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Deciphering principles of morphogenesis from temporal and spatial patterns on the integument

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Abstract

How tissue patterns form in development and regeneration is a fundamental issue remaining to be fully understood. The integument often forms repetitive units in space (periodic patterning) and time (cyclic renewal), such as feathers and hairs. Integument patterns are visible and experimentally manipulatable, helping us reveal pattern formative processes. Variability is seen in regional phenotypic specificities and temporal cycling at different physiological stages. Here we show some cellular / molecular bases revealed by analyzing integument patterns. 1) Localized cellular activity (proliferation, rearrangement, apoptosis, differentiation) transforms prototypic organ primordia into specific shapes. Combinatorial positioning of different localized activity zones generates diverse and complex organ forms. 2) Competitive equilibrium between activators and inhibitors regulates stem cells through cyclic quiescence and activation. Dynamic interactions between stem cells and their adjacent niche regulate regenerative behavior, modulated by multi-layers of macro-environmental factors (dermis, body hormone status and external environment). Genomics studies may reveal how positional information of localized cellular activity is stored. In vivo skin imaging and lineage tracing unveils new insights into stem cell plasticity. Principles of self-assembly obtained from the integumentary organ model can be applied to help restore damaged patterns during regenerative wound healing and for tissue engineering to rebuild tissues.

Keywords

stem cells; hairs; feathers; pattern formation; self-organization; regeneration; hair cycle; systems biology; molecular circuit; modularity

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BIOLOGICAL PATTERN FORMATION

Pattern formation is a process through which order emerges from randomness. It is present in a variety of non-equilibrium systems in both the physical world (such as water waves, rock layers, and sand dunes) and biological world (such as periodic branching of feather, pigment spots and stripes on animal skin, zigzags in the luminal surface of the frog and snake intestine) (Ball, et al. 2009; Inaba, et al. 2012; Shyer, et al. 2013; Lin, et al. 2013a; Mahalwar, et al. 2014). Patterning can occur in the dimensions of space and time, and repeating elements may appear to be identical or accommodate a certain degree of variation (Chuong and Richardson. 2009).

Biological patterns offer crucial functions for the tissues/organs and the organisms at different developmental times. For example, body pigment patterns may be used for camouflage, or to warn or attract others. Intestine luminal patterns increase the absorptive area. Dense hairs and downy feathers can trap air to maintain warmth. Temporal patterns such as the cyclic regeneration of feathers and hairs can replace the worn integuments as well as allow change of integument morphology/pigment patterns based on seasons or physiological developmental stages. Developmental and stem cell biologists have noticed the obvious connections between pattern formation, regeneration and, by extension, tissue engineering. Pattern formation mechanisms could provide insights on developing the correct size, shape and polarity of a population of stem cells and their derivatives in organs, revealing how stem cells maintain their population and multi-potency during tissue homeostasis.

Distinct patterns, like spots, stripes, segments, can be generated by different mechanisms. In non-autonomous pattern formation, spatially localized morphogenetic cues (morphogens) need to be pre-determined at specific sites in the embryo. The diffusive morphogens move across the field of morphogenesis to turn on downstream genes for patterning (Wolpert. 1969; Lander, et al. 2002; Lander. 2013). Documented examples include Drosophila embryo segmentation patterning established by a *Bicoid* gradient (Driever and Nusslein-Volhard. 1988; Houchmandzadeh, et al. 2002), vein formation of imaginal discs in flies (Lander, et al. 2002) and specification of neuronal precursor domains determined by a *Shh* gradient (Dessaud, et al. 2008).

Autonomous pattern formation has been described by two major modeling frameworks. One model is based on spontaneous pattern formation driven by reactions and diffusions of at least two biochemical substances proposed by Alan Turing (Turing. 1952) and its derivative theories (Gierer and Meinhardt. 1972). In such models, one central mechanism driving patterning is based upon short-range activation and long-range inhibition. The second model framework involves mechanics, such as the buckling instability of elastomers (Moulton and Goriely. 2011) in which competition between geometric effects (e.g. the change in tube dimensions) and mechanical effects (e.g. residual stress due to differential growth) create patterns. The details of these theories will be described later in this review.

It is likely that different types of molecular circuits evolved in a convergent manner to produce similar biological patterns. Some molecular circuits may be based on transcription

activity in the genome, some may be based on the threshold response to a morphogen gradient, othersmay be based on the cell interactions in combination with physical-chemical forces. We speculate that the mechanism underlying Drosophila segmentation may be more rigid and specific, since genetic changes are needed to make a new segmentation pattern. While the mechanism regulating feather / hair periodic patterning is more plastic, since the same number of appendage forming progenitors can be modulated to form 10 big hairs or 1000 small hairs, depending on the environmental cues present.

To perfect the outcomes of tissue engineering, we will need to learn more about the principles of morphogenesis, to understand how patterns initiate, develop, and become stabilized at the cellular and molecular circuit levels while the system faces great environmental or genetic fluctuations. The fact that disrupting molecule X interferes with the formation of a certain pattern only indicates that molecule X is involved in this process. To understand the specific role of X we need to detect its spatial distribution, determine which molecules crosstalk with it, and how these molecules are quantitatively affected. This information will reveal the role of X in the context of a specific mechanism. For example, one needs to know if X is an activator, an inhibitor, a modulator for robustness of patterning, or simply a regulator of the activator and/or the inhibitor. In addition, knowledge of the detailed temporal dynamic cellular process becomes very important in obtaining any detailed mechanisms of patterning. Since the integument develops at the body surface and displays a variety of striking patterns that are convenient to observe and experimentally manipulate, as opposed to visceral organs, the integument has become one of the leading model systems for elucidating mechanisms of pattern formation. Next, we discuss pattern formation by using the integument model as a Rosetta stone to decipher the language of morphogenesis.

PERIODIC PATTERN FORMATION IN INTEGUMENTARY ORGANS: MULTIPLICITY ALLOWS VARIABILITY

Integument organs such as hairs, feathers, scales, claws, beaks, teeth, epidermal glands, etc, not only create a boundary between the organism and the environment but also facilitate organismal adaptation to diverse environmental conditions while providing communication between individuals of the same and other species (Fig. 1). Many integumentary organs are composed of several organ primordia that work together as an organ population. For example, there are multiple numbers of mammalian teeth, multiple glands, and thousands of hairs and feathers (Fig. 2B). This multiplicity allows the animal to make variations in different body regions, and thus make integument organs from different regions exhibit regional- and age- specific phenotypes so they adapt to the environment robustly (Chuong et al., 2013). We will first review the current knowledge on how integument organs form a patterned population.

Reaction-diffusion model and beyond

Since integument patterns are often striking and obvious, the integument has become an effective system to observe and analyze patterning during its initial formation, as well as during physiological cycling and injury induced regeneration. More importantly, many

integument patterns (such as the periodic formation of hair follicles or the mosaic color spots on leopard fur) clearly form in an autonomous fashion since the pattern has no correlation with any specific body landmarks (such as the body axes, boundaries) (Lander. 2011). As mentioned previously, Turing type models are representative theories for explaining autonomous biological patterns. The original diffusion-driven theory describes how a homogenous system of two reacting and diffusing chemicals (morphogens) can become unstable such that a small perturbation could result in formation of a periodic chemical pattern over time (Turing. 1952). Turing solved the two morphogen reaction-diffusion (RD) system along an isolated ring and found that the patterns took the form of waves. He discussed six possible states which the pattern could converge toward: (1) stationary case with extremely long wavelength; (2) oscillatory case with extremely long wavelength; (3) stationary case with extremely short wavelength; (4) oscillatory case with extremely short wavelength; (5) stationary case with finite wavelength (this case is widely referred to as the Turing pattern today); (6) oscillatory case with finite wavelength (there are genuine traveling waves across the morphogenetic field) (Turing. 1952; Kondo and Miura. 2010). In order to converge to a stationary pattern, in which the waves created remain in place, the dominant eigenvalue (root) of the system of differential equations must be a real number. Conversely, in an oscillatory pattern, which creates traveling waves, the dominant eigenvalue is a complex number whose complex term determines the frequency of the traveling waves. Some biological examples fitting these cases have been described in a previous review (Kondo and Miura. 2010). The Turing model was later elaborated by Gierer and Meinhardt (Gierer and Meinhardt. 1972). They showed a stable periodic pattern is achievable with a two-morphogen system when one of the morphogens is an activator, meaning it is self-enhancing and thus amplifies its own small perturbations and creates localized peaks. The second morphogen must diffuse quickly and antagonize the production of the activator in order to limit the spread of the peaks formed by the activator. One derivative of the activator-inhibitor model, proposes that the activator also promotes the production of its own inhibitor. A second antagonistic mechanism, known as the substrate-depletion model, introduces a substrate necessary for, and depleted during, the activator's self-enhancement (Gierer and Meinhardt. 1972; Headon and Painter. 2009). The underlying mechanism for both models is short-range activation and long-range inhibition. In the following section we will describe several examples where skin integuments form patterns through molecular interactions predicted by the activator/inhibitor model. We will also show the interesting discoveries from Dr. Kondo's group that morphogen diffusion can be replaced by cell-cell contact over different distances during autonomous patterning (Inaba, et al. 2012; Hamada, et al. 2014a).

Autonomous patterning in feather morphogenesis

Biological evidence supporting the activator/inhibitor model was observed in 1998 (Jung, et al. 1998). In this paper, we showed FGFs work as activators while BMPs function as inhibitors for the periodic formation of feather primordia. The primordia are composed of a thickened epithelium (placode) lying above high cell-density mesenchymal tissue (dermal condensation). Interestingly, unlike the non-autonomous model in which inhibitors would be found in the interbud spacing, here both activators (FGFs) and inhibitors (BMPs) are present in the feather primordia mesenchyme, a property consistent with the Turing mechanism of

autonomous patterning (Gierer and Meinhardt. 1972). In this and subsequent works (Lin, et al. 2006), we learned that a competent cell population responds to the sum of activators and inhibitors that enhance or suppress bud formation, not limited to FGF and BMP. We also found that these molecules can be secreted from either the epithelium or the mesenchyme. Furthermore, subsequent feather reconstitution studies showed that, when mesenchymal cells increase in number, the number but not the size of feather buds increases accordingly. Only changing the activator / inhibitor ratio by mis-expressing noggin or BMP could make buds bigger or smaller (Jiang, et al. 1999). Increasing FGF locally and globally enlarges feather buds at the expense of the interbud along the midline and increased feather bud numbers out toward the younger, lateral edge of the skin (Widelitz, et al. 1996).

The feather branching pattern is also a biological phenomenon that can be explained by Turing diffusion-driven instability mechanisms (Harris, et al. 2005). Harris et al reported a two component activator/inhibitor model that could simulate the periodic branching pattern of downy feathers. With the addition of a third morphogen, a long-acting, local inhibitor, to the activator/inhibitor model, Harris et al 2005 simulated the helical formation of barbs that were assumed to be created by traveling waves. They proposed the notion that location specific increases and decreases of background activator production could simulate a bilaterally symmetric pennaceous feather (a feather containing a central shaft that divided the vane into two halves) with a dorsally positioned rachis (the central shaft) and ventrally positioned barb generative zone (where barbs, or the branches initiate formation). Some evidence suggests Shh and BMP2 are the diffusible activator/inhibitor, respectively (Yu, et al. 2002). PATCHED (membrane-bound Shh receptor) was speculated to be the local inhibitor that can bind Shh molecules diffusing through and disabling them from activating distant cells.

Feather pigmentation pattern formation is a complex process that may involve both autonomous and non-autonomous patterning. Melanocyte progenitors reside in the proximal feather follicle and its activity is regulated by the surrounding niche. In the patterned white regions, melanocytes can be absent or present but fail to differentiate (Lin et al., 2013a). The activation of stem cells is autonomously determined near the bottom of the follicle. The same melanocyte stem cell population in one follicle can form different pigment patterns in different ages or physiological conditions. In the more distal barb forming ramogenic zone, peripheral pulp shows an agouti expression pattern that is complementary to the pigment pattern. Since the mesenchymal agouti pattern dictates melanocyte patterning, this part is a non-autonomous patterning process. However, how agouti is patterned in the pulp is an autonomous patterning process.

Autonomous patterning in hair morphogenesis

Hair follicle patterning is another compelling biological example for Turing diffusion-driven instability (Sick, et al. 2006). It was discovered that Wnt is essential and sufficient for the induction of hair follicles (Gat, et al. 1998; Andl, et al. 2002). Wnt signaling can also induce the expression of its inhibitor DKK (Sick, et al. 2006). Transgenic mice over-expressing DKK2 exhibit decreased hair density (Sick, et al. 2006). Meanwhile Wnt proteins are known to be much larger than DKKs (Maini, et al. 2006). Hence a difference in their diffusion

dynamics is expected. This evidence makes Wnt and DKK good candidates for the activator/inhibitor pair carrying out the Turing mechanism of pattern formation. This finding suggests that skin progenitor cells have equal potential to form hair follicles or interfollicular skin and that the outcome can be determined by tuning the relative activities of Turing model activators or inhibitors in the environment at the time of specification (Maini, et al. 2006).

Another interesting case of the autonomous patterning in hair is the traveling of hair (color) waves (Suzuki, et al. 2003; Plikus, et al. 2009). Hairs in adult mice cyclically regenerate. The regeneration is not synchronous but takes the form of traveling waves over the body. This can be easily observed in *Foxn1* mutant mice whose hair follicles are rapidly discharged after pigment deposition stimulating a new round of regeneration (Suzuki, et al. 2003). The waves can also be observed in normal mice after hair clipping (Plikus, et al. 2009). In *Foxn1* mutant mice the waves have regular spacing, sharing characteristics with the Belousov-Zhabotinskii pattern of reacting and diffusing chemicals (Zaikin and Zhabotinsky. 1970). It is possible that this type of regeneration wave is driven by mechanisms similar to those found in the oscillatory case with finite wavelengths described in the Turing RD model. For the regeneration wave in normal mice the pattern is more complex and a Cellular Automaton model has been proposed to explain it. This model will be discussed further in this review.

Autonomous patterning in fish pigment stripe formation

Studies by Dr. Kondo's group on zebrafish pigment patterns revealed that two types of pigment cells (melanophores and xanthophores) formed a special relationship: Xanthophores promote survival of melanophores at long range while they mutually inhibit each other's growth at short range (Nakamasu, et al. 2009). The short-range repulsive interaction is conducted by xanthophores sending out dendrites to directly contact melanophores (Inaba, et al. 2012), while the long-range survival signals provided by xanthophores to melanophores are through the Delta/Notch interactions on the long projections sent from menanophores to xanthophores (Hamada, et al. 2014b). This relationship is not identical to the activator/inhibitor model but still conforms to the mechanisms of local self-activation paired with long-range lateral inhibition (Meinhardt and Gierer. 2000). In such a system, the diffusion of morphogens was replaced by different types of cell-cell interactions over varying distances.

Mechanical force as a patterning player

Beside the Turing model and its derivatives, the ability of mechanical stress to induce tissue buckling was theorized to explain the autonomous periodic biological patterns such as fingerprints in skin (Kücken and Newell. 2005), brain cortex convolution (Caviness. 1975), mucosal foldings in the airways, blood vessels and gastrointestinal tracts (Moulton and Goriely. 2011), as well as Muri-ori folds on hornbeam leaves (Mahadevan and Rica. 2005). This theory suggests the periodic buckling or folding of tissue results from a trend to minimize internal stress generated by physically restrained tissue growth. For example, the fingerprint pattern might be driven by the intra-epithelial stress induced by the resistance of furrows and creases to the differential growth of the epithelial basal layer (Kücken and Newell. 2005). The idea was supported by computer simulations. Experimental evidence of the contribution of mechanical force to autonomous biological patterning also has started to

emerge. For example a recent study by Tabin and Mahadevan's group demonstrated that removing the compression stresses generated by anisotropic muscle constraints on the growing endoderm-mesenchyme composite (by inhibiting muscle differentiation) can cause the intestine luminal pattern to disappear while reapplying stresses (by putting a silk tube outside the intestine without a muscle layer) could let the pattern re-appear (Shyer, et al. 2013).

Multiplicity allows variability

Periodic patterning allows multiple replicates of an organ to form (ie., feathers, hairs; Chuong et al., 2013). Spatial and temporal variations allow structural and functional variability to occur. The myriad combinations of the above processes allow diverse phenotypes to be generated. This is most obvious in feathers. Temporally, young birds are covered with radially symmetric downy feathers that provide warmth. Later in development feathers within the same tract share common characteristics but these traits can differ between tracts. For instance, downy feathers along the dorsal tract are replaced with bilaterally symmetric feathers. In contrast, downy feathers on the wings are replaced with bilaterally asymmetric feathers that offer advantages in aerodynamics. The distal barbs of both bilaterally symmetric and bilaterally asymmetric feathers are linked to their neighboring barbs by barbules which enable the pennaceous feather vane to efficiently trap the air; however, the proximal regions are downy and plumulaceous. Even within tracts gradual phenotypic changes in size or coloring are observed which offers more opportunities to interact with their environment. This may appear as a gradient of increasing numbers of plumulacous barbs per feather when moving from the wing tip toward the body that serves to retain body temperature. In the following section, we will examine how spatial variation and temporal cycling allows this to happen.

SPATIAL VARIATIONS: LOCALIZED CELLULAR ACTIVITY IN ORGAN PRIMORDIA AND REGIONAL SPECIFICITY ON THE WHOLE BODY INTEGUMENT

Previously we proposed a general scheme where multiple localized cellular activity modules in organ primordia can serve as the foundation to generate complex organ shapes. Different cellular activity modules can be based on highly localized physical processes such as cell polarity, rearrangement, proliferation, apoptosis, and differentiation (Fig. 3). The number, size, position, duration, and spacing of these activity modules can converge to form a spectrum of organ designs (Chuong et al., 2006) suitable for different physiological stages or adaptation to evolutionary needs (Chuong, et al. 2006).

Localized proliferative zone and beak shapes

Avian beaks are a well-known example of evolutionary diversification. Darwin observed that beaks originating from the same kind of finch vary in shape from island to island in Galapagos. Ducks have beaks with wide tips while those of chickens are very narrow. Through short term BrdU labeling we found two localized cell proliferation zones at the lateral edges of the frontonasal mass (one facial prominence involved in beak development)

in Hamburger & Hamilton stage 26 chicken embryos (Hamburger and Hamilton. 1951). These two zones merge into one that later becomes centrally localized. However in ducks the two zones persist in the lateral edge and this causes the widening of the frontonasal mass. Hence the number and topology of the localized cell proliferation zones are major determinants of beak shape. We also discovered that cell proliferation is driven by BMP4 (Fig. 3A) (Wu, et al. 2004b).

Another example of a localized cell proliferation zone controlled by BMP signaling is seen in the developing mouse claw. Normally there is a localized cell proliferation zone located close to the eponychium. In K14-Noggin mice, this zone is split into multiple smaller growth zones, which later differentiate into multiple nail plates (Plikus, et al. 2004).

Localized apoptotic zone and branching morphogenesis

During feather epithelial cylinder branching, we noticed apoptotic cells localized to the marginal plate of the barb ridges. These localized apoptotic zones help sculpt out the space between the barb ridges in a manner similar to the separation of digits during limb development (Zuzarte-Luis and Hurle. 2005). In feathers the localized apoptotic zone is believed to be mainly determined by Shh signaling (Fig. 3B) (Chang, et al. 2004b; Chuong, et al. 2014).

Localized cell rearrangement zone and feather bud orientation

A more recent study of the feather bud elongation process indicates the presence of a localized module of cell rearrangement during feather bud elongation. In development, elongation of feather buds is precise and they are uniformly oriented (Li, et al. 2013a). Through a series of transplantation experiments we discovered the posterior part of the feather dermis is sufficient and necessary for oriented elongation. Within the posterior dermis there is a crescent shaped, spatially well-defined zone composed of nuclear β-catenin positive cells. We demonstrated that β-catenin upregulates the expression of non-muscle myosin IIB (NM IIB) in this zone. As a major cell motor protein NM IIB (Rolo, et al. 2009) drives directional cell motility that leads to the polarized elongation of the feather bud (Li, et al. 2013a). In this case the localized module is based on cell rearrangement behavior.

The continual discoveries of localized modules based on different cell activities suggest this may be a widely adopted strategy in morphogenesis. How do molecular signals regulate the activities, locations, boundaries and durations of these modules? In feather bud elongation, the nuclear β-catenin zone is initiated by Wnt7a emanating from the posterior bud epidermis. Wnt7a protein forms a “noisy” gradient in the dermis (“Noisy” refers to the fluctuations or variability of protein concentration at the micro-scale level), while the nuclear β-catenin zone is quite homogenous in intensity and has a sharp boundary. By combining molecular biology and mathematical modeling we demonstrated the Notch ligand-Notch receptor lateral inhibition which functions downstream of the Wnt pathway is critical for de-noising and establishes the sharp nuclear β-catenin zone boundary, which guarantees the precision of feather bud elongation orientation (Fig. 3C). Other boundary sharpening mechanisms have been reported by studying spinal cord neuron specification (Briscoe, et al. 2000; Todd, et al. 2012).

Regional specificity: different integument organ phenotypes are shaped by topological combinations of different localized cellular activity modules

Specific shapes of an organ primordium can be achieved by combining cellular activity modules in different ways. The above examples show some central signaling pathways (such as BMP, Shh, Wnt) are repetitively involved in setting up localized cell activity zones during organ shaping. Recently we also reported how FGF and Sprouty signaling establish progenitor versus differentiation zones along the proximal-distal feather axis (Yue, et al. 2012). However the details of the gene regulatory network structures (Davidson and Erwin. 2006) and the physical-chemical rules that modulate the spatial and temporal distribution of the cell activity zones can vary in a context dependent manner (Chuong, et al. 2014). The ability to regulate these activities will enable us to engineer the shape, symmetry and size of skin appendages (Lin, et al. 2013b).

A higher level of topological difference can be appreciated in the regional specificity observed in the whole body integument of an individual animal. This is most obvious in the chicken. For example, feathers versus scales, rigid and lengthy flight feathers on the wings versus fluffy downy feathers in the breast region. These regional differences are also obvious in humans, although not so clear in mice. These different regions are derived from the same genome, and yet they are able to exhibit different integument phenotypes, probably via epigenetic mechanisms that remain to be investigated.

TEMPORAL CYCLING: STEM CELLS ARE REGULATED BY IMMEDIATE NICHE AND MULTI-LAYERS OF MACRO-ENVIRONMENTS

Mouse or human epidermis undergoes continuous renewal. Scales in the chicken foot or covering reptile bodies renew continuously, like their epidermis (Wu, et al. 2014). Snake epidermis undergoes synchronous cyclic renewal (Chang, et al. 2009). Integumentary organs with their own stem cells, such as feathers, hairs, and antlers can have their own regenerative cycles (Chuong, et al. 2012). For feathers a regeneration cycle can be divided into two main phases: growing phase (when the stem cells are activated to form feathers) and resting phase (when the stem cells are quiescent) (Lucas and Stettenheim. 1972). In growing phase the feather epithelial stem cells constitute a ring-shaped bulge in the collar region of the proximal feather follicle (Fig. 2A). At resting phase the stem cell ring shifts proximally and is in direct contact with the dermal papilla (DP; Yue, et al. 2005). It is believed that the DP and systemic hormones govern the behavior of stem cells during feather regeneration (Lin, et al. 2013b). Antlers of red deer stags grow and are shed annually in response to testosterone levels brought about by seasonal changes (Chuong, et al. 2012). At growing phase the antler is a bony structure covered by highly vascularized skin. The stem cell niche for the bony part of the antler is believed to be at the cambial layer of the periosteum (Rolf, et al. 2008).

For hairs the regenerative cycle is composed of growth (anagen), degeneration (catagen), and quiescence (telogen) phases under physiological conditions. The cyclic behavior is governed by molecular crosstalk with the surrounding micro- and macro- environments (Muller-Rover, et al. 2001; Millar. 2002; Schmidt-Ullrich and Paus. 2005; Alonso and

Fuchs. 2006; Rendl, et al. 2008). Individual hair follicles contain a stem cell reservoir localized in a specialized region, known as the bulge (Fig. 2A, red), situated directly below the sebaceous glands (Cotsarelis, et al. 1990). These hair follicle stem cells (hfSCs) are quiescent during the resting and telogen phases of the hair cycle, but periodically become activated to become the hair germ which fuels follicle regeneration (Greco, et al. 2009). The regulation of this quiescence and activation is central to cyclic regeneration. This process is regulated by multiple layers of modulators (Fig. 4) (Chen and Chuong. 2012). Many new components have recently been identified which add several additional layers of hfSC regulation. Here, we will discuss these layers of regulatory mechanisms to better understand the reciprocal interactions between the environment and hfSCs during HF regeneration.

The beginning of each anagen phase employs a two-step activation sequence. In the first step, hair germ (HG) progenitor cells, located directly above the DP, start to proliferate with the subsequent second step triggering hfSC activation in the bulge region (Greco, et al. 2009). The HG is a transient region of the HF, derived periodically from the hfSC population during the hair cycle and is located between the DP and bulge hfSCs in telogen follicles (Ito, et al. 2004; Zhang, et al. 2009; Greco, et al. 2009). Interestingly, the HG is biochemically distinct from the bulge and does not express hfSC markers such as NFATC1 and CD34 but instead is enriched for P-cadherin (Greco, et al. 2009). However, at the transcriptional level, HG cells most closely represent activated bulge hfSCs and function as a related extension of the bulge hfSC population (Ito, et al. 2004; Greco, et al. 2009). Both the hfSC and HG populations express the skin SC marker, keratin 15 (K15) (Liu, et al. 2003; Greco, et al. 2009). Despite the inability of HG cells to maintain SC-like characteristics *in vitro*, a recently published study demonstrated that HG cells were capable of functionally replenishing the bulge hfSC population after injury (hair plucking) *in vivo* (Rompolas, et al. 2012; Rompolas, et al. 2013).

Dynamic functional states of intra-bulge hair follicle stem cells

Although hfSCs remain relatively quiescent during most of the postnatal hair cycle, at the beginning of anagen a complex signaling cascade culminates in hfSC activation. hfSC activation occurs through activation of Wnt signaling and inhibition of the BMP signaling pathways which, in effect, stabilize nuclear β-catenin within the HG to promote rapid HF re-growth (Gat, et al. 1998; Botchkarev, et al. 1999; Kobielak, et al. 2003; Van Mater, et al. 2003; Lo Celso, et al. 2004; Andl, et al. 2004; Zhang, et al. 2006; Kobielak, et al. 2007; Ito, et al. 2007; Plikus, et al. 2008; Greco, et al. 2009; Plikus, et al. 2011). During late telogen to early anagen phase transition, HG cells produce increasing levels of Wnt proteins while the DP secretes BMP inhibitory factors such as Noggin (Botchkarev, et al. 1999; Botchkarev, et al. 2001; Greco, et al. 2009) and transforming growth factor-β2 (TGFβ2). These growth factors result in increased TMEFF1 (transmembrane protein with EGF-like and two follistatin-like domains 1) expression in the HG which feeds back to further inhibit the BMP pathway and promote hfSC activation (Oshimori and Fuchs. 2012).

The behavior of slow-cycling hfSCs is tightly regulated by an intricate balance of cell signaling pathways which converge to induce bouts of hfSC quiescence and activation resulting in new hair formation (Blanpain and Fuchs. 2009). One essential pathway required

for proper HF cycling is BMP signaling (Botchkarev, et al. 2001; Kobielak, et al. 2003; Andl, et al. 2004; Plikus, et al. 2004; Zhang, et al. 2006; Kobielak, et al. 2007). BMP ligands bind transmembrane BMP receptor 1A (BMPR1A) on bulge hfSCs (Botchkarev, et al. 2001) during the resting phase of the hair cycle. This induces canonical BMP-signaling to phosphorylate cytoplasmic Smad1, Smad5 and Smad8 (Andl, et al. 2004; Kobielak, et al. 2007). These Smads heterodimerize with Smad4 and translocate to the nucleus to directly transactivate their target genes (Massague, et al. 2005). Thus, ablation of the BMPR1A gene in the skin results in precocious activation of quiescent hfSCs (Kobielak, et al. 2007). Down-regulation of BMP4 transcripts together with up-regulation of a BMP inhibitor, such as Noggin, lead to telogen–anagen transition.

Canonical Wnt signaling is another critical pathway identified to be involved in hfSC homeostasis. β-catenin stabilization is essential to stimulate hair growth (Gat, et al. 1998; Van Mater, et al. 2003; Lo Celso, et al. 2004) and promotes the transition from quiescent hfSCs to proliferating transit amplifying (TA) progeny (Lowry, et al. 2005). Conversely, deletion of β-catenin specifically in hfSCs results in a failure of proper hfSC activation at anagen onset; instead they adopt an epidermal fate with eventual loss of HFs over time (Huelsken, et al. 2001; Lowry, et al. 2005). However, how BMP signaling is precisely integrated to regulate the Wnt pathway as part of a molecular network capable of controlling hfSC activation has just begun to be unveiled. Although the canonical Wnt pathway acting via β-catenin stabilization is well documented to play a significant role in hfSC regulation, until very recently, no specific Wnt ligand(s) and receptor(s) candidates had been proposed as key players in this process (Gat, et al. 1998; Chan, et al. 1999; Reddy, et al. 2001; Lo Celso, et al. 2004; Morris, et al. 2004; Tumbar, et al. 2004; Lowry, et al. 2005). Inhibition of BMP signaling, specifically in the hfSC population, enabled the discovery of a new intrinsic mechanism utilizing BMP/Wnt pathway integration directing hfSCs to adopt an early HG-like fate upon ligand-dependent, canonical Wnt activation. These events result in increased Wnt7a, Wnt7b and Wnt16 expression and β-catenin stabilization in hfSCs which assists self-autonomous regulation, whereas the Wnt antagonist Dkk3 is suppressed (Kandyba, et al. 2013). At the same time, Wnt receptor Fzd10 expression is increased. Functionally, ectopic injection of Wnt7a protein was sufficient to promote hfSC activation and anagen onset in resting telogen HFs (Kandyba, et al. 2013). In contrast, targeted ablation of Wnt7b in bulge hfSCs resulted in perturbed HF cycling with delayed hfSC activation and a shorter growth phase (Kandyba and Kobielak. 2014).

Intra-follicle interactions

Epidermis - dermal papilla—The major source of these hair follicle derived stimulatory factors is the DP (Fig. 2A). Early transplantation studies, demonstrated that the DP regulates hfSC activity during the postnatal HF cycle and coordinates with the epidermis to generate “de novo” hair follicles *in vivo* (Jahoda, et al. 1984; Jahoda, et al. 2001). During the telogen-anagen activation, the DP releases a variety of stimulatory factors such as the BMP inhibitor, Noggin and FGF pathway proteins such as FGF7 and FGF10. These act in concert to stimulate hfSCs toward hair regeneration (Fig.2) (Botchkarev, et al. 1999; Kishimoto, et al. 2000; Rendl, et al. 2005; Rendl, et al. 2008; Driskell, et al. 2009; Greco, et al. 2009). In addition, subcutaneous adipocytes also generate waves of alternating inhibitory and

activating cues which facilitate hfSC niche synchronization within the skin, primarily influencing DP and dermal sheath activity (Plikus, et al. 2008) (Fig. 2A). Dermal sheath cells are adjacent to the outer root sheath keratinocytes and are thought to be able to repopulate the DP (Rahmani, et al. 2014). BMP signaling is also critical for the DP to generate HFs (Rendl, et al. 2008). Modulation of BMP signaling within the DP is responsible for forming different hair types (Driskell, et al. 2009). Thus extensive crosstalk exists between the many cells of the HF niche (Plikus, et al. 2008; Festa, et al. 2011; Plikus, et al. 2011)

Interactions among different epidermal components—More recently, the companion layer, localized between the inner root sheath and outer root sheath (Fig. 2A), was found to act as an important component of the hair follicle niche by producing high levels of fibroblast growth factor 18 (FGF18) and bone morphogenetic protein 6 (BMP6) which promote hfSC quiescence (Fig.2) (Hsu, et al. 2011). Subcutaneous injection of either BMP6 or FGF18 is sufficient to block telogen to anagen transition, demonstrating that the companion layer functions to restrict hfSC activity and assist in maintaining hfSC homeostasis *in vivo* (Blanpain, et al. 2004; Greco, et al. 2009). In contrast, when companion layer cells expressing keratin 6 (K6+) are mechanically removed by hair plucking, bulge hfSCs become precociously activated and the hair cycle prematurely initiates a new hair cycle.

Extra-follicle interactions

Interactions between hair follicles and intra-dermal adipose tissue—By shaving the dorsal skin of mice or rabbits, we can observe regenerative hair waves traversing the skin in living mice (Plikus and Chuong. 2008). In telogen the skin is pink but becomes black as pigmentation is deposited in growing hair follicles during anagen. The pigment continues to be present through catagen and is once again lost at anagen. Watching regions of the skin change color allows us to analyze the behavior of hair stem cell populations, rather than the activation of just a single follicle. Activation of hfSCs induces a new hair cycle beginning with anagen (Plikus and Chuong. 2008). Hair waves were also observed on the backs of Cg-Msx2tm1Rilm/Mmcd mice which show cyclic rounds of growth and alopecia (Plikus and Chuong. 2004).

To explore the mechanism regulating the hair wave we examined fluctuations in molecular expression throughout the progression of the hair cycle. Canonical Wnt signaling is known to regulate the activation of hfSC activation (Millar, et al. 1999). Although essential for the hair cycle, BMP also inhibits hair follicle formation. As demonstrated, waves of BMP expression in the dermis occur out of phase with the Wnt/β-catenin cycle thereby subdividing anagen into propagating (low BMP, high Wnt/β-catenin) and autonomous anagen (high BMP, high Wnt/β-catenin) phases. Telogen was also subdivided into a refractory (high BMP, low Wnt/β-catenin) phase and competent (low BMP, low Wnt/β-catenin) phase for hair regeneration (Plikus, et al. 2008). The duration of these phases could be experimentally manipulated by over expressing exogenous Wnts or suppressing Wnt activity with Dkk1 (Plikus, et al. 2011). Furthermore, the roles of Wnt and BMP signaling

regulating the hair cycle are conserved when applied to the larger dorsal skin of rabbits (Plikus, et al. 2011).

hfSCs can remain dormant after the hair cycle enters the quiescent telogen phase due to the secretion of BMP2 and BMP4 from the DP and dermal fibroblasts directly adjacent to the bulge. In late telogen phase, inhibitory dermal BMP signaling levels decrease and, in turn, Wnt protein expression levels increase (Plikus, et al. 2008; Plikus, et al. 2011). Therefore the local molecular crosstalk between the hfSCs and surrounding niche drive the BMP-high/Wnt-low inhibitory state towards a BMP-low/Wnt-high setting favoring telogen-anagen transition and hair regeneration (Fig. 4).

To further understand how the dynamics of the Wnt/BMP activator/inhibitor pair are linked to the hair regeneration patterns, a cellular automaton (CA) model was introduced (Plikus, et al. 2011). The model correlates the phase-transition ability (e.g. from competent telogen to propagating anagen) of a hair follicle (one automaton) to the different levels of WNT and BMP produced within the follicles (the same automaton) and outside the follicles (adjacent automata). The simulation results could reproduce the regenerative patterns observed in normal mice, WNT pathway perturbed mice, and rabbits. The model reveals fluctuations of activator and inhibitor levels alter the efficiency of coupling hfSCs in neighboring hair follicles and result in changes in the hair regeneration patterns (Plikus, et al. 2011). Cell-lineage models that explicitly incorporate each cell type and feedback regulations may be applied to such systems to further elucidate the underlying mechanisms at the cellular and molecular levels (Lander, et al. 2009; Lo, et al. 2009; Chou, et al. 2010; Ovadia and Nie. 2013; Gord, et al. 2014).

Recently, intradermal adipocyte precursors were shown to actively secrete platelet-derived growth factor-α (PDGFα) which can stimulate hair follicle stem cells to enter anagen (Festa, et al. 2011). Therefore transmission of these accumulating signals to the hfSCs overcomes the strong inhibitory thresholds produced from surrounding niche cells and initiates new HF regeneration (Fig. 4) (Oshimori and Fuchs. 2012).

In conclusion, these integrated models demonstrate how hfSCs, their direct progeny, their niche, as well as micro- and macro-environmental components may counterbalance one another or alternatively, co-operate to maintain stem cells during quiescent homeostasis and activation. The ability of hfSCs to secrete auto-regulatory signals, but still sense surrounding niche environmental cues in a stochastic manner to orchestrate the needs for organ regeneration, may provide a common paradigm for stem cell regulation. Thus, unveiling reciprocal molecular circuits between hfSCs and different niche components might highlight targets for translational applications in future regenerative medicine.

Interactions between hair follicles and body status such as pregnancy and aging—An interesting observation is that the hair waves initially traverse the entire dorsal skin as a single wave but in subsequent hair cycles the waves fragment into smaller domains which are out of synchronization with one another. Furthermore, pregnancy/lactation and skin trauma can reset the fragmented hair cycle domains of the skin back to a single domain (Johnson. 1958). This may be attributable to pregnancy associated changes in hormone

(estrogen, prolactin) levels (Oh and Smart. 1996; Pearson, et al. 1999; Craven, et al. 2001; Foitzik, et al. 2003; Craven, et al. 2006).

Another interesting finding is that the hair cycle slows down in aging mice (> 18 months). Hair wave domains fragment into smaller regions and the hair wave propagation distance decreases. To test whether older skin could respond to signals from younger mouse skin, a piece of old mouse skin was transplanted to a young host. The older skin responded to signals from the younger mouse and restored hair cycling within 3mm of the host site. Follistatin, another BMP inhibitor, was expressed to high levels in late telogen and early anagen. Follistatin can induce hfSC activation and hair wave propagation; however, follistatin levels are significantly reduced in the skin of aged mice (Chen, et al. 2014). The data suggest that follistatin from young mice can induce hair wave propagation in the skin of old mice. Each of these examples demonstrates that the extrafollicular environment can dramatically influence hfSC activation (Chen, et al. 2014).

Multi-scale antagonistic competition between BMP and Wnt signaling

While there are multiple layers of regulation, the above work implies that hierarchical regulatory layers are mainly based on canonical BMP/Wnt signaling (Fig. 4). Such multi-layered control helps ensure that all regulatory information is considered, enabling the hfSC population to review the total activator/inhibitor activity to decide whether to activate or remain quiescent (Kandyba and Kobielak. 2014; Kandyba, et al. 2014). Furthermore, the delay in cell cycle-associated canonical Wnt-dependent target gene activation following hfSCs BMP inhibition implies that BMP inhibition precedes ligand-receptor dependent canonical Wnt up-regulation and hfSCs activation (Kobielak, et al. 2007; Kandyba, et al. 2013; Kandyba, et al. 2014; Kandyba and Kobielak. 2014).

The integration of regulation between extrinsic and intrinsic regulators may work together to either synchronize or desynchronize both networks leading to either accelerated or delayed hfSCs reactivity by changing the length of the refractory and competent telogen phases (Kandyba, et al. 2013; Kandyba, et al. 2014; Kandyba and Kobielak. 2014). Thus, hfSCs can sense suppressive BMP levels from intrinsic or extrinsic sources. A model was developed based upon oscillating adult bulge hfSCs states (Maini, et al. 2006) in response to constant competitive equilibrium between activators and inhibitors which is fundamental for the maintenance of hfSC quiescence or activation. Therefore, the release of hfSC activation cues can reset the balance back towards quiescence within the bulge, thereby achieving a cyclic molecular network (Kandyba, et al. 2013).

“Organ metamorphosis” in feathers is made possible by physiological regenerative cycling

At least four different plumages appear in avian species from hatching to the end of the first annual molting cycle, including natal down, juvenal, alternate and basic plumages (Lucas and Stettenheim. 1972). The plumages present at different physiological developmental stages could have dramatically different shapes and colors. In adult birds, the plume pattern could also vary greatly in response to season changes and gender differences (sexual dimorphism). The metamorphosis of plumage patterns is enabled by the robust and cyclic

regeneration activities of feather stem cells (Chuong, et al. 2012). The DP and associated pulp may serve as a relay center to translate the systematic information into differential feather branching and color patterns. For example, in the presence of extra thyroid substance, as well as progesterone, the DP will expand in size (Shaffner. 1954; Juhn and Harris. 1955; Shaffner. 1955). The seasonal changes of plumage patterns have been suggested to result from changes of photo-stimulation time, metabolic rate, and neuroendocrine regulations (Dawson and Goldsmith. 1983; Nolan Jr and Ketterson. 1990; Kuenzel. 2003; Vézina, et al. 2009). Sexual dimorphism is the systematic difference in phenotype between different genders of the same species driven by the sex hormones (Mayer, et al. 2004). For example, testosterone levels are related to the size of a plumage ornament (‘tail white’) on the dark eyed junco (McGlothlin, et al. 2008). Besides testosterone, previous studies suggested that gonadal steroid hormones (estrogen, pituitary peptide hormone, and luteinizing hormone) are also involved in regulating the differential plumage color patterns in the male and female birds of the same species (Kimball and Ligon. 1999). Therefore environmental signals from inside and outside the body can modulate the feather stem cells and their progenies’ activities to vary feather shapes and color patterns.

PURSUING UNDERSTANDING AT THE GENOMIC LEVEL: SYSTEMS BIOLOGY APPROACHES

There are interesting and important variations of the integument organ in different body regions and at different developmental stages, but how is this temporal-spatial regulation achieved from each individual's genome? The advent of next generation sequencing (NGS) technology provides a large scale, unbiased approach to profile genetic and epigenetic networks in different biological processes. Skin appendages provide a large scale, robust platform to do functional screens of the candidate molecules discovered by NGS derived technologies. The integration of diverse NGS applications, e.g. whole genome sequencing (WGS), RNA sequencing (RNA-seq), chromatin immunoprecipitation followed by sequencing (ChIP-seq), chromosome conformation capture followed by sequencing (3C-seq), bisulfite-seq (BS-seq), and so on, look to be a promising direction in the field of morphogenesis and regeneration (van Dijk, et al. 2014). With the apparent integument pattern, feathers and hairs provide major models for us to understand the genetic and epigenetic basis of integument phenotypes (Hillier, et al. 2004)

Genetic approach

Frizzle chicken—The feathers of Frizzle mutant domestic chickens (Somes Jr. 1990) curl outward and upward due to an altered rachis structure. An interdisciplinary collaboration identified the genetic basis of the frizzle trait (Ng, et al. 2012). Through a whole-mount linkage scan of 5 pedigrees using 2678 SNPs, the genetic locus associated with the frizzle trait was narrowed to a keratin gene enriched region. Further sequence analysis identified the 69 bp in frame deletion in KRT75 gene of the Frizzle chicken. Additionally, mis-expression of the mutated KRT75 could alter the bending of a WT feather rachis, proving this mutation is sufficient to induce the frizzle trait.

Naked neck chicken—The Naked neck chicken lacks feathers on the neck and narrow feather tracks on the body. Work by Dr. Headon's group demonstrated how genomic analysis could help reveal the molecular mechanism regulating feather patterns (Mou, et al. 2011). Genetic fine mapping identified a large genomic insertion from Chromosome 1 to Chromosome 3 that is associated with the Naked neck trait. The neighboring 770 kb region contained 5 candidate genes potentially affected by this insertion. Of these, BMP12 was the only one normally expressed in developing feather buds. Functional perturbation experiments confirmed that elevated BMP signaling was the cause of the Naked neck trait and the regional disparity of the phenotype was due to inhomogeneous sensitivity to BMP signaling across the body. Microarray analysis of the neck vs trunk region identified differential retinoic acid (RA) levels, controlled by RALDH (RA synthetase), modulate the sensitivity of the feather forming skin to BMP signaling.

Rock pigeons—The above two examples relied on traditional markers to map the genomic regions of interest. The introduction of NGS technology reduced the cost of acquiring the whole genomic sequence of a species. It is even feasible to do whole genome sequencing of multiple strains/subspecies to identify the genetic basis of a specific phenotype (Ellegren. 2014). A good example of this is the study of genetic features controlling pigeon head crest phenotypes (Shapiro, et al. 2013). Taking advantage of the great diversity of the feather landscape in pigeons created by artificial selection, Shapiro et al re-sequenced 40 breeds of rock pigeons and found a SNP in the EphB2 gene that was highly associated with the head crest phenotype. Moreover, they reused the same genome re-sequencing data to unveil molecular epistatic relationships underlying color variation in the domestic pigeon (Domyan, et al. 2014).

Importance of the epigenetic process in integument development and regeneration

Epigenetic mechanisms allow cells with identical genomes to achieve diverse phenotypes by regulating gene expression profiles through various histone modifications. Two functionally redundant epigenetic writers, Ezh1 and Ezh2, have been shown to be involved in regulating H3K27me3 for hair follicle homeostasis. Ezh1/2-null skins lost the ability to regenerate hair follicles, due to decreased proliferation and increased apoptosis (Ezhkova, et al. 2011). Another epigenetic writer, DNMT1, is essential to maintain the undifferentiated state of human epidermal progenitor cells (Sen, et al. 2010). The conditional DNMT1 knockout also influences hair follicle regeneration and leads to progressive hair loss in mice (Li, et al. 2012). Similarly, epigenetic erasers, e.g. HDAC1, also play an important role in hair follicle development. The HDAC1 conditional knockout mice exhibited phenotypes of alopecia and hair follicle dystrophy (Hughes, et al. 2014).

Epigenetic states of hair follicle stem cells

Taking advantage of the enormous number of mouse hair follicles and the well-established markers to distinguish hair follicle stem cells and transient amplifying cells, ChIP-seq analysis was used to study adult hfSCs during activation and differentiation (Lien, et al. 2011). The mRNA, H3K4me3 (marker of initiated genes) and H3K27me3 (marker of repressed genes) profiles of quiescent hfSCs, active hfSCs and transient amplifying cells were compared. Several novel findings were made: (i) much fewer bivalent

H3K27me3+H3K4me3 genes are found in hfSCs than embryonic stem cells (ESCs), suggesting the bivalent, or "poised" state may be uniquely tailored for ESC multi-potency. (ii) polycomb group (PcG) complex mediated H3K27me3 is not heavily involved during the activation of hFSCs but it functions to silence the key regulators of stem cells and releases regulators of committed matrix cells during differentiation.

High order chromatin organization

Gene expression can be governed by higher order chromatin remodeling that can bring together seemingly distant gene bodies which are spaced out in one dimension along the DNA sequence (Botchkarev, et al. 2012; Gdula, et al. 2013). One example is the indirect regulation of chromatin modifications by p63 during epidermis development (Fessing, et al. 2011). More recently, P63 and Brg1 were shown to act through the epidermal differentiation complex (Mardaryev, et al. 2014). More studies like this will help us understand how genes are regulated in clusters, or circuits rather than one at a time to alter cell behavior.

NEW INSIGHTS IN CELLULAR PLASTICITY REVEALED BY REAL TIME IMAGING AND CELL TRACKING

Cellular plasticity and phenotypic stability

Cells in different integument regions are endowed with different identities (gene expression profile, cell morphology). Are these identities permanent or reversible? The occurrence of ectopic hair growth in the gingiva of humans, canines, and rodents (Baranov, et al. 2010) may indicate the potential of ectodermal progenitors to switch between different fates. Experimental evidence supporting the idea that ectodermal progenitors could produce different phenotypes upon stimulation by environmental signals started to emerge in the early 90s when Jahoda et al implanted rat vibrissa dermal papilla cells into the ear pinna and induced vibrissa-like hair formation (Jahoda, et al. 1993). There are also reports of ectodermal progenitors forming different integument organs when certain molecular pathways are perturbed. For example, dissociated sweat gland cells can regenerate sweat glands as well as hair follicles and the epidermis when mixed with newborn dorsal skin dermal fibroblasts (Leung, et al. 2013). Also K14-Noggin transgenic mouse sweat glands were observed to trans-differentiate into hairs (Plikus, et al. 2004). In addition, K14-Noggin mouse nipple epithelium converted to hair-forming epithelium, possibly through a reduction in the levels of parathyroid hormone-related protein when BMP signaling is tuned down (Mayer, et al. 2008). In chicken embryos, retinoic acid treatment could induce feather growth from the scale-forming tarso-metatarsal region (Dhouailly, et al. 1980). Similar phenotypes were also seen upon the mis-expression of β-catenin, BMP7, dominant negative BMP receptor, Dll1, etc (Zou and Niswander. 1996; Crowe, et al. 1998; Noramly, et al. 1999; Widelitz, et al. 2000; Prin and Dhouailly. 2004; Lin, et al. 2006). All the above observations indicate that ectodermal progenitors are plastic (at least within a certain time window) to produce different integument phenotypes.

Intravital imaging of hair follicles reveals plasticity to “regenerate” stem cells

Live imaging is essential for analyzing molecular and cellular dynamics during biological processes such as morphogenesis and regeneration. It used to be difficult for skin appendages due to two factors: (i) they are 3-D structures that can grow to very large scale; (ii) the proximal follicle of hairs and feathers are deeply buried in the skin. Hence to do live imaging on skin appendages two prerequisites must be fulfilled: (i) the microscope must be able to see through deep tissue with good resolution; (ii) the target cell or molecule must be labelled to be distinguished from the environment. Recently Dr. Greco's group successfully monitored the behavior of hfSCs and their progeny during physiological hair regeneration and addressed the role of epithelium-mesenchyme interaction in this process (Rompolas, et al. 2012). This was achieved with a (i) two-photon microscope, which uses infrared illumination that could penetrate deeper into the tissue and has better resolution along the z dimension (Kaminer, et al. 2013); (ii) studies were performed using the K14H2BGFP mouse line, whose epithelial cells emit strong nuclear fluorescence that can help distinguish individual cells; (iii) the skin region connecting the ear and the head was used for imaging, which is thinner than the skin from other body regions. Taking advantage of this intravital microscopy setting, they did lineage tracing of hfSCs from different positions of the bulge and found the position could predict the future fate of the bulge stem cells. Through the use of laser ablation they found the bulge stem cells are dispensable for regeneration and can be replaced by neighboring epithelial cells from the interfollicular epidermis, infundibulum and sebaceous glands (Fig. 5A) (Rompolas, et al. 2013). These dynamic features are consistent with the concept that the plasticity of adjacent epithelial progenitors, other than the stem cells, can switch their fate as needed in response to injury, like a river in which the flow can switch directions depending on the changing landscapes (Chuong and Widelitz. 2009).

Genetic lineage reveals dual fate homeostasis of integument progenitors in vivo

A recent study discovered that potential nail stem cells (K15 positive, label retaining cells) are distributed in a ring configuration at the proximal fold region of the nail. Interestingly, lineage tracing experiments show these cells not only contribute to the growth and regeneration of the nail structure itself but also to the peri-nail epithelium. Thus these stem cells are bifunctional (Leung, et al. 2014). Upon nail injury, nail progenitors contribute more to the formation of the nail structure than the peri-nail epithelium (Fig. 5B) (Leung, et al. 2014). Similarly, in an earlier work by Ito's group the hair bulge stem cells were found to not only contribute to the cyclic regeneration of hair but also to the regeneration of interfollicular epidermis upon wounding (Ito, et al. 2005). This phenomenon was also observed in sweat glands, where slow cycling label retaining cells (LRCs) were able to trans-differentiate into the epidermis under prolonged wound healing conditions (Leung, et al. 2013). These discoveries indicate the integument progenitors can assume different paths of differentiation in physiological or pathological conditions. How to link this plasticity of integument progenitors and the epigenetic landscape would be an issue worth further investigation.

PERSPECTIVES

Autonomous patterning can be generated by robotic stem cells

Self-organized biological patterns not only fascinate biologists but also engineers in the field of robotics, as there are a number of applications for building multi-robot systems that are capable of self-assembly and self-healing (without human intervention) so as to detect and recover from unforeseen errors and attacks. The robots in these artificial systems are termed robotic stem cells as they have stem cell like properties that can self-reorganize to repair damage to their swarming organization (Rubenstein, et al. 2009). This type of cross-field research would be mutually beneficial in that the discovered principles of biological pattern formation can inspire novel design of the interaction algorithms of robotic stem cells. One the other hand the effort to alter the algorithms to create different shapes of the robotic swarm may add to biologists' understanding of how biological patterns evolve over time. For example, Turing type activator-inhibitor interactions have been shown to mediate the periodic formation of hairs and feathers (Maini, et al. 2006). Based on this, a "digital hormone model" was developed for a team of robots in a field to form spot or stripe configurations (Jiang, et al. 2004) and demonstrated the novel utility of this mechanism in controlling self-reconfigurable robotic systems (Shen, et al. 2002). Recently a multi-robot system was developed that can self-assemble into complex two dimensional shapes based on algorithms controlling three primitive collective behaviors: edge-following, gradient formation and localization (Rubenstein, et al. 2014). However for this robotic system to form a specific shape a blueprint is still required.

Self-organization of progenitor cells in tissue engineering

One major goal of tissue engineering is to let cells self-organize into complex organs in vitro with structures comparable to those developed in vivo. Pioneering work of self-organizing tissue pattern formation was achieved by Dr. Sasai's group. They developed a 3-D culture system in which mouse embryonic stem cell aggregates could be induced to differentiate into retinal and non-retinal neuroectodermal epithelium. More intriguingly, the retina epithelium could autonomously fold inward to form a shape reminiscent of the embryonic optic cup. Two-photon live imaging was employed to observe and analyze cell shape and dynamics during optic cup morphogenesis. It helped reveal differential cell morphologies at different phases of this morphogenetic process as well as upon inhibition of ROCK kinase activity. Furthermore, they developed a relaxation-expansion model to explain how the tissue internal forces and cell shape changes result in the spontaneous invagination of the neural retina (Eiraku, et al. 2011; Eiraku, et al. 2012). This work demonstrates the great potential of in vitro self-organized pattern formation systems, in combination with the advancing imaging techniques and mathematical modeling that can deepen our understanding of how the patterning process really works from initiation to the steady state.

Evo-Devo of integument organs driven by novel molecular modules that enable new patterning processes

The morphological diversification of the Metazoa (multicellular animals) is attributable to two major mechanisms. One is the emergence of new paths of cell differentiation (e.g. the cells produce new protein products, or alter their shapes); the other is the adjustment or

novel use of molecular modules for pattern formation. It is believed that the genes and gene regulatory networks involved in cell differentiation keep on expanding during evolution while those molecular modules setting up basic biological patterns have minimal changes (Newman, et al. 2009). One example of the first type of mechanism would be the expansion of keratin multigene families and the diversification of integument organs. The expansion of α-keratin genes may have contributed to the independent origin of hair and nails in mammals and baleen in whales (Vandebergh and Bossuyt. 2012). Large-scale expansions of β-keratin genes in birds and turtles may be involved in the innovation of the feathers and turtle shells (Greenwold and Sawyer. 2010; Li, et al. 2013b). In a recent study we carried out an exhaustive search of α- and β-keratin genes in the Galgal4 genome assembly and characterized the expression pattern of some keratin genes. β-keratin genes have diverse expression patterns in the five types of feathers we examined, and within the same individual feather α- and β-keratins are expressed in different regions (Ng, et al. 2014). For the second type of mechanism, components of Wnt, BMP, and Hedgehog pathways have been discovered to interact in a specific manner to generate the periodic patterns in the integument (Maini, et al. 2006). Many of these components constantly work together as a molecular module. For example the Wnt/Notch module is known to define different morphogenetic fields in different biological processes (Newman, et al. 2009; Muñoz Descalzo and Martinez Arias. 2012; Li, et al. 2013a). Although these genes have relatively conserved coding sequences, their regulatory regions may still accumulate changes through evolution as these modules are recruited to a new biological process.

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References

Alonso L, Fuchs E. The Hair Cycle. J Cell Sci. 2006; 119:391–393. [PubMed: 16443746]

Andl T, Ahn K, Kairo A, Chu EY, Wine-Lee L, Reddy ST, Croft NJ, Cebra-Thomas JA, Metzger D, Chambon P, Lyons KM, Mishina Y, Seykora JT, Crenshaw EB 3rd, Millar SE. Epithelial Bmpr1a Regulates Differentiation and Proliferation in Postnatal Hair Follicles and is Essential for Tooth Development. Development. 2004; 131:2257–2268. [PubMed: 15102710]

Andl T, Reddy ST, Gaddapara T, Millar SE. WNT Signals are Required for the Initiation of Hair Follicle Development. Dev Cell. 2002; 2:643–653. [PubMed: 12015971]

Ball, P.; Ball, P.; Ball, P.; Ball, P.; Ball, P. Nature's patterns: a tapestry in three parts. Oxford University Press, Oxford; New York: 2009.

Baranov A, Chernova O, Feoktistova NY, Surov A. A New Example of Ectopia: Oral Hair in some Rodent Species. 2010; 431:117–120.

Blanpain C, Fuchs E. Epidermal Homeostasis: A Balancing Act of Stem Cells in the Skin. Nat Rev Mol Cell Biol. 2009; 10:207–217. [PubMed: 19209183]

Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E. Self-Renewal, Multipotency, and the Existence of Two Cell Populations within an Epithelial Stem Cell Niche. Cell. 2004; 118:635–648. [PubMed: 15339667]

Botchkarev VA, Gdula MR, Mardaryev AN, Sharov AA, Fessing MY. Epigenetic Regulation of Gene Expression in Keratinocytes. J Invest Dermatol. 2012; 132:2505–2521. [PubMed: 22763788]

Botchkarev VA, Botchkareva NV, Nakamura M, Huber O, Funa K, Lauster R, Paus R, Gilchrest BA. Noggin is Required for Induction of the Hair Follicle Growth Phase in Postnatal Skin. FASEB J. 2001; 15:2205–2214. [PubMed: 11641247]

Botchkarev VA, Botchkareva NV, Roth W, Nakamura M, Chen LH, Herzog W, Lindner G, McMahon JA, Peters C, Lauster R, McMahon AP, Paus R. Noggin is a Mesenchymally Derived Stimulator of Hair-Follicle Induction. Nat Cell Biol. 1999; 1:158–164. [PubMed: 10559902]

Briscoe J, Pierani A, Jessell TM, Ericson J. A Homeodomain Protein Code Specifies Progenitor Cell Identity and Neuronal Fate in the Ventral Neural Tube. Cell. 2000; 101:435–445. [PubMed: 10830170]

Caviness VS Jr. Mechanical Model of Brain Convolutional Development. Science. 1975; 189:18–21. [PubMed: 1135626]

Chan E, Gat U, McNiff JM, Fuchs E. A Common Human Skin Tumour is Caused by Activating Mutations in B-Catenin. Nat Genet. 1999; 21:410–413. [PubMed: 10192393]

Chang C, Wu P, Baker RE, Maini PK, Alibardi L, Chuong CM. Reptile Scale Paradigm: Evo-Devo, Pattern Formation and Regeneration. Int J Dev Biol. 2009; 53:813–826. [PubMed: 19557687]

Chang CH, Jiang TX, Lin CM, Burrus LW, Chuong CM, Widelitz R. Distinct Wnt Members Regulate the Hierarchical Morphogenesis of Skin Regions (Spinal Tract) and Individual Feathers. Mech Dev. 2004a; 121:157–171. [PubMed: 15037317]

Chang CH, Yu M, Wu P, Jiang TX, Yu HS, Widelitz RB, Chuong CM. Sculpting Skin Appendages Out of Epidermal Layers Via Temporally and Spatially Regulated Apoptotic Events. J Invest Dermatol. 2004b; 122:1348–1355. [PubMed: 15175023]

Chen C, Chuong C. Multi-Layered Environmental Regulation on the Homeostasis of Stem Cells: The Saga of Hair Growth and Alopecia. J Dermatol Sci. 2012; 66:3–11. [PubMed: 22391240]

Chen CC, Murray PJ, Jiang TX, Plikus MV, Chang YT, Lee OK, Widelitz RB, Chuong CM. Regenerative Hair Waves in Aging Mice and Extra-Follicular Modulators Follistatin, Dkk1 and Sfrp4. J Invest Dermatol. 2014

Chou C, Lo W, Gokoffski KK, Zhang Y, Wan FY, Lander AD, Calof AL, Nie Q. Spatial Dynamics of Multi-Stage Cell Lineages in Tissue Stratification. Biophys J. 2010; 99

Chuong C, Bhat R, Widelitz RB, Bissell MJ. SnapShot: Branching Morphogenesis. Cell. 2014; 158:1212–1212. e1. [PubMed: 25171418]

Chuong CM, Yeh CY, Jiang TX, Widelitz R. Module-Based Complexity Formation: Periodic Patterning in Feathers and Hairs. Wiley Interdiscip Rev Dev Biol. 2013; 2:97–112. [PubMed: 23539312]

Chuong CM, Randall VA, Widelitz RB, Wu P, Jiang TX. Physiological Regeneration of Skin Appendages and Implications for Regenerative Medicine. Physiology (Bethesda). 2012; 27:61–72. [PubMed: 22505663]

Chuong CM, Richardson MK. Pattern Formation Today. Int J Dev Biol. 2009; 53:653–658. [PubMed: 19557673]

Chuong CM, Widelitz RB. The River of Stem Cells. Cell Stem Cell. 2009; 4:100–102. [PubMed: 19200797]

Chuong CM, Wu P, Plikus M, Jiang TX, Bruce Widelitz R. Engineering Stem Cells into Organs: Topobiological Transformations Demonstrated by Beak, Feather, and Other Ectodermal Organ Morphogenesis. Curr Top Dev Biol. 2006; 72:237–274. [PubMed: 16564337]

Cotsarelis G, Sun TT, Lavker RM. Label-Retaining Cells Reside in the Bulge Area of Pilosebaceous Unit: Implications for Follicular Stem Cells, Hair Cycle, and Skin Carcinogenesis. Cell. 1990; 61:1329–1337. [PubMed: 2364430]

Craven A, Ormandy C, Robertson F, Wilkins R, Kelly P, Nixon A, Pearson A. Prolactin Signaling Influences the Timing Mechanism of the Hair Follicle: Analysis of Hair Growth Cycles in Prolactin Receptor Knockout Mice 1. Endocrinology. 2001; 142:2533–2539. [PubMed: 11356702]

Craven AJ, Nixon AJ, Ashby MG, Ormandy CJ, Blazek K, Wilkins RJ, Pearson AJ. Prolactin Delays Hair Regrowth in Mice. J Endocrinol. 2006; 191:415–425. [PubMed: 17088411]

Crowe R, Henrique D, Ish-Horowicz D, Niswander L. A New Role for Notch and Delta in Cell Fate Decisions: Patterning the Feather Array. Development. 1998; 125:767–775. [PubMed: 9435296]

Davidson EH, Erwin DH. Gene Regulatory Networks and the Evolution of Animal Body Plans. Science. 2006; 311:796–800. [PubMed: 16469913]

Dawson A, Goldsmith AR. Plasma Prolactin and Gonadotrophins during Gonadal Development and the Onset of Photorefractoriness in Male and Female Starlings (Sturnus Vulgaris) on Artificial Photoperiods. J Endocrinol. 1983; 97:253–260. [PubMed: 6406634]

Dessaud E, McMahon AP, Briscoe J. Pattern Formation in the Vertebrate Neural Tube: A Sonic Hedgehog Morphogen-Regulated Transcriptional Network. Development. 2008; 135:2489–2503. [PubMed: 18621990]

Dhouailly D, Hardy MH, Sengel P. Formation of Feathers on Chick Foot Scales: A Stage-Dependent Morphogenetic Response to Retinoic Acid. J Embryol Exp Morphol. 1980; 58:63–78. [PubMed: 7441160]

Domyan ET, Guernsey MW, Kronenberg Z, Krishnan S, Boissy RE, Vickrey AI, Rodgers C, Cassidy P, Leachman SA, Fondon III JW. Epistatic and Combinatorial Effects of Pigmentary Gene Mutations in the Domestic Pigeon. Current Biology. 2014; 24:459–464. [PubMed: 24508169]

Driever W, Nusslein-Volhard C. The Bicoid Protein Determines Position in the Drosophila Embryo in a Concentration-Dependent Manner. Cell. 1988; 54:95–104. [PubMed: 3383245]

Driskell RR, Giangreco A, Jensen KB, Mulder KW, Watt FM. Sox2-Positive Dermal Papilla Cells Specify Hair Follicle Type in Mammalian Epidermis. Development. 2009; 136:2815–2823. [PubMed: 19605494]

Eiraku M, Adachi T, Sasai Y. Relaxation-expansion Model for Self driven Retinal Morphogenesis. Bioessays. 2012; 34:17–25. [PubMed: 22052700]

Eiraku M, Takata N, Ishibashi H, Kawada M, Sakakura E, Okuda S, Sekiguchi K, Adachi T, Sasai Y. Self-Organizing Optic-Cup Morphogenesis in Three-Dimensional Culture. Nature. 2011; 472:51–56. [PubMed: 21475194]

Ellegren H. Genome Sequencing and Population Genomics in Non-Model Organisms. Trends in ecology & evolution. 2014; 29:51–63. [PubMed: 24139972]

Ezhkova E, Lien WH, Stokes N, Pasolli HA, Silva JM, Fuchs E. EZH1 and EZH2 Cogovern Histone H3K27 Trimethylation and are Essential for Hair Follicle Homeostasis and Wound Repair. Genes Dev. 2011; 25:485–498. [PubMed: 21317239]

Fessing MY, Mardaryev AN, Gdula MR, Sharov AA, Sharova TY, Rapisarda V, Gordon KB, Smorodchenko AD, Poterlowicz K, Ferone G, Kohwi Y, Missero C, Kohwi-Shigematsu T, Botchkarev VA. p63 Regulates Satb1 to Control Tissue-Specific Chromatin Remodeling during Development of the Epidermis. J Cell Biol. 2011; 194:825–839. [PubMed: 21930775]

Festa E, Fretz J, Berry R, Schmidt B, Rodeheffer M, Horowitz M, Horsley V. Adipocyte Lineage Cells Contribute to the Skin Stem Cell Niche to Drive Hair Cycling. Cell. 2011; 146:761–771. [PubMed: 21884937]

Foitzik K, Krause K, Nixon AJ, Ford CA, Ohnemus U, Pearson AJ, Paus R. Prolactin and its Receptor are Expressed in Murine Hair Follicle Epithelium, show Hair Cycle-Dependent Expression, and Induce Catagen. The American journal of pathology. 2003; 162:1611–1621. [PubMed: 12707045]

Gat U, DasGupta R, Degenstein L, Fuchs E. De Novo Hair Follicle Morphogenesis and Hair Tumors in Mice Expressing a Truncated Beta-Catenin in Skin. Cell. 1998; 95:605–614. [PubMed: 9845363]

Gdula MR, Poterlowicz K, Mardaryev AN, Sharov AA, Peng Y, Fessing MY, Botchkarev VA. Remodeling of Three-Dimensional Organization of the Nucleus during Terminal Keratinocyte Differentiation in the Epidermis. J Invest Dermatol. 2013; 133:2191–2201. [PubMed: 23407401]

Gierer A, Meinhardt H. A Theory of Biological Pattern Formation. Kybernetik. 1972; 12:30–39. [PubMed: 4663624]

Gord A, Holmes WR, Dai X, Nie Q. Computational Modelling of Epidermal Stratification Highlights the Importance of Asymmetric Cell Division for Predictable and Robust Layer Formation. J R Soc Interface. 2014; 11 10.1098/rsif.2014.0631.

Greco V, Chen T, Rendl M, Schober M, Pasolli HA, Stokes N, Dela Cruz-Racelis J, Fuchs E. A Two-Step Mechanism for Stem Cell Activation during Hair Regeneration. Cell Stem Cell. 2009; 4:155–169. [PubMed: 19200804]

Greenwold MJ, Sawyer RH. Genomic Organization and Molecular Phylogenies of the Beta (Beta) Keratin Multigene Family in the Chicken (Gallus Gallus) and Zebra Finch (Taeniopygia Guttata): Implications for Feather Evolution. BMC Evol Biol. 2010; 10 148-2148-10-148.

Hamada H, Watanabe M, Lau HE, Nishida T, Hasegawa T, Parichy DM, Kondo S. Involvement of Delta/Notch Signaling in Zebrafish Adult Pigment Stripe Patterning. Development. 2014a; 141:318–324. [PubMed: 24306107]

Hamada H, Watanabe M, Lau HE, Nishida T, Hasegawa T, Parichy DM, Kondo S. Involvement of Delta/Notch Signaling in Zebrafish Adult Pigment Stripe Patterning. Development. 2014b; 141:318–324. [PubMed: 24306107]

Hamburger V, Hamilton HL. A Series of Normal Stages in the Development of the Chick Embryo. J Morphol. 1951; 88:49–92. [PubMed: 24539719]

Harris MP, Williamson S, Fallon JF, Meinhardt H, Prum RO. Molecular Evidence for an Activator-Inhibitor Mechanism in Development of Embryonic Feather Branching. Proc Natl Acad Sci U S A. 2005; 102:11734–11739. [PubMed: 16087884]

Headon DJ, Painter KJ. Stippling the Skin: Generation of Anatomical Periodicity by Reaction-Diffusion Mechanisms. Mathematical Modelling of Natural Phenomena. 2009; 4:83–102.

Hillier LW, Miller W, Birney E, Warren W, Hardison RC, Ponting CP, Bork P, Burt DW, Groenen MA, Delany ME. Sequence and Comparative Analysis of the Chicken Genome Provide Unique Perspectives on Vertebrate Evolution. Nature. 2004; 432:695–716. [PubMed: 15592404]

Houchmandzadeh B, Wieschaus E, Leibler S. Establishment of Developmental Precision and Proportions in the Early Drosophila Embryo. Nature. 2002; 415:798–802. [PubMed: 11845210]

Hsu YC, Pasolli HA, Fuchs E. Dynamics between Stem Cells, Niche, and Progeny in the Hair Follicle. Cell. 2011; 144:92–105. [PubMed: 21215372]

Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W. Beta-Catenin Controls Hair Follicle Morphogenesis and Stem Cell Differentiation in the Skin. Cell. 2001; 105:533–545. [PubMed: 11371349]

Hughes MW, Jiang T, Lin S, Leung Y, Kobielak K, Widelitz RB, Chuong CM. Disrupted Ectodermal Organ Morphogenesis in Mice with a Conditional Histone Deacetylase 1, 2 Deletion in the Epidermis. J Invest Dermatol. 2014; 134:24–32. [PubMed: 23792463]

Inaba M, Yamanaka H, Kondo S. Pigment Pattern Formation by Contact-Dependent Depolarization. Science. 2012; 335:677. [PubMed: 22323812]

Ito M, Yang Z, Andl T, Cui C, Kim N, Millar SE, Cotsarelis G. Wnt-Dependent De Novo Hair Follicle Regeneration in Adult Mouse Skin After Wounding. Nature. 2007; 447:316–320. [PubMed: 17507982]

Ito M, Liu Y, Yang Z, Nguyen J, Liang F, Morris RJ, Cotsarelis G. Stem Cells in the Hair Follicle Bulge Contribute to Wound Repair but Not to Homeostasis of the Epidermis. Nat Med. 2005; 11:1351–1354. [PubMed: 16288281]

Ito M, Kizawa K, Hamada K, Cotsarelis G. Hair Follicle Stem Cells in the Lower Bulge Form the Secondary Germ, a Biochemically Distinct but Functionally Equivalent Progenitor Cell Population, at the Termination of Catagen. Differentiation. 2004; 72:548–557. [PubMed: 15617565]

Jahoda CA, Oliver RF, Reynolds AJ, Forrester JC, Gillespie JW, Cserhalmi-Friedman PB, Christiano AM, Horne KA. Trans-Species Hair Growth Induction by Human Hair Follicle Dermal Papillae. Exp Dermatol. 2001; 10:229–237. [PubMed: 11493311]

Jahoda CA, Reynolds AJ, Oliver RF. Induction of Hair Growth in Ear Wounds by Cultured Dermal Papilla Cells. J Invest Dermatol. 1993; 101:584–590. [PubMed: 8409527]

Jahoda CA, Horne KA, Oliver RF. Induction of Hair Growth by Implantation of Cultured Dermal Papilla Cells. Nature. 1984; 311:560–562. [PubMed: 6482967]

Jiang TX, Widelitz RB, Shen WM, Will P, Wu DY, Lin CM, Jung HS, Chuong CM. Integument Pattern Formation Involves Genetic and Epigenetic Controls: Feather Arrays Simulated by Digital Hormone Models. Int J Dev Biol. 2004; 48:117–135. [PubMed: 15272377]

Jiang TX, Jung HS, Widelitz RB, Chuong CM. Self-Organization of Periodic Patterns by Dissociated Feather Mesenchymal Cells and the Regulation of Size, Number and Spacing of Primordia. Development. 1999; 126:4997–5009. [PubMed: 10529418]

Johnson E. Quantitative Studies of Hair Growth in the Albino Rat. III. the Role of the Adrenal Glands. J Endocrinol. 1958; 16:360–368. [PubMed: 13539282]

Juhn M, Harris PC. Local Effects on the Feather Papilla of Thyroxine and of Progesterone. Proc Soc Exp Biol Med. 1955; 90:202–204. [PubMed: 13273397]

Jung HS, Francis-West PH, Widelitz RB, Jiang TX, Ting-Berreth S, Tickle C, Wolpert L, Chuong CM. Local Inhibitory Action of BMPs and their Relationships with Activators in Feather Formation: Implications for Periodic Patterning. Dev Biol. 1998; 196:11–23. [PubMed: 9527877]

Kaminer I, Nemirovsky J, Segev M. Optimizing 3D Multiphoton Fluorescence Microscopy. Opt Lett. 2013; 38:3945–3948. [PubMed: 24081095]

Kandyba E, Hazen VM, Kobielak A, Butler SJ, Kobielak K. Smad1 and 5 but Not Smad8 Establish Stem Cell Quiescence which is Critical to Transform the Premature Hair Follicle during Morphogenesis Toward the Postnatal State. Stem Cells. 2014; 32:534–547. [PubMed: 24023003]

Kandyba E, Kobielak K. Wnt7b is an Important Intrinsic Regulator of Hair Follicle Stem Cell Homeostasis and Hair Follicle Cycling. Stem Cells. 2014; 32:886–901. [PubMed: 24222445]

Kandyba E, Leung Y, Chen Y, Widelitz R, Chuong C, Kobielak K. Competitive Balance of Intra-Bulge BMP/WNT Signaling Reveals a Robust Gene Network Ruling Stem Cell Homeostasis and Cyclic Activation. Proc Natl Acad Sci U S A. 2013; 110:1351–1356. [PubMed: 23292934]

Kimball RT, Ligon JD. Evolution of Avian Plumage Dichromatism from a Proximate Perspective. Am Nat. 1999; 154:182–193.

Kishimoto J, Burgeson RE, Morgan BA. Wnt Signaling Maintains the Hair-Inducing Activity of the Dermal Papilla. Genes Dev. 2000; 14:1181–1185. [PubMed: 10817753]

Kobielak K, Stokes N, de la Cruz J, Polak L, Fuchs E. Loss of a Quiescent Niche but Not Follicle Stem Cells in the Absence of Bone Morphogenetic Protein Signaling. Proc Natl Acad Sci U S A. 2007; 104:10063–10068. [PubMed: 17553962]

Kobielak K, Pasolli HA, Alonso L, Polak L, Fuchs E. Defining BMP Functions in the Hair Follicle by Conditional Ablation of BMP Receptor IA. J Cell Biol. 2003; 163:609–623. [PubMed: 14610062]

Kondo S, Miura T. Reaction-Diffusion Model as a Framework for Understanding Biological Pattern Formation. Science. 2010; 329:1616–1620. [PubMed: 20929839]

Kücken M, Newell AC. Fingerprint Formation. J Theor Biol. 2005; 235:71–83. [PubMed: 15833314]

Kuenzel WJ. Neurobiology of Molt in Avian Species. Poult Sci. 2003; 82:981–991. [PubMed: 12817454]

Lander AD, Gokoffski KK, Wan FY, Nie Q, Calof AL. Cell Lineages and the Logic of Proliferative Control. PLoS biology. 2009; 7:e1000015.

Lander AD, Nie Q, Wan FY. Do Morphogen Gradients Arise by Diffusion? Developmental cell. 2002; 2:785–796. [PubMed: 12062090]

Lander AD. How Cells Know Where they Are. Science. 2013; 339:923–927. [PubMed: 23430648]

Lander AD. Pattern, Growth, and Control. Cell. 2011; 144:955–969. [PubMed: 21414486]

Leung Y, Kandyba E, Chen Y, Ruffins S, Kobielak K. Label Retaining Cells (LRCs) with Myoepithelial Characteristic from the Proximal Acinar Region Define Stem Cells in the Sweat Gland. PloS one. 2013; 8:e74174. [PubMed: 24058524]

Leung Y, Kandyba E, Chen YB, Ruffins S, Chuong CM, Kobielak K. Bifunctional Ectodermal Stem Cells Around the Nail Display Dual Fate Homeostasis and Adaptive Wounding Response Toward Nail Regeneration. Proc Natl Acad Sci U S A. 2014

Li A, Chen M, Jiang TX, Wu P, Nie Q, Widelitz R, Chuong CM. Shaping Organs by a Wingless-Int/Notch/Nonmuscle Myosin Module which Orients Feather Bud Elongation. Proc Natl Acad Sci U S A. 2013a; 110:E1452–61. [PubMed: 23576731]

Li J, Jiang TX, Hughes MW, Wu P, Yu J, Widelitz RB, Fan G, Chuong CM. Progressive Alopecia Reveals Decreasing Stem Cell Activation Probability during Aging of Mice with Epidermal Deletion of DNA Methyltransferase 1. J Invest Dermatol. 2012; 132:2681–2690. [PubMed: 22763785]

Li YI, Kong L, Ponting CP, Haerty W. Rapid Evolution of Beta-Keratin Genes Contribute to Phenotypic Differences that Distinguish Turtles and Birds from Other Reptiles. Genome Biol Evol. 2013b; 5:923–933. [PubMed: 23576313]

Lien W, Guo X, Polak L, Lawton LN, Young RA, Zheng D, Fuchs E. Genome-Wide Maps of Histone Modifications Unwind in Vivo Chromatin States of the Hair Follicle Lineage. Cell Stem Cell. 2011; 9:219–232. [PubMed: 21885018]

Lin CM, Jiang TX, Widelitz RB, Chuong CM. Molecular Signaling in Feather Morphogenesis. Curr Opin Cell Biol. 2006; 18:730–741. [PubMed: 17049829]

Lin SJ, Foley J, Jiang TX, Yeh CY, Wu P, Foley A, Yen CM, Huang YC, Cheng HC, Chen CF, Reeder B, Jee SH, Widelitz RB, Chuong CM. Topology of Feather Melanocyte Progenitor Niche Allows Complex Pigment Patterns to Emerge. Science. 2013a; 340:1442–1445. [PubMed: 23618762]

Lin SJ, Wideliz RB, Yue Z, Li A, Wu X, Jiang TX, Wu P, Chuong CM. Feather Regeneration as a Model for Organogenesis. Dev Growth Differ. 2013b; 55:139–148. [PubMed: 23294361]

Liu Y, Lyle S, Yang Z, Cotsarelis G. Keratin 15 Promoter Targets Putative Epithelial Stem Cells in the Hair Follicle Bulge. J Invest Dermatol. 2003; 121:963–968. [PubMed: 14708593]

Lo Celso C, Prowse DM, Watt FM. Transient Activation of Beta-Catenin Signalling in Adult Mouse Epidermis is Sufficient to Induce New Hair Follicles but Continuous Activation is Required to Maintain Hair Follicle Tumours. Development. 2004; 131:1787–1799. [PubMed: 15084463]

Lo WC, Chou CS, Gokoffski KK, Wan FY, Lander AD, Calof AL, Nie Q. Feedback Regulation in Multistage Cell Lineages. Math Biosci Eng. 2009; 6:59–82. [PubMed: 19292508]

Lowry WE, Blanpain C, Nowak JA, Guasch G, Lewis L, Fuchs E. Defining the Impact of Beta-Catenin/Tcf Transactivation on Epithelial Stem Cells. Genes Dev. 2005; 19:1596–1611. [PubMed: 15961525]

Lucas, P.; Stettenheim, A., editors. Avian anatomy. Integument. Agriculture Handbook. US Department of Agriculture; Washington, DC: 1972.

Mahadevan L, Rica S. Self-Organized Origami. Science. 2005; 307:1740. [PubMed: 15774751]

Mahalwar P, Walderich B, Singh AP, Nusslein-Volhard C. Local Reorganization of Xanthophores Fine-Tunes and Colors the Striped Pattern of Zebrafish. Science. 2014; 345:1362–1364. [PubMed: 25214630]

Maini PK, Baker RE, Chuong CM. Developmental Biology. the Turing Model Comes of Molecular Age. Science. 2006; 314:1397–1398. [PubMed: 17138885]

Mardaryev AN, Gdula MR, Yarker JL, Emelianov VU, Poterlowicz K, Sharov AA, Sharova TY, Scarpa JA, Joffe B, Solovei I, Chambon P, Botchkarev VA, Fessing MY. p63 and Brg1 Control Developmentally Regulated Higher-Order Chromatin Remodelling at the Epidermal Differentiation Complex Locus in Epidermal Progenitor Cells. Development. 2014; 141:101–111. [PubMed: 24346698]

Massague J, Seoane J, Wotton D. Smad Transcription Factors. Genes Dev. 2005; 19:2783–2810. [PubMed: 16322555]

Mayer JA, Foley J, De La Cruz D, Chuong CM, Widelitz R. Conversion of the Nipple to Hair-Bearing Epithelia by Lowering Bone Morphogenetic Protein Pathway Activity at the Dermal-Epidermal Interface. Am J Pathol. 2008; 173:1339–1348. [PubMed: 18832580]

Mayer JA, Chuong CM, Widelitz R. Rooster Feathering, Androgenic Alopecia, and Hormone-Dependent Tumor Growth: What is in Common? Differentiation. 2004; 72:474–488. [PubMed: 15617560]

McGlothlin J, Jawor J, Greives T, Casto J, Phillips J, Ketterson E. Hormones and Honest Signals: Males with Larger Ornaments Elevate Testosterone More when Challenged. J Evol Biol. 2008; 21:39–48. [PubMed: 18034801]

Meinhardt H, Gierer A. Pattern Formation by Local Self-Activation and Lateral Inhibition. Bioessays. 2000; 22:753–760. [PubMed: 10918306]

Millar SE. Molecular Mechanisms Regulating Hair Follicle Development. J Invest Dermatol. 2002; 118:216–225. [PubMed: 11841536]

Millar SE, Willert K, Salinas PC, Roelink H, Nusse R, Sussman DJ, Barsh GS. WNT Signaling in the Control of Hair Growth and Structure. Dev Biol. 1999; 207:133–149. [PubMed: 10049570]

Morris RJ, Liu Y, Marles L, Yang Z, Trempus C, Li S, Lin JS, Sawicki JA, Cotsarelis G. Capturing and Profiling Adult Hair Follicle Stem Cells. Nat Biotechnol. 2004; 22:411–417. [PubMed: 15024388]

Mou C, Pitel F, Gourichon D, Vignoles F, Tzika A, Tato P, Yu L, Burt DW, Bed'hom B, Tixier-Boichard M, Painter KJ, Headon DJ. Cryptic Patterning of Avian Skin Confers a Developmental Facility for Loss of Neck Feathering. PLoS Biol. 2011; 9:e1001028. [PubMed: 21423653]

Moulton D, Goriely A. Circumferential Buckling Instability of a Growing Cylindrical Tube. J Mech Phys Solids. 2011; 59:525–537.

Muller-Rover S, Handjiski B, van der Veen C, Eichmuller S, Foitzik K, McKay IA, Stenn KS, Paus R. A Comprehensive Guide for the Accurate Classification of Murine Hair Follicles in Distinct Hair Cycle Stages. J Invest Dermatol. 2001; 117:3–15. [PubMed: 11442744]

Muñoz Descalzo S, Martinez Arias A. The Structure of Wntch Signalling and the Resolution of Transition States in Development. 2012; 23:443–449.

Nakamasu A, Takahashi G, Kanbe A, Kondo S. Interactions between Zebrafish Pigment Cells Responsible for the Generation of Turing Patterns. Proc Natl Acad Sci U S A. 2009; 106:8429–8434. [PubMed: 19433782]

Newman SA, Bhat R, Mezentseva NV. Cell State Switching Factors and Dynamical Patterning Modules: Complementary Mediators of Plasticity in Development and Evolution. J Biosci. 2009; 34:553–572. [PubMed: 19920341]

Ng CS, Wu P, Fan WL, Yan J, Chen CK, Lai YT, Wu SM, Mao CT, Chen JJ, Lu MY, Ho MR, Widelitz RB, Chen CF, Chuong CM, Li WH. Genomic Organization, Transcriptomic Analysis, and Functional Characterization of Avian Alpha- and Beta-Keratins in Diverse Feather Forms. Genome Biol Evol. 2014; 6:2258–2273. [PubMed: 25152353]

Ng CS, Wu P, Foley J, Foley A, McDonald ML, Juan WT, Huang CJ, Lai YT, Lo WS, Chen CF, Leal SM, Zhang H, Widelitz RB, Patel PI, Li WH, Chuong CM. The Chicken Frizzle Feather is due to an Alpha-Keratin (KRT75) Mutation that Causes a Defective Rachis. PLoS Genet. 2012; 8:e1002748. [PubMed: 22829773]

Nolan Jr V, Ketterson ED. Effect of Long Days on Molt and Autumn Migratory State of Site-Faithful Dark-Eyed Juncos Held at their Winter Sites. The Wilson Bulletin. 1990:469–479.

Noramly S, Freeman A, Morgan BA. Beta-Catenin Signaling can Initiate Feather Bud Development. Development. 1999; 126:3509–3521. [PubMed: 10409498]

Oh HS, Smart RC. An Estrogen Receptor Pathway Regulates the Telogen-Anagen Hair Follicle Transition and Influences Epidermal Cell Proliferation. Proc Natl Acad Sci U S A. 1996; 93:12525–12530. [PubMed: 8901615]

Oshimori N, Fuchs E. Paracrine TGF-B Signaling Counterbalances BMP-Mediated Repression in Hair Follicle Stem Cell Activation. Cell Stem Cell. 2012; 10:63–75. [PubMed: 22226356]

Ovadia J, Nie Q. Stem Cell Niche Structure as an Inherent Cause of Undulating Epithelial Morphologies. Biophys J. 2013; 104:237–246. [PubMed: 23332076]

Pearson AJ, Ashby MG, Wildermoth JE, Craven AJ, Nixon AJ. Effect of Exogenous Prolactin on the Hair Growth Cycle. Exp Dermatol. 1999; 8:358–360. [PubMed: 10439275]

Plikus M, Chuong CM. Making Waves with Hairs. J Invest Dermatol. 2004; 122:vii–ix. [PubMed: 15102103]

Plikus M, Wang WP, Liu J, Wang X, Jiang TX, Chuong CM. Morpho-Regulation of Ectodermal Organs: Integument Pathology and Phenotypic Variations in K14-Noggin Engineered Mice through Modulation of Bone Morphogenic Protein Pathway. Am J Pathol. 2004; 164:1099–1114. [PubMed: 14982863]

Plikus MV, Baker RE, Chen CC, Fare C, de la Cruz D, Andl T, Maini PK, Millar SE, Widelitz R, Chuong CM. Self-Organizing and Stochastic Behaviors during the Regeneration of Hair Stem Cells. Science. 2011; 332:586–589. [PubMed: 21527712]

Plikus MV, Widelitz RB, Maxson R, Chuong CM. Analyses of Regenerative Wave Patterns in Adult Hair Follicle Populations Reveal Macro-Environmental Regulation of Stem Cell Activity. Int J Dev Biol. 2009; 53:857–868. [PubMed: 19378257]

Plikus MV, Chuong CM. Complex Hair Cycle Domain Patterns and Regenerative Hair Waves in Living Rodents. J Invest Dermatol. 2008

Plikus MV, Mayer JA, de la Cruz D, Baker RE, Maini PK, Maxson R, Chuong CM. Cyclic Dermal BMP Signalling Regulates Stem Cell Activation during Hair Regeneration. Nature. 2008; 451:340–344. [PubMed: 18202659]

Prin F, Dhouailly D. How and when the Regional Competence of Chick Epidermis is Established: Feathers Vs. Scutate and Reticulate Scales, a Problem En Route to a Solution. Int J Dev Biol. 2004; 48:137–148. [PubMed: 15272378]

Rahmani W, Abbasi S, Hagner A, Raharjo E, Kumar R, Hotta A, Magness S, Metzger D, Biernaskie J. Hair Follicle Dermal Stem Cells Regenerate the Dermal Sheath, Repopulate the Dermal Papilla, and Modulate Hair Type. Developmental cell. 2014; 31:543–558. [PubMed: 25465495]

Reddy S, Andl T, Bagasra A, Lu MM, Epstein DJ, Morrisey EE, Millar SE. Characterization of Wnt Gene Expression in Developing and Postnatal Hair Follicles and Identification of Wnt5a as a Target of Sonic Hedgehog in Hair Follicle Morphogenesis. Mech Dev. 2001; 107:69–82. [PubMed: 11520664]

Rendl M, Polak L, Fuchs E. BMP Signaling in Dermal Papilla Cells is Required for their Hair Follicle-Inductive Properties. Genes Dev. 2008; 22:543–557. [PubMed: 18281466]

Rendl M, Lewis L, Fuchs E. Molecular Dissection of Mesenchymal-Epithelial Interactions in the Hair Follicle. PLoS Biol. 2005; 3:e331. [PubMed: 16162033]

Rolf HJ, Kierdorf U, Kierdorf H, Schulz J, Seymour N, Schliephake H, Napp J, Niebert S, Wölfel H, Wiese KG. Localization and Characterization of STRO-1 Cells in the Deer Pedicle and Regenerating Antler. PLoS One. 2008; 3:e2064. [PubMed: 18446198]

Rolo A, Skoglund P, Keller R. Morphogenetic Movements Driving Neural Tube Closure in Xenopus Require Myosin IIB. Dev Biol. 2009; 327:327–338. [PubMed: 19121300]

Rompolas P, Mesa KR, Greco V. Spatial Organization within a Niche as a Determinant of Stem-Cell Fate. Nature. 2013; 502:513–518. [PubMed: 24097351]

Rompolas P, Deschene ER, Zito G, Gonzalez DG, Saotome I, Haberman AM, Greco V. Live Imaging of Stem Cell and Progeny Behaviour in Physiological Hair-Follicle Regeneration. Nature. 2012; 487:496–499. [PubMed: 22763436]

Rubenstein M, Cornejo A, Nagpal R. Robotics. Programmable Self-Assembly in a Thousand-Robot Swarm. Science. 2014; 345:795–799. [PubMed: 25124435]

Rubenstein M, Sai Y, Chuong CM, Shen WM. Regenerative Patterning in Swarm Robots: Mutual Benefits of Research in Robotics and Stem Cell Biology. Int J Dev Biol. 2009; 53:869–881. [PubMed: 19557691]

Schmidt-Ullrich R, Paus R. Molecular Principles of Hair Follicle Induction and Morphogenesis. Bioessays. 2005; 27:247–261. [PubMed: 15714560]

Sen GL, Reuter JA, Webster DE, Zhu L, Khavari PA. DNMT1 Maintains Progenitor Function in Self-Renewing Somatic Tissue. Nature. 2010; 463:563–567. [PubMed: 20081831]

Shaffner C. Progesterone Induced Molt. Poult Sci. 1955; 34:840–842.

Shaffner CS. Feather Papilla Stimulation by Progesterone. Science. 1954; 120:345. [PubMed: 13186852]

Shapiro MD, Kronenberg Z, Li C, Domyan ET, Pan H, Campbell M, Tan H, Huff CD, Hu H, Vickrey AI, Nielsen SC, Stringham SA, Hu H, Willerslev E, Gilbert MT, Yandell M, Zhang G, Wang J. Genomic Diversity and Evolution of the Head Crest in the Rock Pigeon. Science. 2013; 339:1063–1067. [PubMed: 23371554]

Shen W, Salemi B, Will P. Hormone-Inspired Adaptive Communication and Distributed Control for CONRO Self-Reconfigurable Robots. Robotics and Automation, IEEE Transactions on 18. 2002:700–712.

Shyer AE, Tallinen T, Nerurkar NL, Wei Z, Gil ES, Kaplan DL, Tabin CJ, Mahadevan L. Villification: How the Gut Gets its Villi. Science. 2013; 342:212–218. [PubMed: 23989955]

Sick S, Reinker S, Timmer J, Schlake T. WNT and DKK Determine Hair Follicle Spacing through a Reaction-Diffusion Mechanism. Science. 2006; 314:1447–1450. [PubMed: 17082421]

Somes Jr R. Mutations and Major Variants of Plumage and Skin in Chickens. Developments in Animal and Veterinary Sciences (Netherlands). 1990

Suzuki N, Hirata M, Kondo S. Traveling Stripes on the Skin of a Mutant Mouse. Proc Natl Acad Sci U S A. 2003; 100:9680–9685. [PubMed: 12893877]

Todd KJ, Lan-Chow-Wing N, Salin-Cantegrel A, Cotter A, Zagami CJ, Lo R, Stifani S. Establishment of Motor Neuron-V3 Interneuron Progenitor Domain Boundary in Ventral Spinal Cord Requires Groucho-Mediated Transcriptional Corepression. PloS one. 2012; 7:e31176. [PubMed: 22363571]

Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E. Defining the Epithelial Stem Cell Niche in Skin. Science. 2004; 303:359–363. [PubMed: 14671312]

Turing AM. The Chemical Basis of Morphogenesis. Phil Trans R Soc B. 1952; 237:37–72.

van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C. Ten Years of Next-Generation Sequencing Technology. Trends in Genetics. 2014

Van Mater D, Kolligs FT, Dlugosz AA, Fearon ER. Transient Activation of Beta - Catenin Signaling in Cutaneous Keratinocytes is Sufficient to Trigger the Active Growth Phase of the Hair Cycle in Mice. Genes Dev. 2003; 17:1219–1224. [PubMed: 12756226]

Vandebergh W, Bossuyt F. Radiation and Functional Diversification of Alpha Keratins during Early Vertebrate Evolution. Mol Biol Evol. 2012; 29:995–1004. [PubMed: 22046002]

Vézina F, Gustowska A, Jalvingh KM, Chastel O, Piersma T. Hormonal Correlates and Thermoregulatory Consequences of Molting on Metabolic Rate in a Northerly Wintering Shorebird. Physiological and Biochemical Zoology. 2009; 82:129–142. [PubMed: 19199554]

Widelitz RB, Jiang TX, Lu J, Chuong CM. Beta-Catenin in Epithelial Morphogenesis: Conversion of Part of Avian Foot Scales into Feather Buds with a Mutated Beta-Catenin. Dev Biol. 2000; 219:98–114. [PubMed: 10677258]

Widelitz RB, Jiang TX, Noveen A, Chen CW, Chuong CM. FGF Induces New Feather Buds from Developing Avian Skin. J Invest Dermatol. 1996; 107:797–803. [PubMed: 8941663]

Wolpert L. Positional Information and the Spatial Pattern of Cellular Differentiation. J Theor Biol. 1969; 25:1–47. [PubMed: 4390734]

Wu P, Alibardi L, Chuong CM. Regeneration of Reptilian Scales After Wounding: Neogenesis, Regional Difference, and Molecular Modules. Regeneration. 2014; 1:15–26. [PubMed: 24665362]

Wu P, Hou L, Plikus M, Hughes M, Scehnet J, Suksaweang S, Widelitz R, Jiang TX, Chuong CM. Evo-Devo of Amniote Integuments and Appendages. Int J Dev Biol. 2004a; 48:249–270. [PubMed: 15272390]

Wu P, Jiang TX, Suksaweang S, Widelitz RB, Chuong CM. Molecular Shaping of the Beak. Science. 2004b; 305:1465–1466. [PubMed: 15353803]

Yu M, Wu P, Widelitz RB, Chuong CM. The Morphogenesis of Feathers. Nature. 2002; 420:308–312. [PubMed: 12442169]

Yue Z, Jiang TX, Wu P, Widelitz RB, Chuong CM. Sprouty/FGF Signaling Regulates the Proximal-Distal Feather Morphology and the Size of Dermal Papillae. Dev Biol. 2012; 372:45–54. [PubMed: 23000358]

Yue Z, Jiang TX, Widelitz RB, Chuong CM. Mapping Stem Cell Activities in the Feather Follicle. Nature. 2005; 438:1026–1029. [PubMed: 16355227]

Zaikin A, Zhabotinsky A. Concentration Wave Propagation in Two-Dimensional Liquid-Phase Self-Oscillating System. Nature. 1970; 225:535–537. [PubMed: 16056595]

Zhang J, He XC, Tong W, Johnson T, Wiedemann LM, Mishina Y, Feng JQ, Li L. Bone Morphogenetic Protein Signaling Inhibits Hair Follicle Anagen Induction by Restricting Epithelial Stem/Progenitor Cell Activation and Expansion. Stem Cells. 2006; 24:2826–2839. [PubMed: 16960130]

Zhang YV, Cheong J, Ciapurin N, McDermitt DJ, Tumbar T. Distinct Self-Renewal and Differentiation Phases in the Niche of Infrequently Dividing Hair Follicle Stem Cells. Cell stem cell. 2009; 5:267–278. [PubMed: 19664980]

Zou H, Niswander L. Requirement for BMP Signaling in Interdigital Apoptosis and Scale Formation. Science. 1996; 272:738–741. [PubMed: 8614838]

Zuzarte-Luis V, Hurle JM. Programmed Cell Death in the Embryonic Vertebrate Limb. 2005; 16:261–269.

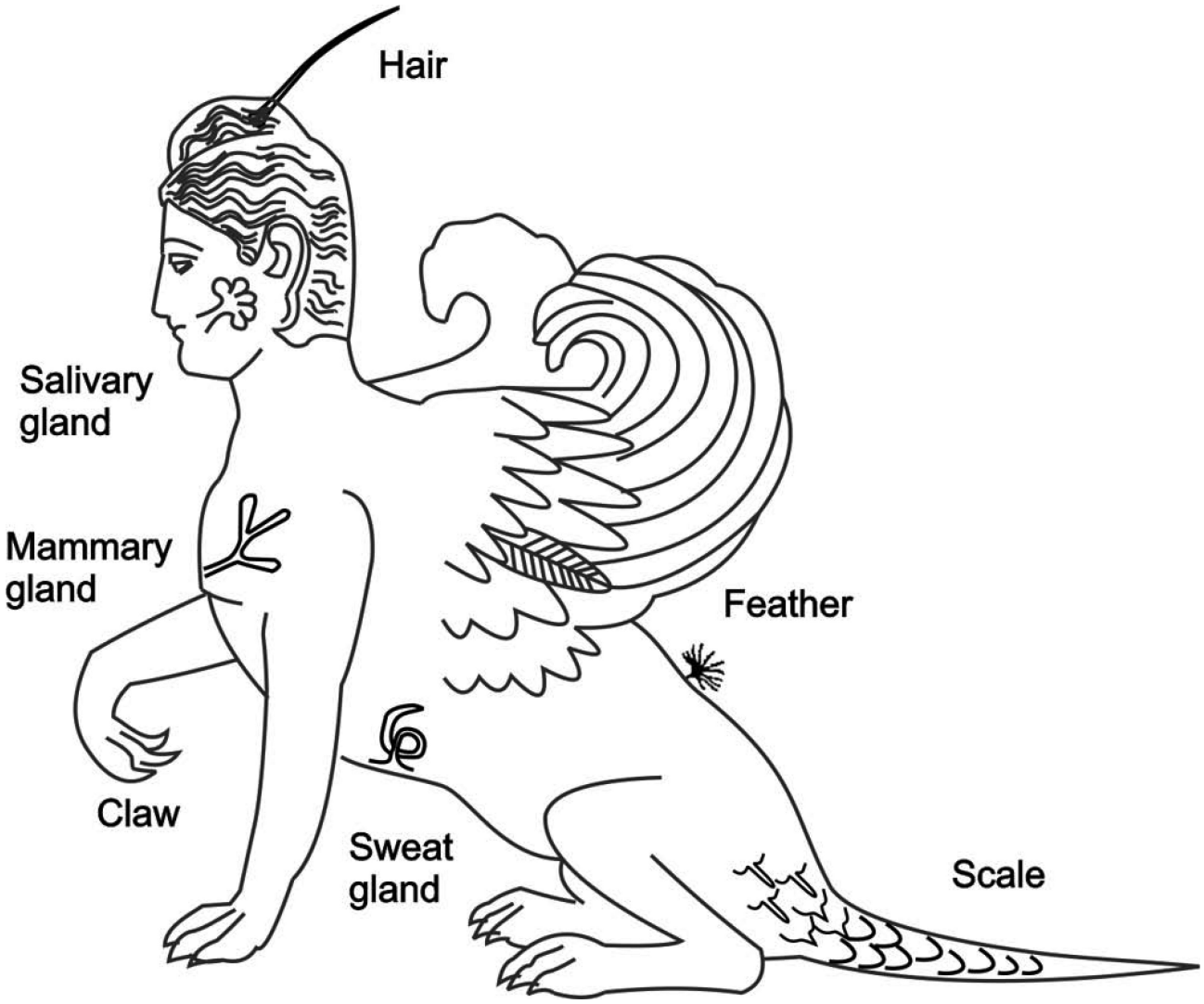

Fig. 1. Sphinx of integumentary organs

A Greek version of a Sphinx is used to symbolize the riddle on how different integument organs (hair, feather, scale, claw, sweat gland, mammary gland and salivary gland) are expressed in different body surface regions. Conversion from the scale to feather is also depicted.

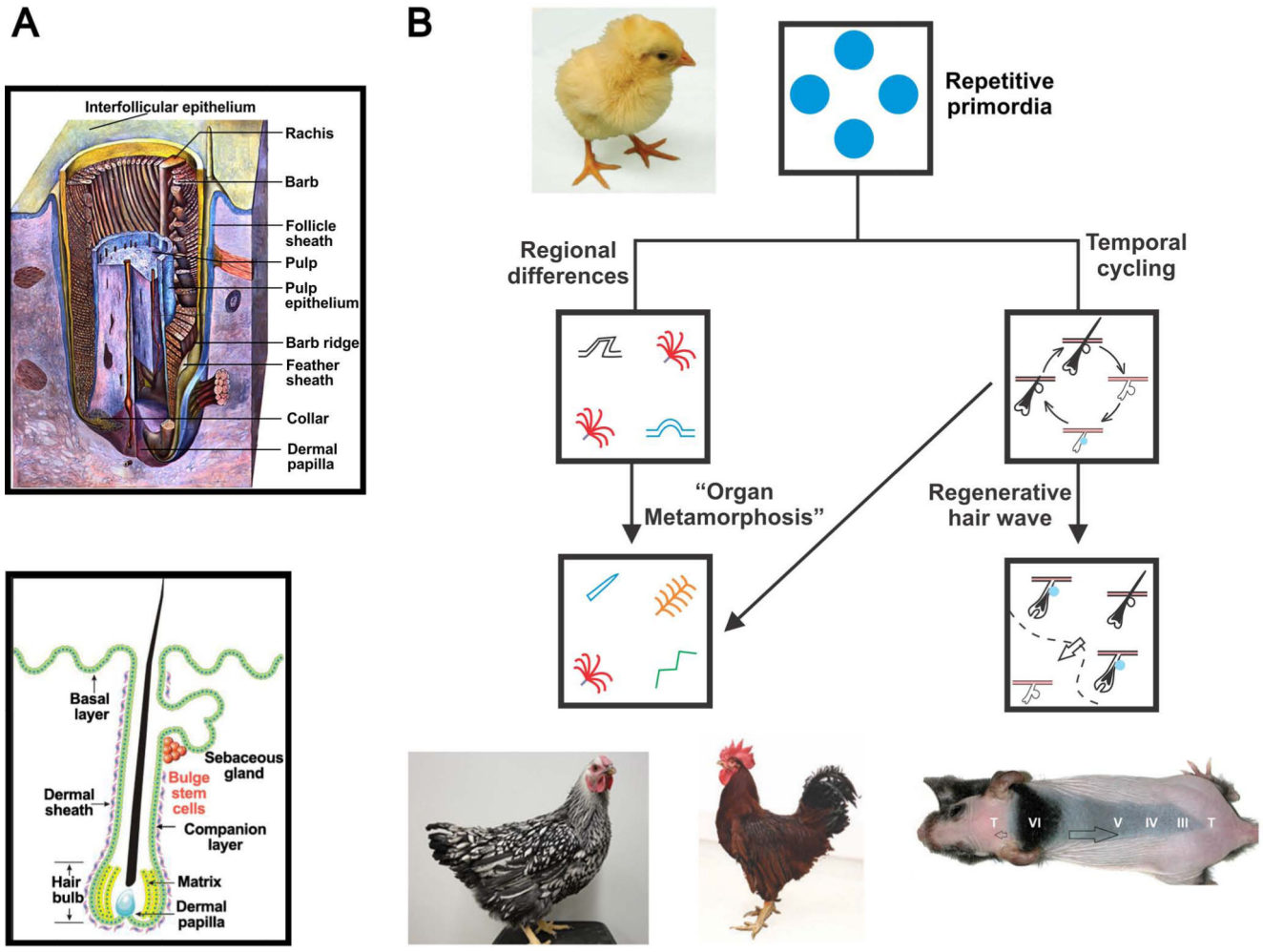

Fig. 2. Diagram depicting variations in spatial and temporal patterns of integument organs

A: Schematic drawing showing the growth phase of the feather follicle (Lucas and Stettenheim. 1972) and hair follicle (Chen and Chuong. 2012). Note in both follicles, they undergo regenerative cycling through growing, resting and molting phases, thus giving the follicle an opportunity for phenotype renewal. **B:** For integument patterning first the integument primordia form regularly spaced arrays over the morphogenetic field. Different phenotypes are generated through a process akin to metamorphosis that occurs at the organ level. Individually they undergo temporal cycling and as a population they can form a regenerative wave. Newly hatched chicks have most parts of the body covered by feathers of similar morphology; whereas adult chickens show regional specificity of feather morphology. The figure is modified from (Wu, et al. 2004a; Chuong, et al. 2013). Photo credit: female Silver Laced Wyandotte by Doug and Pete Akers; male Silver Spangled Hamburg by Jim Legendre.

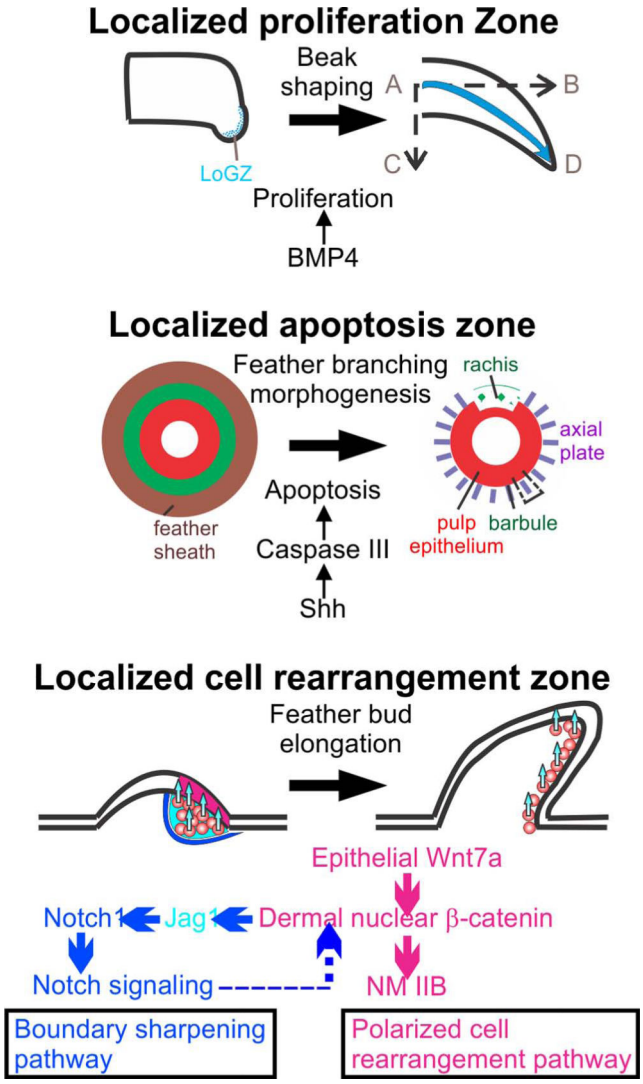

Fig. 3. Combination of localized cell activity zones can lead to organ shaping

A: Position and number of localized cell proliferation zones modulate the shape of avian beaks; **B:** Cell apoptosis zones induced by Shh signaling give rise to periodically branched barbs; **C:** Posteriorly localized cell rearrangement zones inaugurate the directional elongation of feather buds. The three panels are modified from Wu et al (Chang, et al. 2004a; Wu, et al. 2004b; Li, et al. 2013a), respectively.

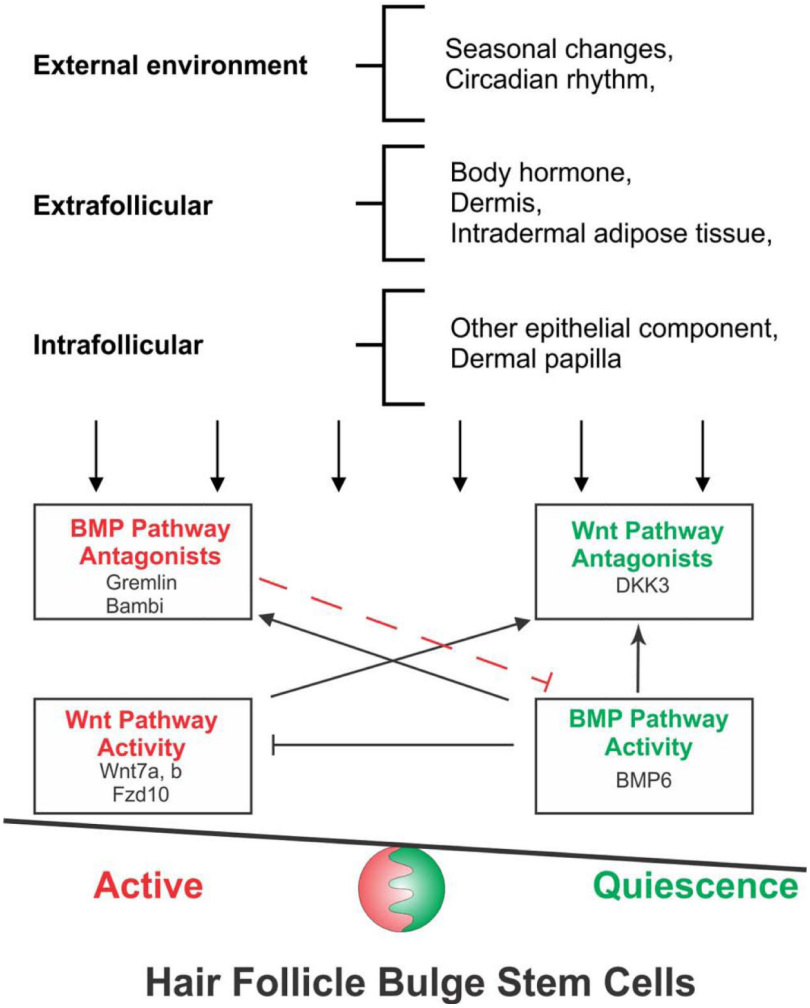

Fig. 4. Hierarchical regulation of stem cell activation and quiescence

Hair follicle bulge stem cells are in a quiescent (green) or active (red) state via balanced molecular signaling (mainly Wnt and BMP) influenced by the external environment, extrafollicular and intrafollicular factors.

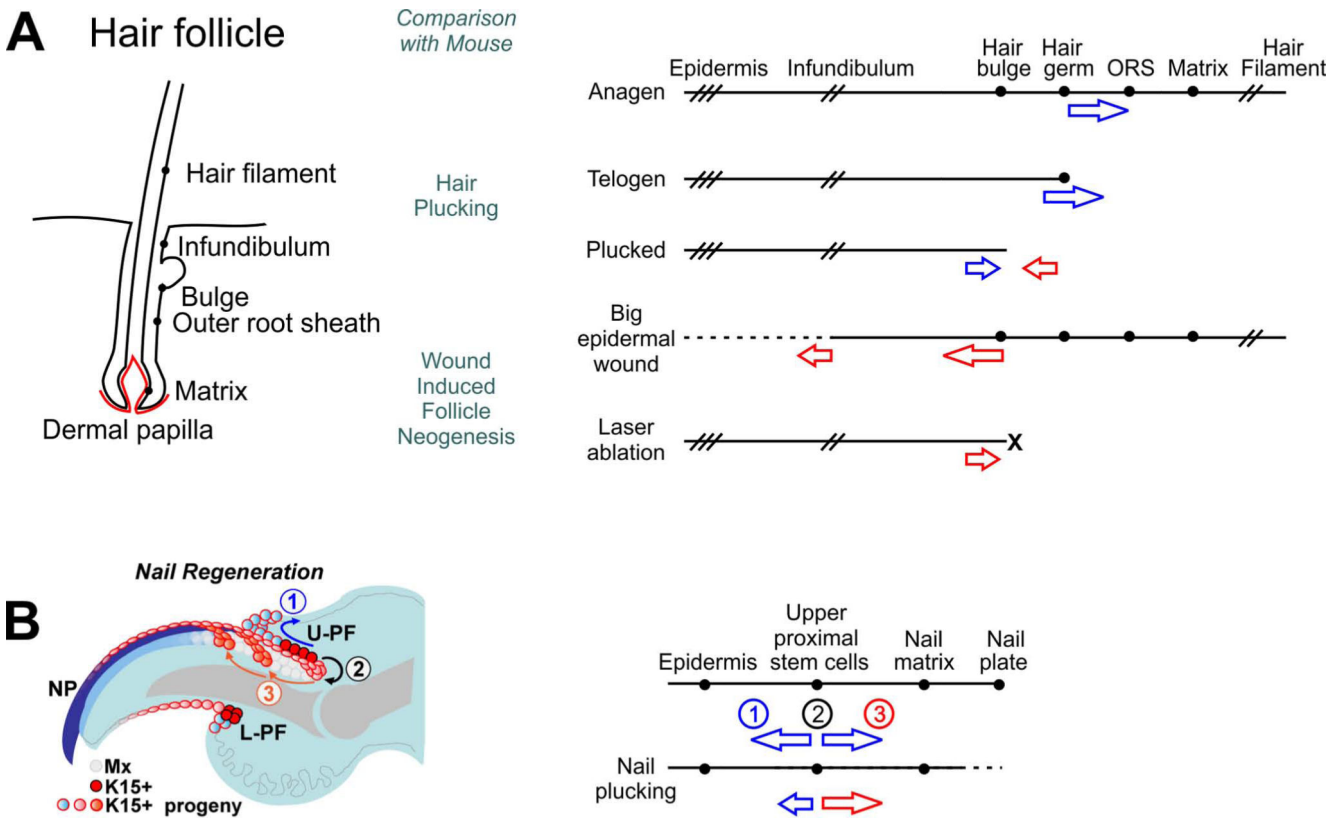

Fig. 5. Flow of stem cells can change in physiological growth and after injury, implying the concept of "River of Stem Cells" (Chuong and Widelitz, 2009)

Hair and nail are used as examples. **A:** Schematic drawing of hair follicles showing cellular flow under physiological conditions (anagen and telogen), after plucking, after the formation of a large wound and laser ablation. **B:** Schematic drawing of cell flow in the nail during growth and regeneration. Blue arrows: cell flow during growth; red arrows: cell flow during regeneration. Flow ① is to peri-nail epidermis. Flow ② is for self-renewal of nail LRCs. Flow ③ is to the nail matrix. Panel B is modified from (Leung, et al. 2014).