Module Based Complexity Formation: Periodic Patterning in Feathers and Hairs

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Abstract

Patterns describe order which emerges from homogeneity. Complex patterns on the integument are striking because of their visibility throughout an organism's lifespan. Periodic patterning is an effective design because the ensemble of hair or feather follicles (modules) allows the generation of complexity, including regional variations and cyclic regeneration, giving the skin appendages a new lease on life. Spatial patterns include the arrangements of feathers and hairs in specified number, size, and spacing. We explore how a field of equivalent progenitor cells can generate periodically arranged modules based on genetic information, physical-chemical rules and developmental timing. Reconstitution experiments suggest a competitive equilibrium regulated by activators / inhibitors involving Turing reaction-diffusion. Temporal patterns result from oscillating stem cell activities within each module (micro-environment regulation), reflected as growth (anagen) and resting (telogen) phases during the cycling of feather and hair follicles. Stimulating modules with activators initiates the spread of regenerative hair waves, while global inhibitors outside each module (macro-environment) prevent this. Different wave patterns can be simulated by Cellular Automata principles. Hormonal status and seasonal changes can modulate appendage phenotypes, leading to "organ metamorphosis", with multiple ectodermal organ phenotypes generated from the same precursors. We discuss potential evolutionary novel steps using this module based complexity in several amniote integument organs, exemplified by the spectacular peacock feather pattern. We thus explore the application of the acquired knowledge of patterning in tissue engineering. New hair follicles can be generated after wounding. Hairs and feathers can be reconstituted through self-organization of dissociated progenitor cells.

Introduction

Patterns describe order which emerges from homogeneity. They may appear in spatial arrangement or temporal sequence. They can be repetitive elements which are identical or with variations. Patterns exist in the physical world as well as in living systems. Biological pattern formation is a physical process that was adopted by biological systems during early times in evolution (Fig. 1A). With increasingly complex life forms, periodic patterns became more prevalent, helping organisms adapt to diverse environmental conditions[1]. Among the myriad of biological contexts in which periodic patterns are found, the ectodermal organs are probably the most conspicuous and intriguing. They often display diverse and delicate patterns that are robust enough to withstand the wear and tear organisms are subject to in their daily interactions with their external environmental. Thus there is significant selection pressure for ectodermal organs to regenerate and to be adaptive. Also, many ectodermal organs are found on the body surface and therefore are most visible. From teeth, to scales, feathers, hairs and pigmentation patterns, these spectacular examples of morphogenesis have long fascinated biologists.

Patterns can emerge both spatially and temporally (Fig. 2A). Patterns that are spatially repetitive have several advantages[2]. First, having a large number of patterning units allows

damaged units to be repaired or replaced without sacrificing overall function[3]. Second, a population of patterning units can acquire emerging properties that are not easily achieved by a singular unit. For example, numerous hairs form a coat that traps air for endothermy and the array of feathers on a male bird can form a stunning visual pattern to attract a mate. Third, it allows units in different body regions to generate different skin appendage phenotypes which can then be selected to best fit the possible functional needs of that organism. The same set of patterning units can be used to generate distinct phenotypes by changing the characteristic of single units or the rules by which these units interact without requiring an overall redesign. For example, a bird grows flight feathers on the wing but downy feathers on its belly. This results from a combination of periodic patterning and regional specificity (Fig. 2B).

There are also advantages to having patterns that are temporally repetitive, i.e., cycling. Instead of continuous renewal, skin appendages undergo repetitive renewal, and in each cycle they regenerate most of the organ. Cyclic molting and regeneration provide the opportunity to discard worn or injured appendages and remake a fully functional new organ[4]. Cycling also enables the coupling of regeneration among adjacent follicles, making a higher level meta-patterning (or an organization of patterns) possible in a hair follicle population. For example, the regenerative hair wave results from coupling the activation of stem cells in adjacent single hair follicles to become a collective regenerative behavior that spreads over the hair population[5, 6]. Finally, the regeneration of skin appendages gives the organism an opportunity to make new organ phenotypes that meet the needs of changes in the seasons or at different life stages[7]. These advantages would not be possible without repetitive patterning that allows one ectodermal organ (skin appendage) to become a population of repetitive organs.

1. HOW DO PERIODIC PATTERNS FORM?

How repetitive patterns form at the cellular and molecular level is of major interest to developmental biologists. In different animals, repetitive patterns may be generated by different mechanisms. In regard to how information is given during pattern formation, in general there are two modes: *self-organizing* and *instructional*. In the first mode, physical-chemical rules of interaction dictate how cells can self-organize into periodic patterns[6, 8]. The molecular clock mechanism in establishing somite segmentation belongs to this category as well[9]. In the second mode, changes in patterns are effected according to a specific blueprint. The work in the segmentation of Drosophila larvae led many to think that even vertebrate cells were designated with a molecular zip code system directing cells to form patterns. However, if a blueprint is needed, this mode will require a large volume of information. In scenarios where precise placement of repetitive patterns are required, a blue print model offers higher fidelity but self-organization is a more economical design requiring less DNA information.

To explain pattern formation, Alan Turing proposed a reaction-diffusion model. According to this model, periodic patterns can arise from a homogeneous chemical state through interaction between a short range activator and a long range inhibitor (Fig. 1A)[10]. The activator is autocatalytic (therefore self-amplifying) and can induce the production of the inhibitor. The inhibitor has a longer-range of diffusion and suppresses the activator. The short-range positive feedback enables a system to deviate from the initial homogenous state through minor instabilities, while the long-range negative feedback ensures spacing between activator peaks. These rules are first demonstrated in non-biological systems, most notably chemical reactions[11]. Theoretical biologists have modified aspects of this model for application to biological patterning and its implication was broadened by the use of an activator / inhibitor concept[3], among them hair germ formation[3, 12] and feather barb branching[13, 14]. Most recently, Kondo has developed simulation software, which enables

investigators to alter parameters for the RD model to generate many two-dimensional patterns observed in real biological systems, including pigment patterns found on seashells and the skin of the popper fish. More strikingly, the dynamics that are described by mathematical models can be clearly visualized on the skin of zebrafish where pigmentation patterns are locally ablated by a laser and allowed to reform[15].

Some researchers address the pattern formation problem by invoking the concept of a pre-pattern. For example, through differential adhesion, cells can sort themselves and form organized cell aggregates with patterns[16]. While this led to the study of a state before patterns are clear, it did not really solve the problem because it starts with cells with inherently different adhesivity. To go after the origin of pattern formation, we must start from the ground state in which all progenitor cells are equivalent.

2. PERIODIC PATTERNING IN ECTODERMAL ORGANS

2.A. Repetitive patterning during feather morphogenesis

Periodic patterning of feather germs: Avian skin is divided into tracts. Thousands of feathers develop across the avian skin. Within each tract, feathers have similar attributes but may vary and form a gradient of size and shape[17]. Each developing feather germ within a tract is equidistant from its neighbors, forming a periodic array with each feather germ surrounded by a hexagon of neighboring germs[18]. This is seen clearly in the spinal tract. Because of the exquisite pattern and sequential formation, this process has led scientists to propose models that the formation of new lateral row buds used the prior medial row formed earlier as a template or mechanical cue[19]. While the exquisite hexagonal pattern appears to be similar, the exact pattern from one embryo to another is not identical (Fig. 1B), implying that pattern formation is based on stochastic, self-organizing processes rather than a pre-patterned blueprint.

We take this opportunity to clarify some terminology. While functionally the skin is considered as one organ, each hair or feather follicle has also been considered as a "mini-organ" because architecturally it is self-sufficient and separated by inter-follicle spacing. Along this line, the primordium of each feather is called a feather germ when it is flat and a feather bud when it protrudes out of the skin surface.

Restrictive versus de novo molecular expression patterns: We searched for molecules that appear early in the periodic patterning process. In the early developmental stages of skin, prior to feather morphogenesis, certain molecules were expressed at moderate levels throughout the epithelium of the skin (Fig. 3E). Their expression was then up-regulated in regions destined to become feather germs and down-regulated in regions that would become inter-germ. We called this a restrictive expression pattern. Examples of genes that show a restrictive expression pattern include Wnt7a, β-catenin, L-fringe, NCAM, etc[20–23]. We also saw some genes which showed the reciprocal expression pattern. They are also expressed at a median level all over, but then become increased in the inter-germ and decreased in the bud epithelium. Gremlin[24, 25] and Wnt11[26] showed this type of restrictive expression pattern. Genes showing the restrictive pattern may be involved in patterning the placement of feather germs and may also be involved in stabilizing the feather bud – interbud border later.

Some genes were not present at the earliest stages of feather germ formation but were induced after the feather germs form. We call this pattern the de novo mode of expression. Genes which showed the de novo mode of expression include sonic hedgehog, Msx2, etc[23, 27–29]. These genes are involved in stabilizing the boundary of feather germs or later stages of feather bud morphogenesis, but not the initial setting of periodic patterning.

The dynamics of the patterning process from the homogeneous field is best visualized by the expression of the beta-catenin transcript (Fig. 3A). Feather germs begin to form along the midline of the skin and subsequent rows are laid out progressively bilaterally over time. The pattern that forms shows that each feather germ is surrounded by a hexagonal array of neighboring feather germs. As a feather germ (about 200 μm in diameter, with stronger beta-catenin staining) emerges from the homogeneous field, it creates a halo that is negative for beta-catenin (Fig. 3B, B', C). The inhibitory zone, about 200 μm in diameter, is concentric with the feather germ, suggesting that it receives molecular cues from the newly formed feather germ. Most interestingly, in the nascent buds, the feather germ is in direct contact with the inhibitory zone of the previous germ. Then it creates its own inhibitory zone and two feather germs are pushed apart with an inhibitory zone equivalent to the width of the initial feather bud plus that of its neighbor (compare bud 2, 3 in Fig. 3B, B', C). A similar restrictive mode during bud emergence with the creation of a lateral inhibitory zone can be seen in immunostaining for pERK (Fig. 3D;[30]). The ectodysplasin (eda) receptor (edar) is expressed downstream of beta-catenin[31]. Experimental mis-expression of Eda activity can alter the size of feather buds but not interfere with the periodic patterning process itself[32].

Molecules involved in periodic patterning of feather germs in chicken skin: The formation of feather germs can be explained by the balance of activators and inhibitors. Molecules such as Fibroblast Growth Factors (FGFs)[33, 34][35] and Bone Morphogenetic Proteins (BMPs) play a role[37–39] in establishing the periodic patterning of feathers. Their specific role can vary in different stages of embryogenesis as well as in different skin regions. In the very early stage when feather tracts are forming, inhibition of BMP sigaling in the mid-ventral apterium can induce ectopic tracts[39], but BMP-4 coated beads were also reported to induce additional tracts in the dorsal-scapular semi-apterium[40]. Once the tracts form, BMP is shown to inhibit periodic patterning of individual buds[37, 38]. Addition of FGFs in the early stage of patterning can lead to the formation of many small-size feather buds. On the other hand, addition of FGF at later stage leads to the formation of enlarged feather buds[34]. Once the initial feather germ pattern is set, each germ initiates a lateral inhibitory zone mediated by delta 1[41] and a bud tip growth zone mediated by Shh[27, 42].

More recently, a mutation leading to the up-regulation of BMP12 in the neck of the naked neck chicken was also found to function as an inhibitor of feather formation. Furthermore, retinoic acid expression in the neck skin sensitized this region to the inhibitory effects of BMP12[43]. However, BMPs do not always serve the role of an inhibitor. BMP7 which signals through the activin rather than BMP receptors[44, 45] can stimulate feather germ formation[46]. BMPs were also reported to serve as activators of feather formation in ventral skin[40, 47]. This may be due to different functions of BMP in a different context.

Thus tilting the balance of activators and inhibitors can alter the induction of feather tracts or modulate the number and size of feather germs that can form from a homogeneous tract field. The activity of known molecular signalling pathways is context dependent, depending on the timing and location within skin regions. However, the result is consistent with a reaction-diffusion mechanism for feather germ formation[38]. The function of activator – inhibitor pairs was further tested in vitro using reconstituted dorsal skin explant cultures[20]. Here, the mesenchyme is separated from the epithelium. The mesenchyme is then dissociated into a single cell suspension before plating at high cell density. The intact epithelium is then layered on top of the mesenchyme and the reconstituted explant is cultured. In this experimental model, all molecular signalling disappears and the cells are reset to a primordial state where each cell is equivalent. Cells randomly migrate in and out of the presumptive feather primordia[20, 48] in a competitive equilibrium until stable cell aggregates form. Surprisingly all new buds appeared synchronously under these

experimental conditions, thus uncoupling the periodic patterning process from the sequential bud forming process. In another study, feather germs were found to disappear from intact stage 30 chicken embryo skin explants after 12 hours in culture and reformed 6 hours later[49]. This interesting observation suggests parallel events occur in intact and reconstituted skin explants cultures.

In this in vitro system, one can vary parameters and evaluate what controls the number and size of feather germs. Under these conditions the size of feather germs is constant and the number of feather germs is determined by the mesenchymal cell number. Below a certain threshold of mesenchymal cell number, no feather germs form. Above the threshold, feather buds start to appear randomly. The final hexagonal pattern is the result of highest packaging, not pre-patterned codes (Fig. 3F). To increase the size, one has to alter the activator / inhibitor ratio. Increasing the levels of BMP receptor within the mesenchymal cells reduced the size of feather primordia, while suppressing BMP expression with exogenous Noggin increased feather bud size[20].

The molecular gradient itself is not sufficient to establish periodically arranged feather primordia. Cells need to be reorganized in response to chemo-attractant and chemo-repulsive signals[30]. Treating developing chicken skin with U0126, a MAPK inhibitor, caused individual buds to merge, thus changing spots into stripes. This occurred in a dose- and stage-dependent manner[30]. Thus, by modifying downstream signalling from the FGF receptor we were able to change the shape as well as the orientation of feather primordia.

The importance of periodic patterning can also be appreciated in scaleless chickens. In this mutant, the beta-catenin expressing tract field forms normally, however, periodic patterning does not occur (Fig. 3A"). Eventually, feathers fail to form. FGF coated beads are able to rescue this process[35, 41], implying the restoration of activator activity can again elicit this periodic patterning process.

Periodic patterning of feather branches: Periodic patterning is again invoked later in feather development during the process of branching morphogenesis. Branching morphogenesis allows for remarkable diversity in feather structures (Sidebar 1). Similar activator / inhibitor principles may be involved in determining the number of barb ridges. Shh and BMP may work as activators and inhibitors (Fig. 5,[51, 52]), while a Wnt 3a gradient converts the radial symmetrically arranged barb ridges into bilateral symmetry[53]. This is another example of periodic pattern formation process.

2.B. Repetitive patterning during hair morphogenesis—In earlier theoretical works, Nagorcka showed how reaction-diffusion can account for many aspects of hair pattern formation[13]. However, it was not until recently that supporting experimental results started to emerge. During embryogenesis and early post-natal life, the succession of three inductive waves leads to the development of three types of hairs (guard, awl, and zigzag). During hair formation, the factors that promote hair follicle fate include Fgf, Wnt and Eda signals while inhibitory factors include Bmp and Dkk[60]. The involvement of dynamic interaction between activators and inhibitors in establishing hair patterns during the successive inductive waves are not immediately obvious but can be clearly demonstrated through computer simulation using an R-D based model. Sick and colleagues used a transgenic mouse line that expresses excess Dkk2, a Wnt inhibitor, in the epithelium after the first hair inductive wave to test the proposed involvement of the R-D mechanism in hair patterning. This transgenic mouse line revealed patterns predicted by computer simulation using a Wnt-Dkk-specific reaction-diffusion model[61]: 1) In addition to blocking the formation of new hair follicles, the excess inhibitor invokes ring-like zones of high activator activity around follicles established during the first induction wave. 2) Given that the pre-

existing follicles are insensitive to the activator and inhibitor, this ring-like zone is converted to discreet new follicles surrounding the pre-existing one, thus forming hair clusters. The authors further showed that the ring-like zone of high activator activity is not caused by direct activation of Wnt by Dkk2 in the absence of Krm2, a concern raised by Stark et al.[62], because Krm1 is expressed in the epithelium and the Foxn1-Dkk1 mouse exhibited similar phenotypes as the Foxn1-Dkk2 mouse[63]. These results provide compelling evidence that Wnt-Dkk work as the activator-inhibitor pair in an R-D scheme to set up the inter-follicular space during hair development.

Another piece of evidence comes from analyzing the effect of excess activator. A transgenic mouse line carrying stabilized β-catenin in the basal layer of the epithelium demonstrated precocious follicle formation and an increased number of hair placodes[64, 6564, 65]. Most importantly, excess β-catenin leads to elevated mesenchymal Wnt activity, as well as increased BMP2, BMP4 and Dkk1 levels in hair placodes. This is compatible with autocatalysis of the activator and induction of inhibitors by activators that are described in the reaction-diffusion mechanism. Sostdc1, a soluble inhibitor of BMP and modulator of Wnt activity, is also increased in the mutant mouse. The authors proposed a model in which the interplay between Wnts and BMPs are involved in establishing the spacing between hair follicles: epithelial Wnt signal induces mesenchymal Wnt activity. Together, they activate BMPs, which suppress nearby cells through Lef1 and Edar. The boundaries of placodes are further strengthened by the BMP antagonist activity of Sostdc1.

In addition to spacing between hair follicles, hair patterning also involves aligning the hair bulb and shaft so as to achieve the maximal covering efficiency. The collective orientation of the hair follicle is one example of planar cell polarity. It was shown that prenatally, there is a Frizzled 6-dependent event to setup the global hair follicle orientation and postnatally, there is a Frizzled 6-independent mechanism to align neighboring follicles to achieve a more fine-tuned hair pattern[66].

Together, these experiments addressed the interaction between activator and inhibitor in an R-D scheme. Proving other features of an R-D mechanism, including the autocatalysis of activator, differential diffusion of activator and inhibitor still remain a challenge to experimental biologists[67, 68]. It is not surprising that redundancy and extra layers of control mechanisms are built into this putative feedback network. Multiple Wnts, BMPs, DKKs, as well as antagonists of BMPs and DKKs are implicated in this patterning process.

3. EVOLUTION OF COMPLEX INTEGUMENT PATTERNS

3.A. Cyclic temporal patterns: regenerative hair wave in adult animals—The
hair follicle undergoes a cyclic degeneration and regeneration cycle throughout life. The length of growing (anagen) and resting phase (telogen) can determine hair length. A long resting phase can lead to shorter hairs or no hair, i.e., alopecia. We have developed ways to visualize hair stem cell activation over the entire skin in living mice by shaving the hair and examining the initiation of pigmentation as follicles enter anagen. We found cyclic BMP signalling from the subcutaneous adipose layer regulates stem cell activation during hair regeneration (Fig. 4B,[5]. More molecular analyses showed Wnts serve as activators. These studies revealed that hair growth patterns can be governed by simple rules based on a pair of activator/inhibitor signals. Regeneration in a population of hair follicles spreads like a chain reaction, forming diverse wave patterns[6]. Mathematical modelling reveals the self-organizing and stochastic nature of this behavior. It emerges at the population level (Fig. 4A), allowing hair regeneration to become a very adaptable trait. These variations are seen among different animal species that have different needs for hair: robust spreading in rabbits, gradual wave-like spreading in mice, and random growth with loss of coupling

among follicles in human skin (Fig. 4D). The hair wave can also vary under different physiological conditions of the same individual, such as in puberty, pregnancy and aging.

Cellular automata models have been used to explain many biological phenomena (Fig. 4C)[69, 70]. In a general cellular automata model, the field is divided into a number of discrete "cells", which evolve through a number of time steps, according to a set of rules based on the states of neighboring "cells". Each "cell" of the model corresponds to an area of the pattern field and information on this area is stored as the "state" of the cell. To simulate the regenerative hair wave, principles of cellular automata are used[6]. The self-organizing pattern is based on simple rules and probability, so wave patterns under similar activator / inhibitor conditions are similar but non-identical. The spectrum of regenerative hair wave patterns in human (less coupling), mouse, and rabbit (highly coupled) in the space of single follicle cycling (X axis) and follicle coupling (Y axis) can be seen in Fig. 4D.

Change in light/dark cycles produced by the seasonal lengthening and shortening of days or changes in temperature can alter the type of skin appendages. In nature, changes in the length of the light period are translated into changes in the plasma melatonin and prolactin levels which can trigger animals to produce a longer/shorter or whiter/darker coat to improve their chances for survival during a given season[71]. Now that we know that human and rodent skin and hair follicles are extra-pituitary sites of melatonin synthesis[72–74], one wonders to what extent environmental cues (such as the length of the light period) can also affect seasonal changes in skin and skin appendage patterns. A recent work shows hair regeneration is delayed after plucking in mice with a mutation in bmal1, a molecule involved in circadian rhythms[75].

3.B. Organ "metamorphosis": new skin appendage phenotypes in adult animals—The temporal cycling of hair and feather follicles allows each follicle to molt and regenerate a new appendage and also provides an opportunity to alter the appendage phenotype to adapt to seasonal needs or changes in life stages under physiological conditions[7]. Baby chicks have downy feathers with a similar appearance all over their bodies which mainly function in maintaining warmth and sometimes for maternal bonding. As the bird grows, flight feathers emerge so the growing chicks can leave the nest. Upon puberty, sexual dimorphism becomes apparent[76]. In crown, tail, saddle and many body regions, feathers grow into specific shapes and lengths for communication purposes: to attract birds of the opposite sex or to scare away birds of the same sex. Some feathers also change their pigmentation patterns.

"Metamorphosis" is a change in the phenotype of an individual that gives the organism a new lease on life to adapt to different environments. Since their genotype is unchanged, mechanisms regulating this expression must be epigenetic. The most dramatic example of metamorphosis is from the aquatic tadpole to a terrestrial frog. In the skin, the small radial symmetric downy feathers in chicks can be replaced by feathers with bilateral symmetry, sometimes growing up to a meter in length, as in the adult peacock (Fig. 2B). The pigmentation of these new feathers can also be highly ornamental compared to their predecessors. These differences take place despite the fact that the cells share the same genome and are produced from the same follicle. What might control this difference in gene expression? We presume that macro-environmental changes have altered the follicle micro-environment resulting in altered gene expression. In this way the follicle stem cells are modulated to form different types of ectodermal organ phenotypes. We see parallels between morphogenetic changes which take place at the organ and organism levels and name the former "organ metamorphosis". The repetitive nature of ectodermal organs which occurs in space and time enables these changes to take place (Fig. 2A). Spatial redundancy allows some appendage follicles to rest while the general function at the organism level is

continued by other follicles. The temporal cycling allows a follicle to re-invent itself with a mechanism that we still do not completely understand – but is likely to involve some hormonal regulation and epigenetic changes.

An obvious example of organ metamorphosis occurs during postnatal chicken development[17]. Similar changes can also be seen in mammals. In the human, hair follicles in beard, axilla and genital regions are transformed from vellus to terminal hairs during puberty[7, 77]. In aging, the reverse tends to occur in the scalp of some males, leading to androgenic alopecia. The mechanism controlling how scalp and occipital hairs respond to sex hormones is still unknown but appears to be mediated by differences in dermal papillae which exhibit a varied response to stimulation with androgens or estrogens[78–80].

Sexual dimorphism in many mammalian and avian species can be striking. Such differences are generated by the combinatorial use of principles described above (Fig. 2A). During organ regeneration, new phenotypes can be made from the same follicle. Androgenic and estrogenic hormones can then modulate skin appendage stem cells to different modes of morphogenesis and growth. Since these changes are region-specific, the process must also rely on region-specific mechanisms. Thus the repetitive nature of ectodermal organs sets up the modular basis of morphogenesis that allows temporally and spatially complex integument patterns to form. These, in turn, can help animals to be better suited to their environment.

Peacock feathers illustrate the most extraordinary example of biological pattern formation (Fig. 2B). The complex pattern is built through several layers of patterning processes. The downy plumage of a young male peacock chick undergoes molting at sexual maturity. At this time, a much larger feather grows from the same follicle. The nearly concentric pigment pattern on each feather is likely set up by molecular gradients. Within a feather tract, each feather is positioned with regular spacing from its neighbors and grows to a specific length. In other body regions, there are fan shape feathers that show both chemical and physical colors. Further, these processes do not occur in females, whose feathers are short and brownish. The complex pattern here is made possible by the use of modular principles.

3.C. Evolution of complex integument patterns—How do these patterns evolve? We can track these changes back to the origin of feathers at the time of dinosaurs[2, 50, 82]. The Jehol Biota (120–145 million years ago, spread across Northern China) provides some clues (Fig. 5a). *Sinosauropteryx* had fuzzy fibers with a short shaft and primitive barbs covering its body. These primitive skin appendages appear to be the same all over the body withoutregional differences (Fig. 5b). The branches may have helped to trap air and prevent heat loss in this possibly endothermic creature (Sidebar 1).

Caudipteryx exhibits bilateral symmetric feathers (Fig. 5d) with well formed feather vanes (pennaceous feathers). Furthermore, regional differences where feathers in the body, forelimb and tail are different, imply functional specialization. This variety of feather phenotypes helps to warm the body while providing communications through its tail and forelimbs. However, the bilaterally symmetric nature of its feathers suggests that it could not fly.

Microraptor gui hadboth fore- and hind-limbs covered with pennaceous feathers (Fig. 5e). Feathers at the distal limb positions had asymmetric vanes, suggesting effective aerodynamic function. Those studying it proposed that it moved by gliding.

Longirostravis (Fig. 5g)[83] represents early wading birds. It had feathers with an asymmetric vane in the lateral wing, implying that it was a reasonably good flyer. It also shows specialization of its crown feathers.

Thus a gradual evolution of complex feather patterns from dinosaurs to birds seems to have occurred. First, the primitive branching in *Sinosauropteryx* was probably selected because effectively provided endothermy. A follicle structure suggests that it was subject to cyclic renewal. Later in evolution, *Caudipteryx* showed hierarchical branching (rachis, barb and barbule structures), the formation of open vanes and clear regional differences in morphology. Sexual dimorphism is observed in *Confucisornis*. Thus, the repetitive arrangement and cyclic regenerative nature of integument appendages provide the modules that can be altered in different body regions at different physiological stages to allow an organism to evolve complex integument appendage patterns, which are then selected over evolutionary time to increase survivability in its environment.

4. ENGINEERING OF THE PERIODIC PATTERNING PROCESS

Periodic pattern formation used to be considered as a basic science issue. We now have learned that the ability to initiate this process is also essential for the regeneration of new hairs. First, we ought to differentiate between two major categories of hair regeneration. One is the regeneration of the hair shaft from existing hair follicles. This is the behavior described in the regenerative hair wave section, in which the total number of modules (hair follicles) does not change, but the cyclic growth / resting states change. A Cellular Automata model is effective in describing this behavior and can also suggest parameters that might lead to androgenic alopecia.

The other kind is the regeneration of new modules (new hair follicles). Here reaction-diffusion is involved as described in the periodic patterning session. This, of course, is much more challenging, as it is equivalent to making an organ de novo. However, by opening a large wound (> 1 cm diameter after wound contraction) in the back of a mouse, remarkably, new hair germs formed[84]. Interesting, the new hairs formed in the center of the wound, about 300 μm away from the wound margin. Repair and regenerative processes may be in competition. A repair response is favored near the wound margin, while cells in the wound center are free to regenerate[85]. Once the regenerative response is elicited, cells self-organize into periodically arranged hair germs, without a need to implant individual hair follicles as seen in the practice of hair transplantation, or the use of pre-patterned tissue scaffolds. In patients with severe wounds (such as burns), no hairs or other skin appendages are able to regenerate. It remains to be seen whether such a phenomenon can occur in humans with proper management.

To learn how to generate new skin appendage germs for the purpose of regenerative medicine, we need to reconstitute them from dissociated single cells. We have previously done so for feather germs[20]; Fig. 6A). Recently, we used a similar process to generate reconstituted new hairs[86]. While earlier work showed new hair formation, the process can be laborious[87] or may frequently form hair cysts[88]. By mixing dissociated epithelial and mesenchymal cells and allowing them to interact on a flat 3D matrix on the back of a SCID mouse, the reconstituted skin is able to grow hairs that are evenly spaced on a plane and point outwards (Fig. 6B, C).

It is important that the number, size and spacing of skin appendage germs are properly regulated. By applying what we learned in periodic pattern formation, we can modulate this process for different applications. For example, with 10 million cells competent to form hairs, we should be able to guide them to form 10 big hairs or 1000 small hairs, depending on the needs of the patients.

Conclusion

The periodic patterning process allows repetitive elements to form with even spacing. Each element then becomes a module that can be modified differently in different regions, forming the basis of region specific skin appendages. Stem cells allow for the cyclic molting and regeneration of each module, forming the basis of physiological regeneration. The combination of these processes and regulation by body hormones make "organ metamorphosis" possible.

We can appreciate that periodic patterning is an efficient design principle which has emerged early in evolution to allow functional redundancy and phenotypic variation. The "module" principle is like using Legos to build different structures, and making the exchange of modular units possible. While many of the regulatory mechanisms remain to be investigated, skin appendage modules serve as a Rosetta stone for us to understand the fundamental principles of patterning morphogenesis.

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Basics structure of feathers

Branching morphogenesis allows for remarkable diversity in feather structures. Feathers develop within tracts on the body surface. Each feather within a tract has common features, although they may differ in size and color; often forming a gradient. For example, ventral body feathers have a larger plumulaceous component, while wing feathers have a larger pennaceous component. The ventral body feathers are symmetric in that the length of the barbs on either side of the central feather backbone (rachis) are of equal length; whereas, flight feathers are bilaterally asymmetric and produce greater lift and aerodynamic stability. Other tracts, such as the tail feathers (rectrices) are used for both flight stability and communication and have different attributes when compared to the ventral body or flight feathers.

Branching can produce radially symmetric downy feathers which provide warmth to young birds. Later in development the downy feathers molt and are replaced by more complex bilaterally symmetric juvenal feathers and then they are replaced later in development by adult feathers. Even in adult feathers, the base often has a downy (plumulaceous) component that continues to provide warmth. A second function that feathers can provide is communication. This is produced by the shape, movement and sexually dimorphic coloring of the feathers and may be used to scare adversaries or to attract a mate. Third, the feathers enable flight which provides access to a totally new environment, the sky. In flight feathers (remiges), neighboring barbs are linked together by barbules. This produces pennaceous wing feathers which can move the air and produce an aerodynamic surface.

Further Reading/Resources

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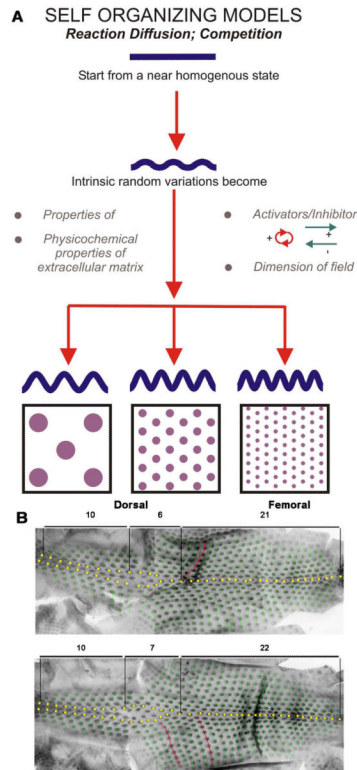
Figure 1.
A. Homogeneously distributed cells through random interactions form unstable aggregates. This forms random variations which are amplified above a threshold at which the patterns become set. Distinct patterns are formed by competition between intrinsic factors (properties of the membranes and extracellular matrix), concentrations of activators and inhibitors and the size of the primordia field. From Jiang et al., 2004. B. Feathers from 2 similarly aged chicken embryos demonstrate stochasticity in the placement of feather buds. The feathers show a similar yet non-identical pattern. Numbers of feathers in each region are indicated. Yellow dots highlight feathers along the midline. Green dots highlight subsequent feather buds. Red dots show the relative position of sequential feather buds.
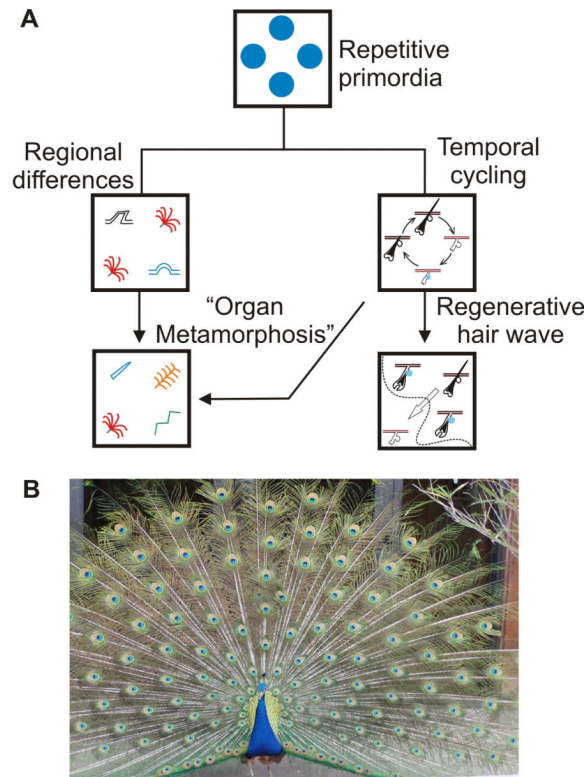
Figure 2.
A. Diagram depicting how repetitive primordia in different regions can serve as "modules" and assume different characteristics. Through a process akin to metamorphosis that occurs at the organ level they can develop into different ectodermal organs. Individually they undergo temporal cycling and as a population can form a regenerative wave. B. A peacock shows the stunning complex skin appendage pattern occurring in feather size, shape, arrangement, and pigmentation. These are also sexually dimorphic. Feather patterning uses all the module variation principles discussed here.
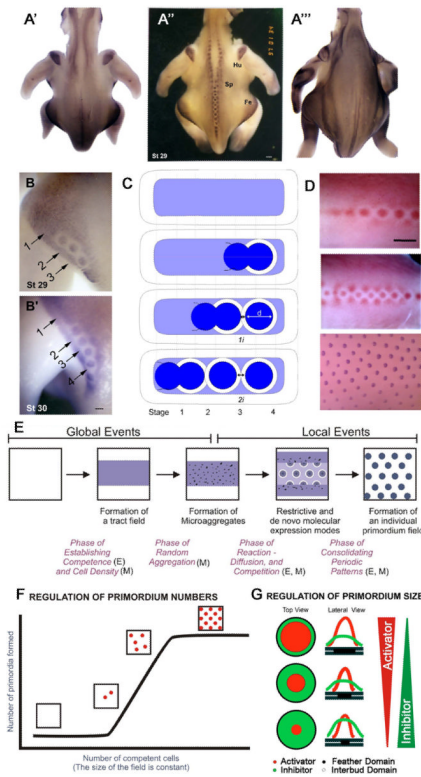
Figure 3.
Cellular and molecular events during periodic formation of feather primordia. A-A'''. In situ hybridization for β-catenin demonstrates a restrictive expression pattern. A''. In normal chicken skin development there is moderate staining in each of the feather tracts. As feather buds form, staining increases in the buds and decreases in the surrounding interbud region. The pattern appears normal in scaleless chickens at stage 29 (A') but fails to progress in a restrictive manner at stage 30 (A''). B. In the femoral tract, the β-catenin-free spacing between buds increases over time. C. Schematic of increasing feather bud spacing during early stages of feather morphogenesis. From Widelitz et al., 2000. D. pERK immunostaining in chicken embryos from stage 29, 32 and 35. From Lin et al., 2009. pERK also demonstrates a restrictive expression pattern. E. Schematic of early acting global and later early events during feather morphogenesis. From Jiang et al., 2004. F. Reconstituting skin explants showed that the size of the feather buds increased with increasing numbers of dissociated mesenchymal fibroblasts. Approximately equal spacing between buds was observed. G. Model depicting how the relative activator / inhibitor activities regulate feather bud size and spacing. From Jiang et al., 1999.
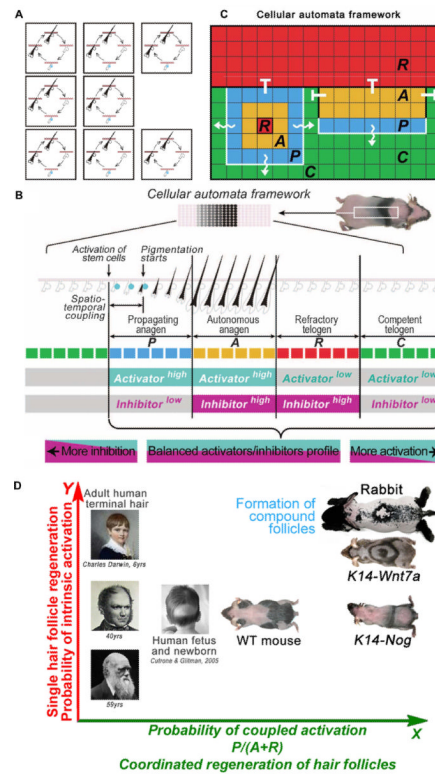
Figure 4.
A. Schematic illustration of hair cycling in a population of hair follicles. B. A two-dimensional CA model can predict regenerative patterns in a large population of hair SCs. Skin pigmentation patterns result from color changes of many HFs when they collectively cycle through four phases: P (blue)→A (yellow)→R (red)→C (green). Distinct hypothetical activator/inhibitor signaling profiles can be assigned to all four phases. C. Cellular Automata model for the observed hair wave. Wiggly white lines indicate the spreading of the hair wave from hairs in propagating anagen to hairs in competent telogen, whereas hairs in autonomous anagen or propagating anagen cannot initiate the hair cycle in hair follicles that are in refractory telogen. D. Hair follicles may regenerate in response to intrinsic signals which drive each individual follicle or to extrinsic signals which can couple the activation of the hair cycle in neighboring follicles. By modulating the strength of intrinsic stem cell activation (Y axis) and the probability of coupled activation (X axis), different animals or different physiological conditions in the same animal can significantly alter the global dynamics of hair regeneration. As a result, versatile hair growth patterns in rabbits, mice, normal and alopecic human scalps can be explained within the same patterning framework based simply on how hair stem cell activities are "managed". Panels B, C and D are from Plikus et al., 2011.
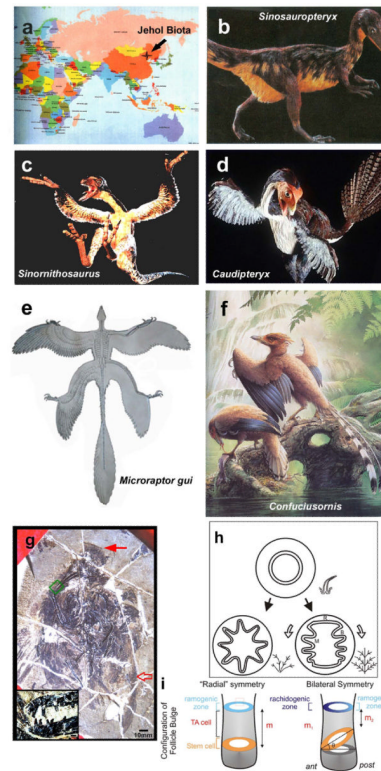
Figure 5.
Representatives of feathered dinosaurs and Mesozoic birds from Jehol Biota. a. Map showing the location of the excavated site. b. Sinosauropteryx. c. Sinornithosaurus. d. Caudipteryx. e. Microraptor gui. f. Confuciusornis. Panels b–d reprinted with permission from National Geographic[89]. Panel e is from Xu et al., 2003[90]. Panel f is from Hou et al., 2003[91]. g. Longirostrornis fossil, primarily in dorsal view. Inset, close up of beak and teeth. h. Model of branching morphogenesis responsible for radial vs bilateral symmetric feathers based on feather cross sections. It shows the barb ridge formation is basically a periodic patterning process. See text for more. i. Schematic depicting the tilting of feather stem cell ring (orange color) leads to the symmetric breaking at ramogenic zone (region where periodic patterning of barb ridges takes place, blue color), thus making radial vs bilateral symmetric feathers.
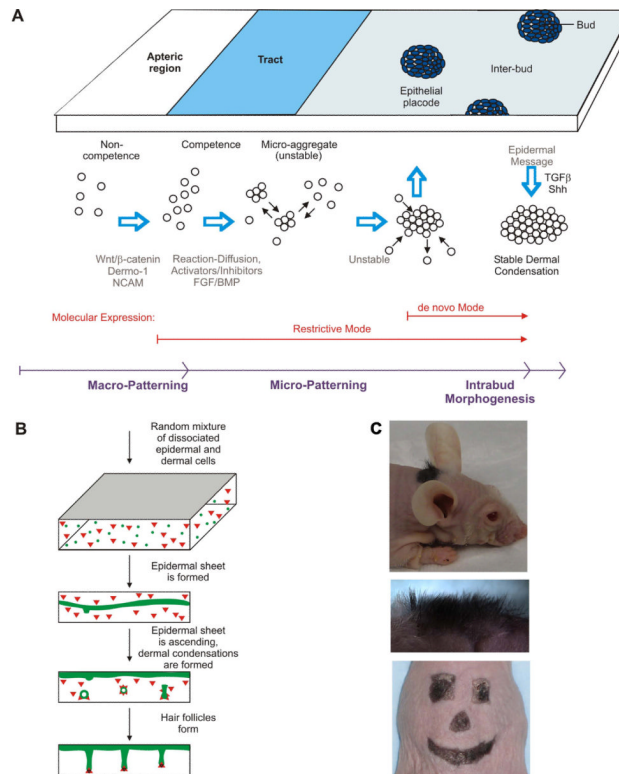
Figure 6.

A. Schematic summary showing the periodic patterning process during feather morphogenesis. Epithelial – mesenchymal signaling fosters the formation of competent epithelium. Spatially distributed activators and inhibitors of feather formation promote the expression of adhesion molecules which lead to the formation of unstable microaggregates. In these early stages, FGF acts as an activator while BMP acts as an inhibitor. The expression of FGF/pERK later promotes chemotaxis toward a signaling center in this patterning process leading to the formation of stable epithelial placodes and dermal condensations. The placode boundary is unstable at fitow but then becomes stabilized. Adapted from Lin et al., 2009. B. Schematic drawing of the hair reconstitution procedure. Green, epidermal cells; red, dermal cells. Cells are mixed randomly in the three dimensional matrix. Epidermal cells sort themselves out and coalesce to form a layer first near the bottom of the matrix which then rises to the top surface. Dermal cells form condensations adjacent to the epidermis. Hair germs appear periodically and progress to form hair pegs and later, hair follicles. The morphogenetic process occurs between about day 5 to 12 after grafting. C. Reconstituted hair follicles form normally. Using a reasonably stiff matrix to hold multipotential cells, the graft can be cut to specific shapes and sizes for cosmetic applications. Panels B and C are from Lee et al., 2011.