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Physiological Regeneration of Skin Appendages and Implications for Regenerative Medicine

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The concept of regenerative medicine is relatively new, but animals are well known to remake their hair and feathers regularly by normal regenerative physiological processes. Here, we focus on 1) how extrafollicular environments can regulate hair and feather stem cell activities and 2) how different configurations of stem cells can shape organ forms in different body regions to fulfill changing physiological needs.

Regenerative medicine has great potential. The main challenge is how to elicit and harness the power of regeneration. Currently, the major issues are how to obtain stem cells, how to pattern stem cells into organized tissues and organs, and how to deliver stem cell products to patients. Although human beings have very limited powers of regeneration, many animals have robust regenerative powers, distilled and selected over millions of years of evolution. Here, we review fundamental principles of regenerative biology learned from nature in the hope that they can be applied to help the progress of regenerative medicine.

Several reviews have covered how lower animals, such as hydra, planaria, salamanders, and lizards regenerate after injury (1, 9, 69). Injuries can lead to repair or regenerative responses. In repair, the wound is covered to produce a protective shield without complete functional restoration, whereas in regeneration the original tissue is reformed and restores its initial function. Examples of regeneration are plentiful, such as in the amphibian limb (63) and the lizard tail (2), where blastema are induced. Regeneration can occur in mammals e.g., in the injured mouse liver (22) or in large skin wounds that result in *de novo* hair regeneration (34). In contrast, large human skin wounds produce scarring. Hence, there is a great need to understand the mechanisms employed by organs in higher vertebrates that can undergo regeneration to improve the quality, functionality, and appearance of healed wounds in humans and gain insights for regenerative medicine.

Hair and Feather Follicles Are Leading Models of “Physiological Regeneration” in Higher Vertebrates

Here, we focus on “physiological regeneration” in which organs in higher vertebrates undergo episodic loss and regeneration under normal physiological conditions, whereas their organ stem cells undergo cycles of activation/quiescence (FIGURE 1, A AND B).

Regenerative skin appendage cycling provides a powerful mechanism for the skin to change its appearance and functions in accordance with stages of postnatal and sexual development or seasonal environment (FIGURE 1C). In adult mammals, hair follicles go through regenerative cycles of growth (anagen), regression (catagen), rest (telogen), and hair shaft shedding (exogen) phases (FIGURE 1A; Refs. 31, 37, 50, 67). Feathers also go through growth and rest periods. In every cycle, hair or feathers are lost and their follicles greatly reduced before a new organ structure regenerates; the lower follicle must be rebuilt from hair/feather follicle epithelial stem cells through interactions with the mesenchyme-derived dermal papilla (12). Therefore, these skin appendages are excellent models to learn how to activate stem cells in adults and shape them into a new organ.

The integument forms the interface between an organism and its environment. Skin appendage organs take the opportunity of physiological regeneration to remake their organ phenotypes. These changes help animals adapt to their environments or to different stages of their lives. Animals grow and change from inconspicuous babies dependant on their parents to sexually mature animals that need to attract mates; they then reach the time when they breed their own offspring and eventually become aged. The appearance and characteristics of their coats (hair/feather length and colour) at these different life cycle stages can be entirely different, selected during evolution to improve their chances of survival and reproductive capabilities (FIGURE 1D). For example, some birds show bright colors during the breeding season but otherwise have a less conspicuous coloration to reduce detection by predators. Similarly, human hair patterns clearly distinguish adults from children and between the adult sexes; loss of hair color characterizes the elderly. In addition, hair is changed biannually in many mammals to match changes in their environment; e.g., northern hares

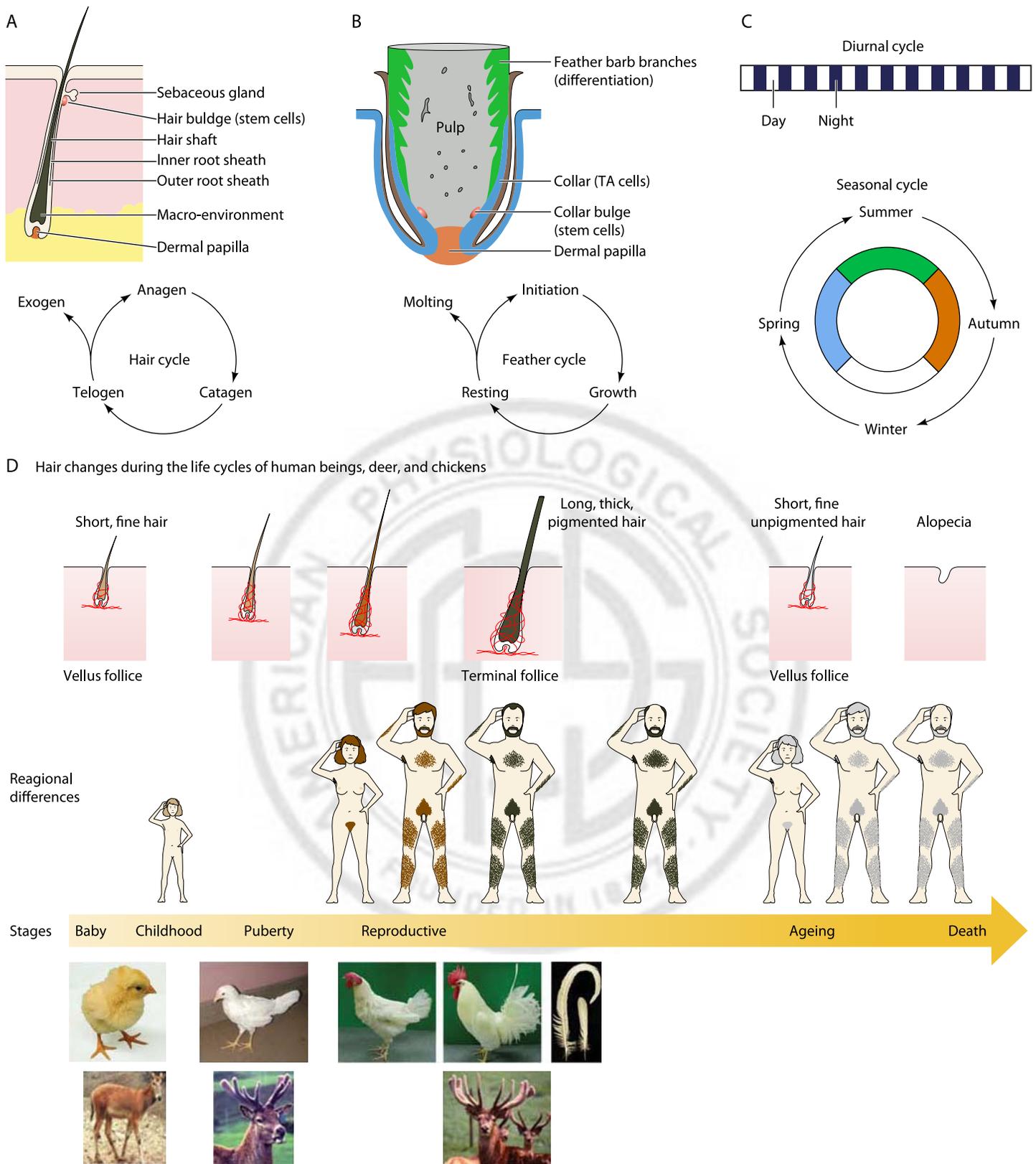


FIGURE 1. Physiological regeneration of hairs and feathers

A: hair follicle and cycling. B: feather follicle and cycling. C: hair follicle cells show circadian fluctuation (day and night are shown as alternating black and white bands) (40). The length of the hair cycle is affected by seasonal changes so that the phenotypes of hairs and feathers can change under different physiological conditions depending on the need for thermoregulation or attracting mates. D: each animal goes through physiological changes during different stages of its life cycle. Human beings, chickens, and deer (*Cervus elaphus*) all have different skin appendage patterns (spatial differences) in youth and adult stages (temporal differences). Different size and pigmented skin appendages are generated from the same follicle. How the hair/feather cycle integrates with different physiological signals is a fundamental biological question, and the mechanisms remain to be worked out. Understanding this process is critical to managing these regenerative ectodermal organs after illness, injury, or disease.

have a thick white coat in the snowy winter but a shorter, brown coat in the summer (20). The ability of ectodermal organs to reform with different shapes, sizes, and colors provides a unique experimental opportunity for us to learn how stem cells sense various physiological conditions, communicate with their external environment, and generate different functional forms, a wonderful, comprehensive, and accessible model for the study of system biology (3).

In the past decade, major progress has been made gaining new molecular understanding of hair (reviewed in Refs. 7, 21) and feather follicles (16, 39) by focusing on how skin appendages form during development, where the stem cells are, and how the stem cell niche regulates the activity of stem cells within the context of a single follicle (24, 48). Excellent reviews also cover the intrafollicular signaling and regulation of the various types of stem cells within hair follicles ranging from epithelial to mesenchyme-derived cell types (5, 6, 17, 21, 24, 64). Since these intra-hair follicle molecular mechanisms have already been reviewed in detail, they will not be repeated here. Instead, in this perspective, we aim to provide a more global conceptual network of extrafollicular factors regulating hair stem cells. Since this is relatively new, many molecular mechanisms have not been fully mapped. Indeed, it is our intention to stimulate more work in this direction.

Our work on the interaction of hair follicles and intradermal adipose tissue has revived interest in the macroenvironmental extrafollicular regulation of hair stem cells (53). After initially establishing bone morphogenetic protein (BMP) as an extrafollicular modulator, as well as an intrafollicular inhibitor, we later found that Dickkopf (DKK) and soluble frizzled related proteins (sfrps) also fulfilled this role (51). Platelet-derived growth factor (PDGF) and injected adipocytes were found to further affect hair cycling (5, 6, 17, 19, 19), and nerve-derived sonic hedgehog (SHH) was also shown to define the function of a subpopulation of hair stem cells (10, 58). These new findings led to recent perspectives that highlight interactions between hair follicles and other tissues (35).

Physiological Regulation of the Activities of a Population of Skin Appendage Stem Cells

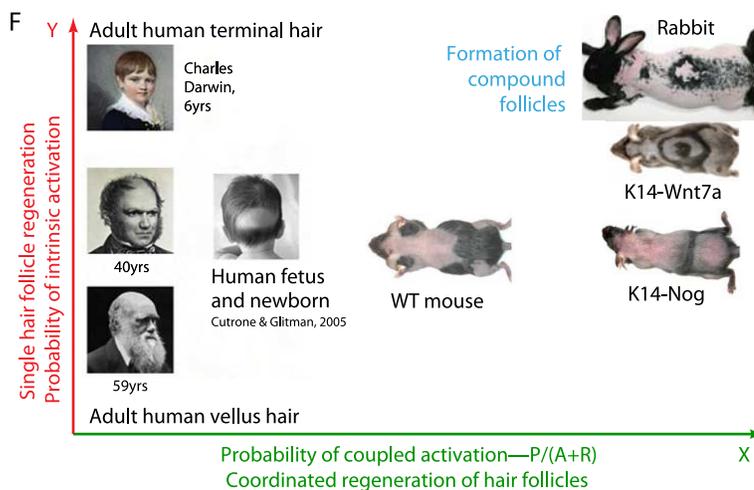
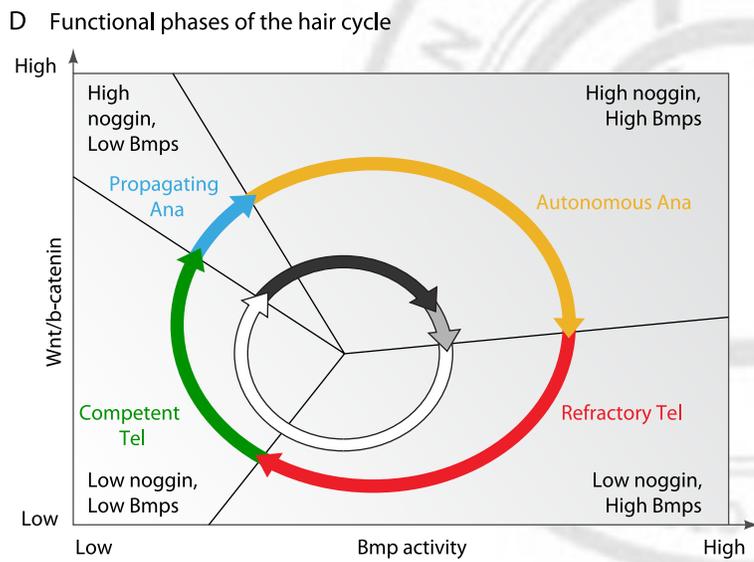
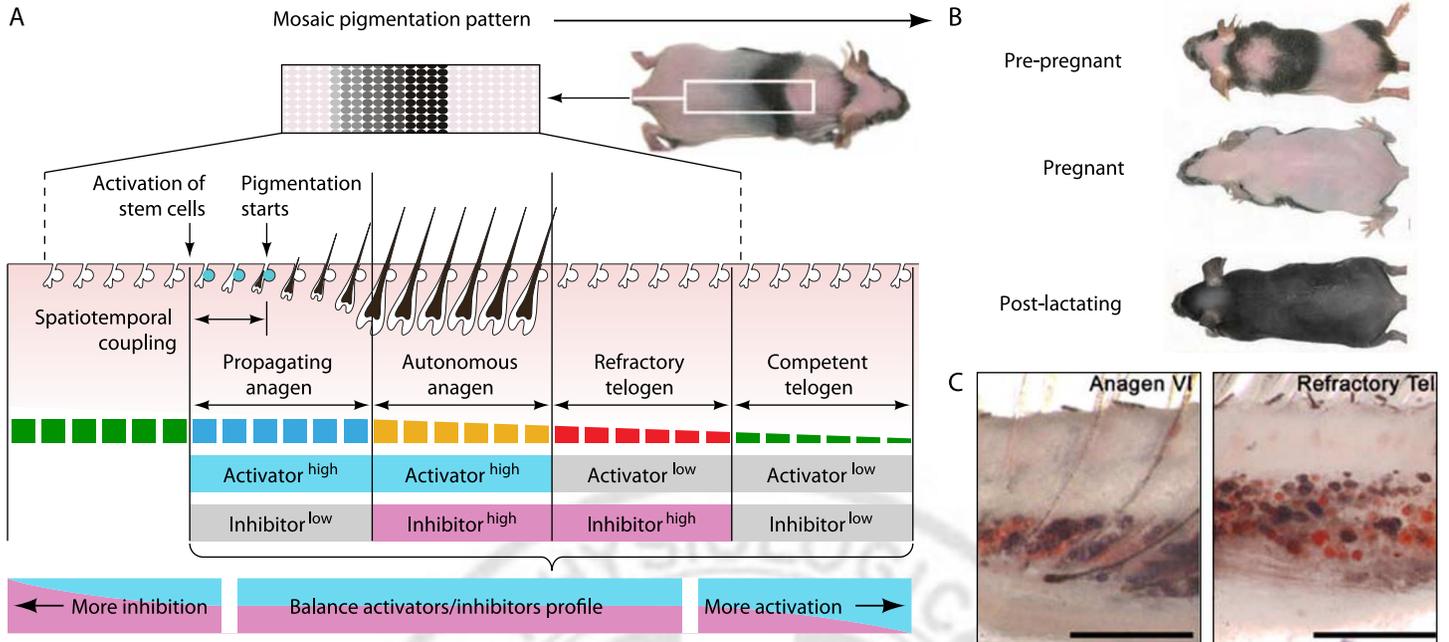
Here, we focus on the behavior of a population of follicles, i.e., a group of regenerating units, each capable of oscillating through several functional states. In this population, each regenerative unit may cycle randomly (completely autonomous), synchronously (completely coupled), or in a coordinated fashion to form transient waves and cycling

domains (FIGURE 2, A AND B). However, it has been difficult to visualize and analyze these patterned changes in vivo and to decipher their underlying mechanisms. In mouse skin, hair cycling is patterned and appears coordinated, allowing us to visualize macroscopic changes in hair growth states clearly on a manageable time scale. Hair cycling patterns are complex and change over time. Although present in wild-type mice, they are most obvious in mutant mice with the cyclic alopecia phenotype (45, 47, 72) and in the traveling stripes on a special strain of nude mice (68).

Visualizing hair waves is facilitated by simply clipping hairs in black mice (e.g., C57Bl6) with pigmented hairs and nonpigmented interfollicular skin. After hair was clipped (using barber clippers, not plucking or wax stripping, which cause injury and hair regeneration), anagen skin shows black pigmentation from the developing hair, whereas telogen skin remains pink (52). Using this improved non-invasive method, we can examine hair follicle stem cell activity in normal mice under different physiological conditions. We observed that hair regenerative waves sweep across mouse skin continuously; however, sometimes they can propagate, and sometimes they cannot pass certain domain boundaries (FIGURE 2, A AND B). We demonstrated that these domains are refractory because regenerative hair waves cannot pass through these regions. Further molecular studies showed that they are high in BMP2, which oscillates in the interfollicular dermis, adjacent to the cycling hair follicles (51–53). However, this dermal BMP oscillation is out of phase with Wnt oscillation within the hair follicle (FIGURE 2A). The asynchrony of these two oscillating cycles divides telogen into refractory and competent phases and anagen into propagating and autonomous phases (FIGURE 2D). *KRT14-noggin* transgenic mice exhibit both decreased BMP activity at the epithelial-mesenchymal interface and a reduced duration of refractory telogen (53), whereas *KRT14-wnt 7a* mice show increased Wnt activity at the epithelial-mesenchymal interface and a lower threshold for anagen propagation (51).

In rabbits, each follicle becomes a compound follicle with multiple bulge stem cells, hair germ cells, and dermal papilla, which are activated in a time sequence. This configuration increases the ability of follicles to initiate and propagate the hair wave, leading to a fractal-type wave pattern (FIGURE 2F; Ref. 9).

Interestingly, in normal female mice, we found the regenerative hair wave is on hold at telogen during periods of pregnancy and lactation. After lactation, the hair wave starts to move again (FIGURE 2B). Furthermore, we observe that the



dermal BMP is located in the subcutaneous adipose tissue (FIGURE 2C). Initially, we were puzzled by this finding. However, when we speculate that both subcutaneous adipose tissue and hairs originally evolved for endothermy, it becomes unsurprising that these two organs interact. This data allows us to develop a broader model that highlights the importance of the follicle macroenvironment on hair follicle stem cell activation. The macroenvironment is defined as factors extrinsic to the hair follicle, including those from the dermis and systemic hormones, as well as the influences exerted from the external environment, such as seasonal variations in day length and temperature. The microenvironment is defined as factors intrinsic to the hair follicle, such as epithelial and mesenchymal cells adjacent to bulge stem cells, the follicle sheath, dermal papilla, etc. (see FIGURE 4, A AND B). In human scalp, hair follicles are normally asynchronous with their neighbors, but during pregnancy they are maintained in anagen, and after the birth and during lactation many enter catagen and telogen, causing a synchronized partial shedding or moult (44). Thus macroenvironments also influence human hair follicles.

The fantastic phenomena here point to the importance of how hair follicle stem cells “sense” the body’s physiological status and the external environment (FIGURE 2E). Seasonal changes in day length alter melatonin secretion by the pineal gland in many species including sheep, hamsters, minks, and rodents. In this way, nerve impulses stimulated by light are transduced to endocrine signals (18, 58). The changes in melatonin secretion are generally translated to the follicle via the hypothalamus-pituitary route, although there can be direct effects of melatonin on the pituitary (42). Prolactin is strongly implicated as a major effector hormone at the hair follicle level in many species, although other factors are probably also involved (57). There are also seasonal changes in hair growth throughout the year in humans living in temperate regions. These include annual cycles in scalp hair growth in men and women with an autumn moult (13, 49, 58), whereas androgen-dependent beard and thigh hair grow faster in men in the summer (58). The endocrine control of this is

not established, although the rise of androgen levels in the summer may influence the latter (57).

Hormonal Dependence of Organ Phenotypes

Changes during the life of an individual such as puberty or pregnancy also lead to significant hormone changes, particularly in sex hormones. Although hormones circulate in the whole body, we know that only certain skin appendages respond to these physiologically induced changes. Indeed in some species, the responses of follicles in different body areas to the same hormones, androgens, vary dramatically. Androgen effects range from marked stimulation of growth in some areas like the manes of male red deer and lions and the human beard, through to no effect such as on the flanks or eyelashes, contrasting with inhibition on human scalp (41, 55, 57, 73). How do these skin appendage stem cells “read” the signal? The full molecular basis of this regional specificity remains to be solved, although most studies point to a key role for the dermal papilla cells in interpreting these hormonal signals for other follicular cells (55, 56).

In birds, feather moulting can be halted during seasonal migration and resumed when birds arrive at their destination. This also indicates that the regenerative cycle is under a systemic control. However, moulting progresses in waves in vivo, and flight feathers from both the left and the right wings moult in a coordinated fashion to maintain balance in flight. This indicates that there may be local coordinated signals between adjacent follicles. Making new feathers or hair involves a significant energy cost to the individual; sparse, brittle hair is a characteristic of human starvation and disease (8). Therefore, it is not surprising that local mechanisms have evolved to prevent external signals such as daylight changes interpreted via altered systemic hormone levels, which reach all tissues simultaneously, from forcing an animal to remake all its skin appendages at the same time. A requirement for local factors derived from the adipose tissues or dermis in addition to a circulating trigger would concur with an energy-conserving system, particularly with the amount of adipose reflecting an animal’s access to sufficient food.

FIGURE 2. Macroenvironmental regulation of mouse, hair cycles

A: progression of regenerative hair waves is affected by the ratio of activators and inhibitors in the dermal macroenvironment. B: hair waves are reset during pregnancy and restart after lactation. C: many of the macroenvironment modulators such as BMP2 (blue) are present in the subcutaneous, sudan red positive adipocytes (red). D: schematic representation of the hair cycle rhythm (black) and the dermal rhythm (red), which define the four functional stages of the hair cycle (catagen is omitted for simplification). E: a bison with thick fur in Yellowstone during winter. F: regenerative hair wave patterns in different animals. Y-axis is the frequency of cycling of a single follicle. X-axis shows the level of coupling in a hair follicle population, i.e., how much the cycling stages of one hair follicle affect the cycling behavior of adjacent follicles. Toward the right, there is much higher coupling. This means that a hair follicle can enter anagen either via intrafollicular or extrafollicular signals. Toward the left, follicles are not coupled and behave independently. Therefore, follicle stem cells can only be activated via their own intrafollicular signals. In human babies, hair waves are seen in occipital regions. P, propagatory anagen; A, autonomous anagen; R, refractory telogen; C, competent telogen. A and F are from Ref. 51, and B–D are from Refs. 52 and 53 and used here with permission. Photograph in E is by Cheng-Ming Chuong.

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From our mouse studies, we can see that their regenerative hair wave is very responsive to the macroenvironment. In people, hair follicles cycle in waves in newborns, but in adults each hair follicle cycles randomly (67). This could be explained as an extreme form of the wave model, with a loss of the signaling network for propagatory anagen. Since hair or feather follicles can go through regenerative cycles, a completely new type of skin appendage can reform with a new shape and size through this mechanism. At puberty, skin appendages in specific regions are transformed in response to circulating sex hormones in both birds and mammals; this is particularly obvious in the tail feathers of hens/roosters and peahens/peacocks and the beards of men (FIGURES 1D AND 3C; Refs. 46, 55, 57).

The visible protective scalp, eyelash, and eyebrow hairs of children are supplemented by pubic and axillary hair in both sexes, signaling sexual maturity and beard development, whereas the beard and greater body hair distinguishes men (FIGURE 1D). This androgen-dependent replacement of tiny, pale vellus hairs by long, thick, darker terminal ones is highly area specific (55, 57). In marked contrast, androgens can also cause the reverse transformation stimulating gradual replacement of large scalp hairs by tiny vellus ones causing balding. These changes involve macroscopic alternations in size and color requiring major cell biological and biochemical changes to occur in a highly controlled manner. These generally require the follicle to undergo more than one cycle to complete the transformation. Such responses require functional androgen receptors within the follicle, particularly in the regulatory dermal papilla. The levels of these receptors vary with androgen-sensitive follicles, e.g., beard and balding scalp follicles containing higher levels than nonbalding scalp (28, 61). In red deer stags, only androgen-dependent mane follicles contain androgen receptors (70), and none are detectable in the androgen-insensitive flank follicles.

In addition, the ability to metabolize testosterone to more potent 5α -dihydrotestosterone is seen in male-specific, androgen-sensitive follicles but not in the bisexual axillary and pubic follicles (4, 25, 71). Androgen effects within follicles also differ. For example IGF-1 is stimulated by androgens in beard cells (33), contrasting with TGF- β in balding scalp (30, 32), whereas SCF production is highest in cells from darker beard follicles and lowest in those from pale balding scalp follicles (29, 60). Therefore, specificity of responses not only requires differential expression of androgen receptors and metabolizing enzymes but also androgen-regulated genes within specific follicles. Presumably, such differential expression is the consequence of

region-specific developmental programming during embryonic development. This is similar to the metamorphosis from tadpole to frog or larva to butterfly, yet it is at the individual organ level. This area requires more research to clarify the specificity of response to circulating hormones in the macroenvironment.

Within the areas affected by androgens, there appears to be a spreading of the changes that could be thought to resemble the waves seen in mice. Beard growth generally starts above the mouth and on the chin and extends across the face over several years; similarly androgenetic alopecia frequently progresses gradually over many years in men in a specific pattern from the front of the hairline and the central vertex to the whole top of the scalp (27). These slow changes may be due partially to the long anagen phase on the human scalp of ~ 2 –5 yr (37), since such major transformations generally require more than one hair cycle. Whether these gradual spreads are due to dermal factors facilitating/inhibiting the androgen-stimulated responses or the necessity for some additional local factors to pass from affected follicles to the next unaffected area or some other factor is not yet clear. One interesting point concerns the intrinsic regional differences of the human scalp. In most androgenetic alopecia cases, follicles in the occipital region retain large, thick hairs, which are not affected by testosterone. Even upon transplantation to the front of the head, they keep this property. Based on studies in birds, the embryological origin of mesenchymal cells may have an effect on regional specification (14), but the molecular mechanism remains to be investigated.

Similarly another form of human hair loss, alopecia areata, also involves spreading of the lesion outward in a circular pattern (59); whether this is due to dermal factors and/or the spread of attacking immune cells is unclear.

Androgen effects on several tissues increase with age/exposure to the hormone.

In the prostate, another androgen-sensitive tissue that involves mesenchymal-epithelial interactions, androgens cause hypertrophy in many men over 40 and prostate carcinoma in older men. Similarly, the human body continues to replace many hairs with larger ones in later life in response to androgens; beard and chest hair are not fully developed until the mid-30s and hair in the ear canal continues developing until much later in life (26). In red deer stags, the whole antler is regenerated and shed annually in response to seasonal changes in testosterone (FIGURES 1D AND 4C); the new antlers are larger each year, identifying the older, more dominant males (23, 41).

Topology of Stem Cell Microenvironment and Organ Shaping

Skin is the organ with the largest spectrum of morphological variations. Skin appendages vary in diameter, length, shape, color, texture, patterning, function, etc. in both birds (FIGURE 3A) and mammals. It is not easy to do research in mammals other than mice, but a mouse's gender cannot be determined from the morphology of their hair, which does not change greatly in shape during their lifetime. In contrast, bird feathers and human hairs do show sexual dimorphisms. The large size of a feather follicle makes it an ideal model to analyze this "bigger" microenvironment. The feather follicle at growth phase is a vase-shaped structure with a cylindrical epithelial wall, a base made of the dermal papilla, and a core made of feather pulp (FIGURES 1B AND 3B; Ref. 74). In feathers, the

cycle can be divided into growth (G), resting (R), and initiation (I) phases (FIGURE 1B), in parallel to the anagen and telogen phases of the hair cycle. In growing stages, proliferating cells are in the thick layer of epidermis around the dermal papilla and thus are named the collar. A small cluster of slow cycling stem cells are located in the collar region and face the pulp (FIGURE 1B; Ref. 76). As feathers mature from the distal end, the basal epithelium, together with the mesenchymal pulp, retreats toward the proximal end and forms periodically positioned pulp caps (43). This involves the apoptosis of pulp cells, allowing the filament cylinder to open and the feather vane to form. The mature feather, the part protruding out of the body surface, is composed of cells that have keratinized and died. It is still connected to the follicle through the proximal end of the calamus (feather shaft proximal to the vane). Toward the end of the growing phase, feather stem cells descend

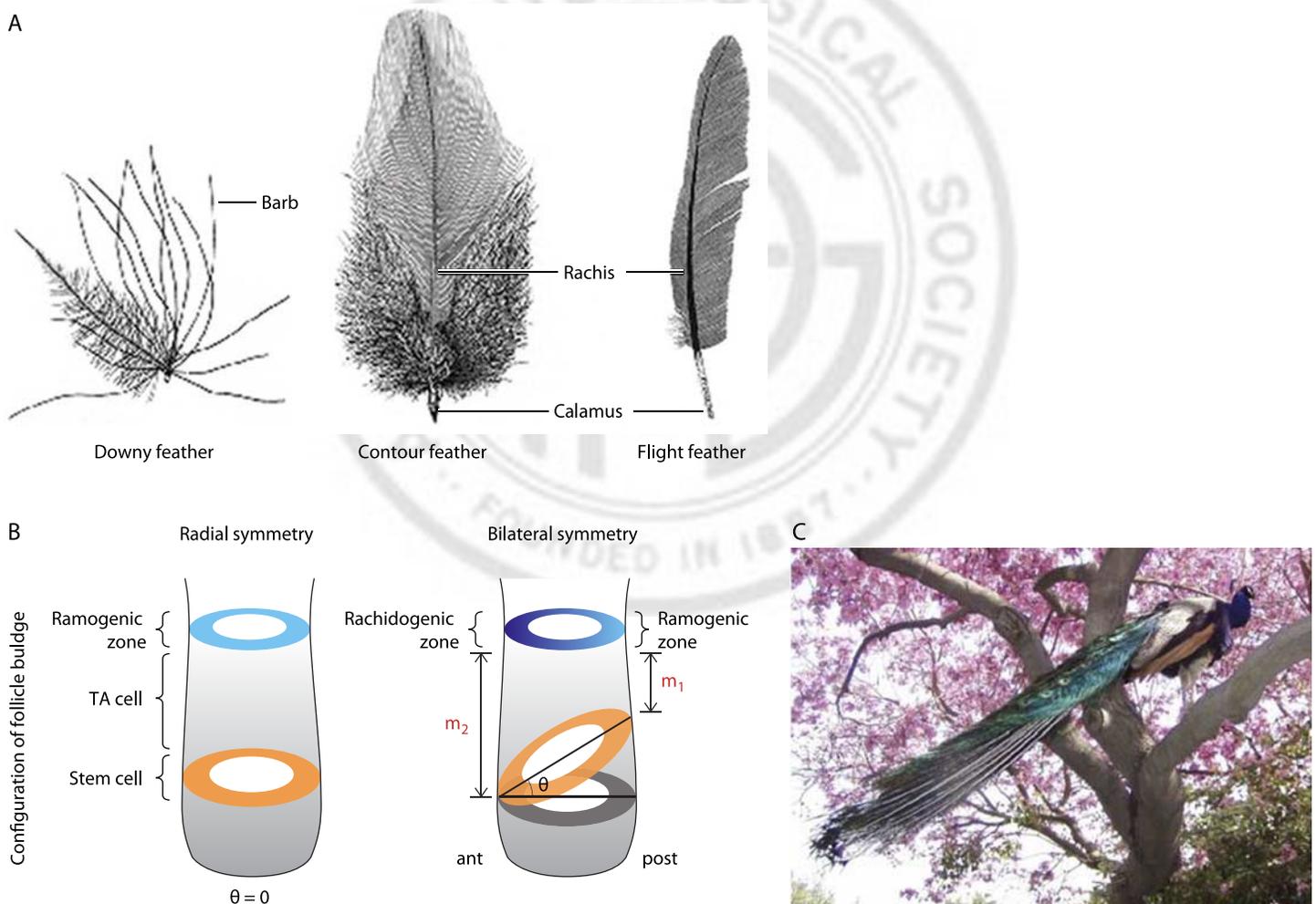


FIGURE 3. Microenvironmental shaping of feather morphology
 A: feather morphology can vary from radially symmetrical (i.e., downy feathers) to bilaterally symmetrical (i.e., contour feathers) to asymmetrical (i.e., flight feathers). B: in radially symmetrical feathers, the feather stem cell ring is horizontally placed at the base of the follicle. In bilaterally symmetrical feathers, the stem cell ring is tilted to a certain angle. This breaks the symmetry, and TA cells (transient amplifying cells) become asymmetrical at the ramogenic or rachidogenic zones where the rachis and barbs (feather branches) form. C: a male adult peacock in the blossoming Los Angeles Arboretum shows off spectacular plumages in different body regions. A is from Ref. 43 and B is from Ref. 75 and used here with permission. Photograph in C is by Cheng-Ming Chuong.

from the collar bulge to be in close contact with the dermal papilla (76), here called the papilla ectoderm. Eventually, the collar epidermis thins and the growth phase transits to the resting phase. In feathers, the resting phase can last for several months, depending on the body region, season, and ages of the chicken.

After hatching, downy feathers are formed all over the baby chick (FIGURE 1D). There are no apparent differences among feathers from different body regions. After a couple of moltings, the adult bird, in this case a peacock, looks completely different (FIGURE 3C). Feathers of distinct morphology and with specialized functions cover different parts of the bird: body feathers for thermoregulation, tail and crown feathers for communication, and wing feathers for flying. Not only does each of these feathers have a unique shape and texture, but they are also positioned in specific body regions to maximize their effectiveness in adapting each bird to its environment. There are also radially symmetrical body feathers and bilaterally symmetrical flight feathers (FIGURE 3A). The mechanisms underlying these differences are probably similar to those used to regulate regional differences in size and shape found in human hairs; hence, chicken feathers may be an experimental model for this important area.

It is amazing that feather stem cells can be moulded into different forms by changes in the follicle microenvironment. Stem cells are located as a concentric ring sitting at the bottom of the feather follicle. Interestingly, in radially symmetrical feathers, the stem cell ring is placed horizontally. In bilaterally symmetrical feathers, the stem cell ring tilts toward the anterior rachis position (FIGURE 3B; Ref. 51). In bilaterally symmetrical feathers, we found a Wnt3a gradient from anterior to posterior positions, which does not exist in radially symmetrical feathers. Flattening the Wnt 3a gradient converted bilaterally symmetrical feathers to radially symmetrical feathers (75). Furthermore, swapping dermal papillae between radial and bilaterally symmetrical feathers produced chimeric feathers whose phenotype is in accord to the origin of the dermal papilla. This mirrors the experiments in hair follicles that have established that the dermal papilla and connective tissue sheath determine the type of hair produced (62).

New Insights Learned From Regenerative Biology

1) Learn to modulate hair regeneration by changing the extrafollicular environment rather than hair stem cells themselves

In the lifespan of an animal, ectodermal organs like feather and hair follicles undergo physiological

regeneration and change phenotypes in different body regions (e.g., downy feathers in the abdomen, flight feathers in the wing) and in different periods of the animal's life (i.e., replacing vellus hairs with a beard at puberty). From chick to adult or even in different seasons when adult feathers change shape (FIGURE 1D), this change seems to be mediated locally by alterations in the dermal papilla. Interestingly, different regions of the skin are responsive to hormonal changes leading to sexual dimorphisms in the color, length, and shape of feathers and hair. Although hormones circulate in the blood of the whole animal, the regional responses may differ significantly. Therefore, the specificity must reside locally within the follicle. How this information is stored or altered needs further investigation. However, the results reported above suggest that a single follicle can be modulated to grow big or small hairs/feathers using novel strategies to modify the follicle environment.

When seasons change, how do skin appendage stem cells communicate with their macro-environment? Changes in light/dark cycles produced by the seasonal lengthening and shortening of days or alterations in temperature can alter the type or coloring of skin appendages (FIGURES 2E AND 3C). This can be seen in the seasonal (summer/winter) coat variation of birds and mammals. As well as the well established circulating hormone regulation discussed earlier, we also know that human and rodent skin and hair follicles are sites of local synthesis and metabolism of many hormones (38, 57, 65, 66). This would fit with the mouse results, implicating that, in addition to the central control, there might also be peripheral control, which may link systemic physiology to the energy demands of hair cycling.

2) Learning the principles of morphogenesis by studying how different forms of feathers/hairs are built and rebuilt during regeneration under physiological conditions

How do the changes in the appendage follicle mould the shape of appendages? Although we have learned hair/feather stem cells can regenerate and make new filaments, we still have a lot to learn about how to guide stem cells to form the shape, size, and orientation of the ectodermal organs. Once we understand environmental regulation of skin appendage morphology, we can use this knowledge to prepare the local macroenvironment so future stem cell products can be implanted and assimilated into hosts successfully.

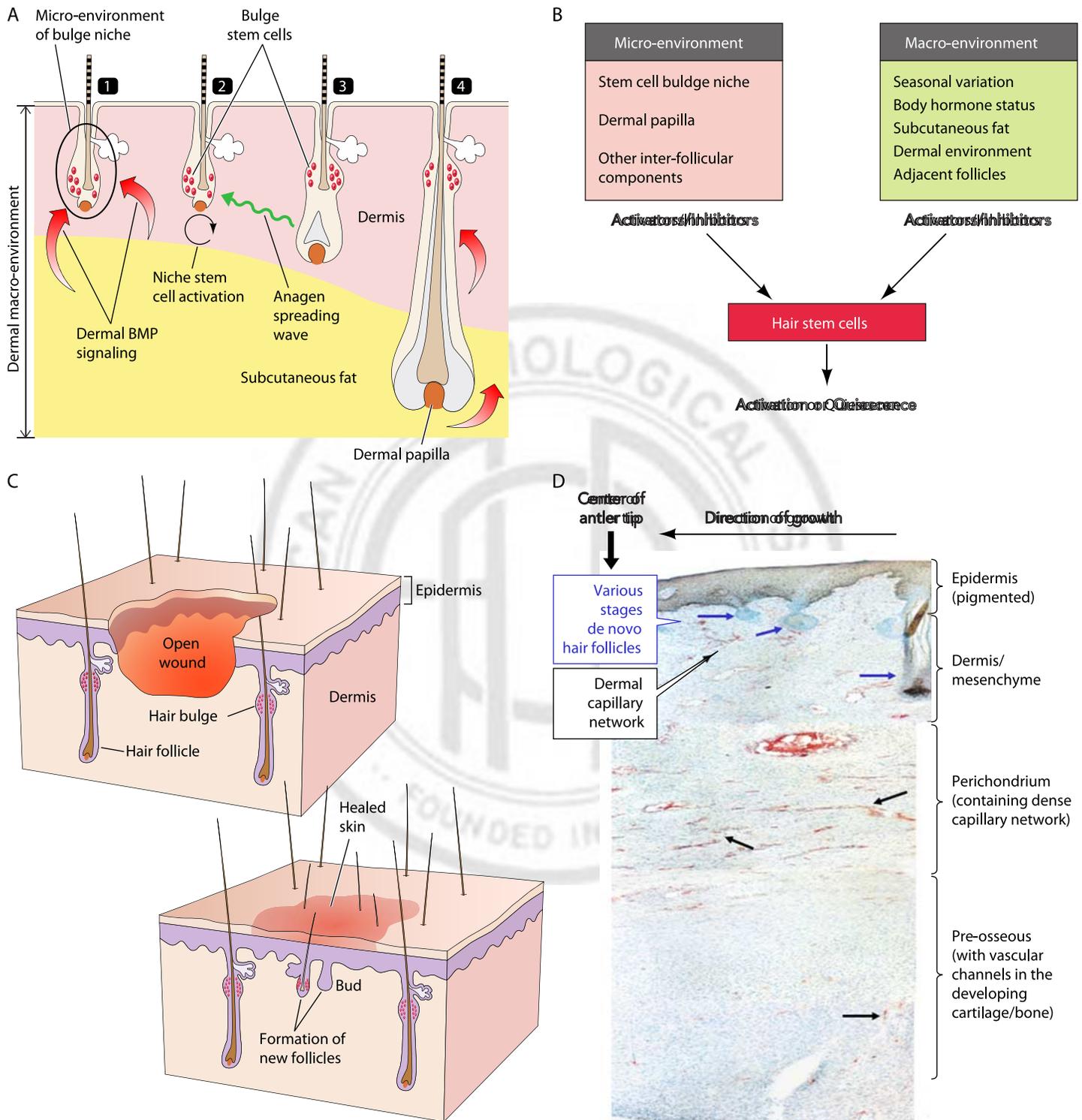


FIGURE 4. Intrafollicular regeneration and regeneration of new follicles

A: hair and feather cycling involves only regeneration of stem cells in that particular follicle. B: in addition to the influence of the intrafollicular microenvironment, the regeneration of hair stem cells is modulated by the extrafollicular macroenvironment, which includes the subcutaneous adipose layer, body hormone conditions, as well as seasonal variation in temperature and light/dark cycles. C: following the formation of a large wound in the mouse, new hair follicles can reform through the hair germ stage. D: deer antlers regenerate every year as seen in FIGURE 1D. It is another splendid example of physiological regeneration. It involves both cartilage regeneration and regeneration of new hair follicles. A is from Ref. 52, C from Ref. 11, and D is modified from Ref. 15; all are used here with permission.

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3) learn to reprogram cells endogenously (without having to add exogenous molecules as seen in ips) for new hair follicle formation after severe injury

Another aspect of regeneration is to form both intrafollicular and follicular regions of the skin. Up to this point, we have discussed regeneration within a follicle whereby appendage epithelial stem cells, located in the hair bulge or feather collar bulge, are used to rebuild an appendage in that follicle, although different appendage phenotypes can be formed. This seems to be an evolutionary achievement as well since the unit-based regeneration can leave other parts of the integument intact. However, a more drastic type of regeneration involves making new skin with hairs. Here, hair follicles are produced anew. This was demonstrated in a big wound on the back of a mouse (FIGURE 4C; Refs. 11, 34), where the hair-producing cells are supposedly reprogrammed from suprabasal cells. This appears to be an “extreme makeover” after severe injury.

Yet, this type of regeneration is a physiological event in deer. The annual regeneration of deer antlers (36, 54) is obviously a physiologically triggered shedding of the old antler. A big open wound is produced at the original insertion sites of the antler. A blastema forms and re-epithelization occurs. Although the antler proper is made of cartilage, it is covered by velvet, a full new skin with new hair follicles. Histological observations show that new hair follicles are generated from the more central and distal part of the growing antler (FIGURE 4D; Ref. 15). Therefore, an array of hair germs, from early to mature stages, lay out nicely from the distal to proximal antler.

In contrast to mice or deer (FIGURE 4D), after a similar big injury, human skin only forms scars without the formation of new hair follicles. If we can learn more about how this remarkable “endogenous reprogramming” takes place, we may apply this knowledge to turn on molecular pathways to regrow hairs or digits in the context of regenerative medicine.

Summary

Physiological regeneration represents the process in which regeneration takes place under normal conditions, not in response to injury. Taking advantage of this new lease on life, ectodermal organs are regularly re-made, often with different phenotypes at various times of an animal’s life to serve distinct functions. The characteristics of these phenotypes have been selected over generations to boost the fitness of the organism.

The status of the macroenvironment is modulated throughout the duration of an animal’s life, including changes in development, seasonal changes, pregnancy, and aging. The key scientific question here is how changes in the macroenvironment convey their message to specific appendage follicles in certain body regions. Another key question is how the changes in the follicle mould the shape of appendages.

Using the episodic regeneration of skin appendages as a clear readout, we have the opportunity to understand and modulate the behavior of adult stem cells and organ regeneration at a level heretofore unknown. Through this work, we hope to be able to establish or improve the stem cell environment so it can be applied to regenerative medicine.

In conclusion, we think it will be very productive to learn how nature manages the physiological regeneration process. This is a reprogramming process in which the genetic and epigenetic events converge to generate complex functional forms, depending on the physiological need in different parts of the body and at different stages of life. Principles learned from regenerative biology can then be applied toward regenerative medicine. ■

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