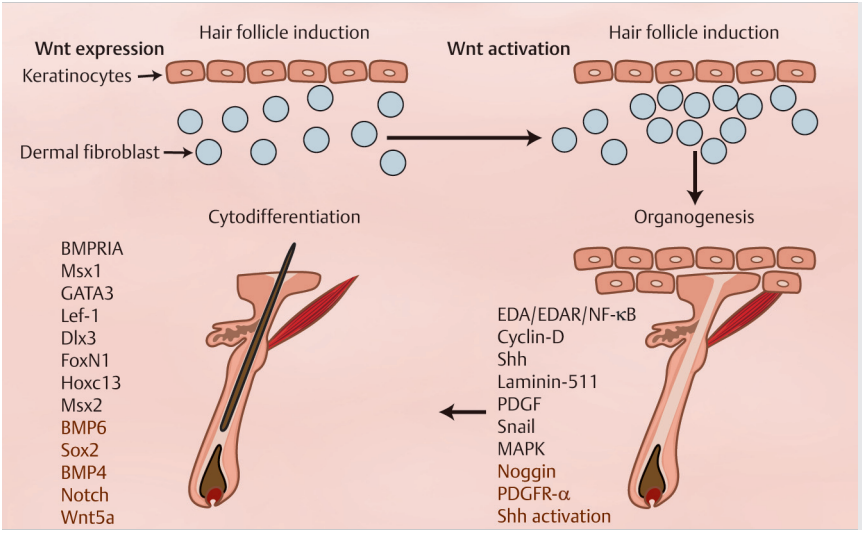

Fig. 4.1 The stages of morphogenesis are broadly classified into: induction, organogenesis, and cytodifferentiation, and its proper development involves a strong interplay between Wnt, Notch, Hedgehog, and bone morphogenetic protein signaling pathways.

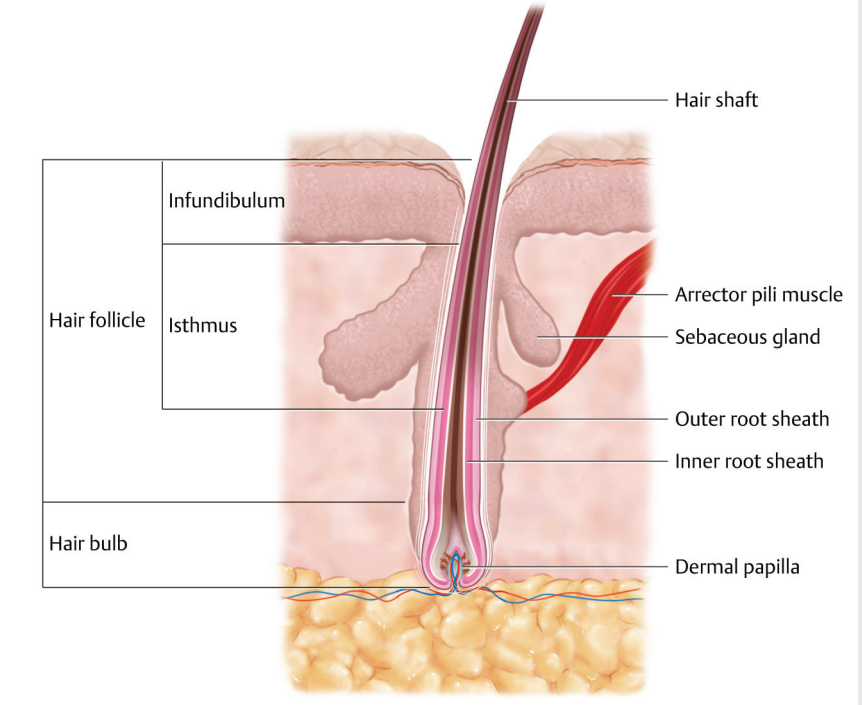

Fig. 4.2 The hair follicle is part of the pilosebaceous unit that contains the sebaceous gland and arrector pili muscle. It is composed of two main compartments: the upper part (infundibulum and isthmus) and lower part (bulb, matrix, and dermal papilla).

4.3.1 Anagen

Anagen is the growth phase, and bulge region stem cells differentiate to all hair lineages, resulting in hair elongation. During this active growth phase, a hair fiber is produced, as the HF enlarges and reaches its characteristic onion shape.

Anagen can be divided into six stages (I–VI). During anagen I–V (proanagen), hair progenitor cells proliferate, envelope the growing DP, grow downward into skin, and begin to differentiate into the hair shaft and inner root sheath (IRS). The newly formed hair shaft then develops, and hair matrix melanocytes show pigment-producing activity. In anagen VI (metanagen), full restoration of the hair fiber–producing unit occurs, and is characterized by formation of the epithelial hair bulb surrounding the DP, which is located deep in subcutaneous tissue, and the new hair shaft appears from the skin surface. The duration of anagen varies according to anatomic location, with this phase in the scalp lasting an average of 3 to 4 years but in some individuals it may last up to 8 years; in contrast, anagen lasts only 3 months in the eyebrow.[1,2,5,6]

4.3.2 Catagen

Catagen is the regression phase, and starts when anagen ends. During catagen, differentiation and proliferation of hair matrix keratinocytes decreases, melanocytes stop pigment production, and hair shaft production is completed.

At this stage, there is substantial decrease in cell cycling because of increased apoptosis in epithelial cells of the bulb, outer root sheath (ORS), and outermost epithelial layer. Club hairs are formed, with the keratinized brush-like structure at its base anchoring it to the telogen follicle. The DP is

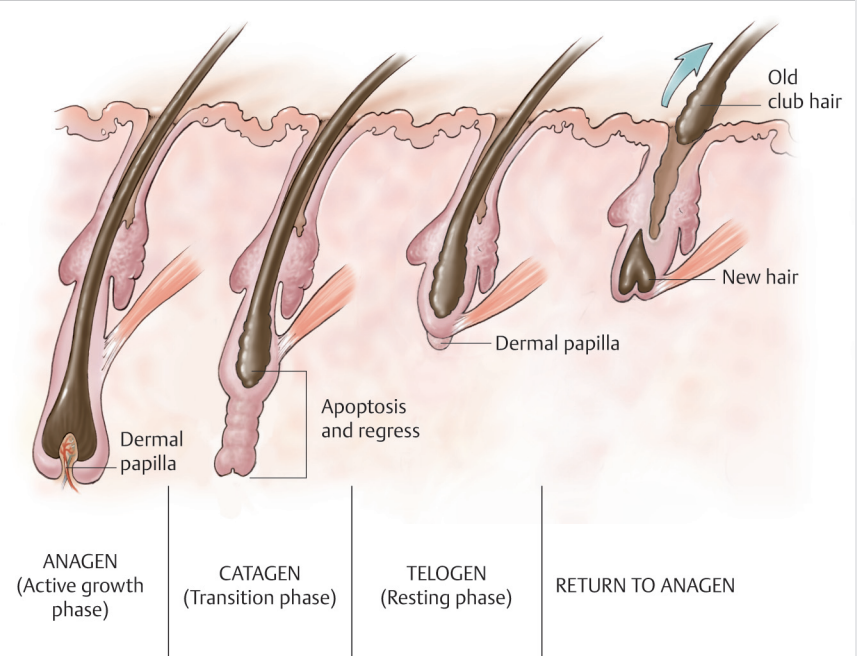

Fig. 4.3 The characteristic growth and retraction phases that a hair follicle cycles through is known as the hair cycle. This includes three distinct phases, namely anagen (growth phase), catagen (regression phase), and telogen (resting phase). (Reproduced with permission from Barrera A, Uebel CO. Hair Transplantation: The Art of Follicular Unit Micrografting and Minigrafting. 2nd ed. Thieme Publishers; 2013.)

transformed into a cluster of quiescent cells closely adjacent to the regressing HF epithelium and travels from the subcutis to the dermis/subcutis border to maintain contact with the distal part of the HF epithelium, including the secondary hair germ and bulge. This phase lasts a few weeks. As majority of HF cells undergo apoptosis, there is shortening of the lower compartment, and DP cells are brought closer to the bulge. This upward movement of the follicular papilla during catagen is crucial for re-establishing follicular papilla–bulge cell contact and induction of a new hair cycle.

Molecules that promote catagen induction have been identified as p75, p53, TGF-β1, FGF5, BDNF, and BMPRIa. It is the exchange of signals between the papilla and bulge that regulates catagen duration. The cells that escape apoptosis during this phase comprise the reservoir that leads to the next anagen.[1,2,5,6]

4.3.3 Telogen

Finally, telogen begins and the hair goes into resting phase. This phase in the scalp generally lasts 3 months, but may last up to 8 months in some; in contrast, telogen lasts only a few weeks in the eyelashes. Cells enter a quiescent state waiting for signals to re-enter anagen, with an estimated 5 to 15% of scalp HFs remaining in telogen at any time point. Telogen HFs lack pigment-producing melanocytes and IRS. Their DP is closely attached to a small cap of secondary hair germ keratinocytes containing HF stem cells.

At the end of telogen, the hair sheds (exogen). The HF subsequently re-enters the growth phase a few weeks later by stimulating bulge stem cells. The bulge activation theory proposes that bulge stem cells proliferate after signals from the DP. Bulge cell proliferation is the cellular source of the entire HF structure, including hair matrix cells. These daughter cells are transient amplifying cells, which can undergo only a limited number of mitoses, thus establishing the length of anagen and onset of catagen.[1,6]

Telogen-to-anagen transition is, however, dependent on many factors, and since the HF strongly expresses estrogen receptors during the telogen phase, binding of 17-β-estradiol to these receptors prevents HFs from exiting the telogen phase to enter anagen phase.

Disruption of the processes involved in the hair cycle can therefore lead to various hair growth disorders, and further understanding is essential for the development of more effective therapeutics.

4.4 Hormones and Hair Follicles

The effects of neurohormones on HF growth are complex and strongly dependent on hair cycle stage. A close localization of autonomic and sensory nerve fibers and the bulge area suggests that neuropeptides may influence stem cells and modulate the hair cycle. It is also now clear that HFs are not only a target of neuromediators, but its keratinocytes, melanocytes, and fibroblasts also synthesize neurohormones.

Several studies showed the expression of a neuroendocrine system in the human HF. In particular, the expression of urocortin, corticotropin-releasing hormone (CRH) and CRH receptors, proopiomelanocortin-derived neuropeptides (alpha-melanocyte-stimulating hormone [α-MSH], β-endorphin, adrenocorticotropic hormone [ACTH], thyrotropin-releasing hormone, melatonin), and their associated receptors has been reported.

The role of neurohormones and neuropeptides in human HF pigmentation extends beyond the control of melanin synthesis by α-MSH and ACTH, and includes melanoblast differentiation, reactive oxygen species scavenging, and HF pigmentary unit remodeling.[6]