Distinct mechanisms underlie pattern formation in the skin and skin appendages

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Summary: Patterns form with the break of homogeneity and lead to the emergence of new structure or arrangement. There are different physiological and pathological mechanisms which lead to the formation of patterns. Here we first introduce the basics of pattern formation and their possible biological basis. We then discuss different categories of skin patterns and their potential underlying molecular mechanisms. Some patterns, such as the lines of Blaschko and naevus, are based on cell lineage and genetic mosaicism. Other patterns, such as regional specific skin appendages, can be set by distinct combinatorial molecular codes, which in turn may be set by morphogenetic gradients. There are also some patterns, such as the arrangement of hair follicles (hair whorls) and fingerprints, which involve genetics as well as stochastic epigenetic events based on physical-chemical principles. Many appendage primordia are laid out in developmental waves. In the adult, some patterns, such as those involving cycling hair follicles, may appear as traveling waves in mutant mice. Since skin appendages can renew themselves in regeneration, their size and shape can still change in the adult via regulation by hormones and the environment. Some lesion patterns are based on pathological changes involving the above processes and can be used as diagnostic criteria in medicine. Understanding the different mechanisms which lead to patterns on the skin will help us appreciate their full significance in morphogenesis and medical research. Much remains to be learned about complex pattern formation, if we are to bridge the gap between molecular biology and organism phenotypes.

Introduction to Pattern Formation

What is a pattern? Patterning can be considered as the loss of homogeneity, when small, random perturbations to a system are amplified through a number of local processes and iterations to form recognizable structure or order (Meinhardt, 1982; Murray, 2003; Chuong et al., 2006). For example, one of the simplest forms of patterning is the asymmetric conversion of part of a homogenous field (Fig. 1A, gray) to a different state (Fig. 1B, black). The new pattern can be generated as dots, stripes, patches, segments, branches, etc (Fig. 1C-E), and can be arranged at random or periodically.

What are the mechanisms of biological pattern formation? In some cases, they may be based on the distribution of cell lineage so that cells strictly follow their fates genetically (Fig. 1F). In other cases, it may be based on combinatorial molecular coding which can be interpreted at the enhancer / transcription factor level (Small and Levine, 1991) or at the cell adhesion level (Steinberg, 1996) (Fig. 1G, H). These molecular changes usually appear before the real morphological changes and are referred to as pre-patterns (Nagorcka and Mooney, 1992; Forgacs and Newman, 2005). These can explain many downstream phenomena which follow the generated pre-pattern, but they do not explain the upstream issue - we do not know how these molecular codes are set up. For example, morphogenetic gradient models have been proposed in which cells can interpret their position within a morphogen gradient as Wolpert (1969) has proposed in the French flag model (Fig. 1C). Cells can enter a new state in a concentration dependent manner (Ashe and Briscoe, 2006; Fig. 1I, J). This can explain many examples of how molecular codes are set, but still does not resolve the issue as to the origin of the pattern - we still do not know what set up the molecular gradient, for example, how the exact morphogen and its point of secretion are determined. This is where self-organization comes into play: stochastic events combined with physico-chemical principles can increase the order and /or structure of a system, perhaps resulting in emergent events, without being guided by an external source (Camazine et al. 2003; Newman and Comper, 1990; Newman et al. 2006). In other words, patterns at the global level may solely arise as a result of interactions between lower level components.

Reaction diffusion models following the method first outlined by Turing (Turing, 1952) have been applied to explain many biological periodic patterning processes (Gierer and Meinhardt, 1972; Fig. 1K, L). In this model, the morphogenetic field starts with a homogenous distribution of cells, activators and inhibitors, and random fluctuations initiate the periodic patterning process. The activators and inhibitors undergo a series of interactions, which can include self- and cross- activation and inhibition; both can diffuse, with the inhibitor diffusing further than the activator. With time, patterns in the form of dots or stripes in activator and inhibitor concentration gradually emerge, with the pattern depending on the ratio of activators to inhibitors and the size and shape of the pattern field. This mechanism has been proposed to explain the formation of hair follicles (Nagorcka and Mooney, 1992) and feather patterning (Jung et al., 1998).

Cellular automata models have also been proposed to explain many biological phenomena (Fig. 1M; Wolfram 1992; Deutsch et al., 2004). 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. Along this line, a digital hormone model has been developed to explain the formation of dermal condensations by feather mesenchymal cells (Shen et al., 2004).

Oscillation is another important property which may be used in patterning. The oscillation can occur at the level of a single cell, or at the level of an organ (Fig. 1P, S, T, e.g. hair and feather follicle). A clock and wavefront mechanism involving cellular oscillation has been used to explain the formation of somites (Fig. 1N-P; Pourquié, 2003). Oscillation of hair follicles in hair cycling becomes very visible in nude mice and Msx2 null mice (Miilitzer, 2001; Suzuki et al., 2003; Ma et al., 2003), and a model based on the Belousov-Zhabotinski reaction was recently suggested to explain this phenomena of wave formation, although no underlying molecular basis was identified (Fig. 1R, Fig. 5; Suzuki et al., 2003).

Of course, the physical-chemical events are still genetically based since the biological patterns are species-specific. A way to conceive this is that DNA gives rise to RNA and proteins which build cells with unique physical-chemical properties. At this level, groups of cells interact with outcomes based on these properties and the surrounding environment - not just on the molecule itself. Therefore, the pattern formation process is best appreciated as a combination of genetic and epigenetic events, and the results are both deterministic and stochastic, as seen in the fingerprints of homozygotic twins: similar but non-identical (reviewed in Jiang et al., 2004).

Organs can also grow and change their shape, size and organization during development (Fig. 1S, T). Another level of variation is that the morphogenetic field (in this case, the surface of the animal body) changes in size and shape during development (Fig. 1U-X). The way in which these changes take place can influence patterns which are at a formative stage. For example, during the expansion of the skin, new dermal and/or epidermal cells may be added to specific regions such as those receiving cells from the dermatome or the advancing ventral body folds. In other cases, new cells may be inserted randomly all over the developing skin (Fig. 1U-X). These growth modes can have different consequences for patterns forming on the skin. Similarly, the shape of the morphogenetic field may change, even sometimes reducing in size, and this also may lead to variation in patterning, for example the formation of stripes rather than spots (Murray, 2003; Fig. 1Y, Z).

Our aim is to try to analyze these pattern formation processes and identify the biological bases underlying them, but much remains to be learned. We have described above only some examples and they certainly do not exhaust all models which have been proposed for patterning. We can also contemplate that complex pattern formation is generated through a combination of the above processes, which perhaps results in patterns that are more robust to genetic and environmental perturbations. We will point out these patterns and the processes that may be involved in the following sections.

Development Based and Unalterable

Although the appearance of integuments of mammals, birds and reptiles can be very different (Fig. 2A), the development of their skin and skin appendages share similar hierarchical morphogenetic processes (Fig. 2B). On some occasions, different types of skin appendages appear ; while on other occasions, the patterns of similar types of appendages are arranged differently (Ball, 1999). We think that this is controlled through genetic and epigenetic controls that operate at different levels (Jiang et al., 2004). Here we take a closer look at these regulatory processes.

Regional specificity: Regional specificity implies that different skin regions such as the scalp, beard, eyebrows, face, lips, palms, nails, mammary glands, sweat glands, etc have different characteristics. Epidermal precursors (or stem cells) are initially multi-potent and competent to form all these different structures. During development, special domains of the dermis begin sending specific messages to the epidermis. Through a series of epithelial-mesenchymal interactions, these different skin domains with special structures and functions gradually emerge. The integument diversifies to endow different functions to different parts of the human skin. An example of regional specificity can be seen by comparing the different types of feathers present on the breast, wing, tail, etc of birds. This is most evident in pheasants as shown in Fig. 2A.

How these dermal specificities and epidermal competence are set up is mostly unknown. A model based on a skin Hox code was proposed suggesting that different combinations of Hox gene expression may be the basis of skin regional specificity; setting up the subsequent differences in diffusible morphogens and adhesion molecules (Chuong, 1993). Different Hox expression patterns are shown in different regions of chicken skin (Duboule, 1998; Kanzler et al., 1997; Reid and Gaunt, 2002). Indeed there are spatiotemporally defined, specific HOX expression patterns in human skin (Stelnicki et al., 1998) and the Hox expression patterns of dermal cells derived from different topological skin regions in humans are different (Chang et al., 2002).

Most interestingly, the characteristics of these different skin regions can be trans-determined. For example, the engrailed pathway was shown to be involved in defining the mesenchymal characteristics of the ventral versus the dorsal paw (Loomis et al., 1996). Tbx4 and Tbx5 are shown to be involved in defining the identity of the chicken leg versus wing and hence scale or feather forming dermis (Rodriguez-Esteban et al., 1999). Epidermal cells can trans-differentiate and convert hairs into glands or scales into feathers under the influence of retinoic acid, or by ectopic expression of specific molecules such as beta-catenin (Robinson et al., 1990; Dhouailly et al., 1980; Widelitz et al., 2000). A recently engineered K14 - noggin transgenic mouse shows that sweat glands are transformed to hairs (Plikus et al., 2004), while noggin overexpression under the neuron-specific enolase promoter can convert outer root sheath keratinocytes into sebocytes (Guha et al. 2004). An adult cornea can also be diverted to form pilosebaceous units when they are confronted with embryonic hair forming dermis (Pearton et al., 2004). These observations imply that the specific combinatorial molecular codes may specify phenotypes of skin and skin appendages (Chuong, 1993; Prin and Dhouailly, 2004). Altering these codes may lead to a resetting of the phenotypes. The upstream question concerning how the molecular codes are set remains unanswered.

Developmental wave: During skin development, hair or feather primordia are laid out in a temporal order as they gradually acquire competence (reviewed in Dhouailly et al., 2004). Their arrangement and orientation is reflected as a propagating wave of skin appendage formation. This process is clearly shown in the chicken skin in Fig. 3A. In the spinal tract of the chicken, the formation starts at the midline and spreads bilaterally. At the lateral edge, feather primordia are at the induction stage. Toward the midline, they progress to form short feather buds, long feather buds, feather filaments with branching morphogenesis, etc. A morphogenetic wave sweeping from the midline to the lateral has been inferred (Sengel, 1990). However, while the lateral row appear after the more medial row, the formation of the lateral row does not really have to depend on the medial row (Jiang et al., 1999). While this sequential appearance may be perceived as a "gradient", it is actually a temporal wave since the lateral feather buds will eventually also go through feather bud and filament stages. For a tract, there has to be a primary row before these sequential appearance take place. The gradual emergence of buds along the primary row in the midline can be readily visualized by in situ hybridization staining for beta-catenin (Fig. 3B). The molecular basis of these process remain unknown.

In humans, this is manifested as hair whorl patterns in the occipital region (Fig. 1Q; reviewed in Plikus and Chuong, 2004). In human fetuses lanugo hairs form whorl patterns both on the scalp and trunk skin (Gworys and Domagala, 2003). On the thoracic wall there are lanugo whorls that begin bilaterally over the nipples. The whorls collide and merge along the midlines (Domagala, personal communication). In adults, whorl patterns are distinct only on the parietal scalp. Is the whorl pattern genetically controlled? A pair of homozygotic twins was shown to have one and two whorls, respectively (Paine et al., 2004). Therefore there must be an epigenetic component in hair whorl determination. While conserved molecular pathways underlie all hair follicle formation, local environmental and fortuitous factors can influence the final hair pattern.

In the mouse, transgenic mice which lose frizzled 6 show the formation of multiple whorls (Guo et al., 2004), suggesting the involvement of the Wnt pathway in this process. Interestingly, some strains of guinea pigs also show multiple whorls on their skin.

Periodic patterning: This mechanism is most obvious in the formation of skin appendages and pigment patterns. During the formation of feather primordia, the epithelium has to become competent to respond to induction signals (forming a field). A reaction diffusion mechanism, involving activators and inhibitors is proposed to operate in the dermal mesenchyme (Jung et al., 1998; Jiang et al., 1999). Through this mechanism, cells are set to become the primordia of skin appendages, stochastically. This then leads to the formation of the feather or hair primordia, evenly spaced with interfollicular skin. Similar processes were proposed for hair / wool formation (Meinhardt and Gierer, 2000; Nagorcka and Mooney, 1985; Moore et al., 1998). It should be emphasized that the process of periodic patterning can be uncoupled from the developmental wave process discussed in the last paragraph. The sequential appearance of feather buds is so exquisite (Fig. 3A) that it led scientists to propose models which are based on the use of previous buds as templates (Murray and Oster, 1984). The experiments by Jiang et al., 1999 showed that in a reconstitution situation in which mesenchymal cells are dissociated into single cells, the periodic patterns will reform simultaneously. So the sequential appearance results from a global competence wave imposed on the local periodic patterning process.

Another dramatic example often referred to is the dissolution of pigment cells that lead to the formation of stripes on zebra fish or zebras and the formation of pigment ducts on fish or leopards. This was addressed earlier by Murray (1993). Recently, Prum also applied a reaction diffusion mechanism to make a theoretical model of feather pigment patterns (Prum and Williamson, 2002). However, some pigment patterns are controlled by enhancer regions genetically as shown by differences in the Droopy Ear mouse mutant. Here the normal, sharp delineation between dorsal and ventral pigmentation patterns is disrupted. This is produced by a loss of function mutation in TBox 15 which then allows agouti to be expressed further dorsally (Candille et al., 2004).

Do these patterns result from genetic coding or stochastic events? In fact, it is likely to be both..For example consider patterns such as fingerprints. They are used for individual identification because the ridge width and possibility for branching nodal points provide ample possibility for endless variation. Fingerprints among monozygotic twins have more similar attributes (similar width, organization plan) than with unrelated individuals, but they are non-identical and are sufficiently different to be used as individual identifiers (Jain et al., 2002). Thus there is a non-genetic component at this level of tissue morphogenesis, where molecular codes become indirect and cells interact based on physical-chemical rules.

Morphogenetic gradient: We acknowledge the importance of molecular codes and have proposed the skin Hox code hypothesis for regional specificity of skin and skin appendages (Chuong, 1993). Yet, how are these molecular codes set up? A morphogenetic gradient, such as a Shh gradient, has been used to explain dorsal-ventral spinal cord determination (Monsoro-Burq and Le Douarin, 1999) and anterior-posterior limb bud patterning (McGlinn and Tabin, 2006). Here we will use a recent example describing how a Wnt 3a gradient in adult feather follicles pattern epithelial stem cells to form either radial or bilateral, symmetric feathers.

In the adult bird, there are radially symmetric body feathers and bilaterally symmetric flight feathers. Feathers do not contain the bulge structure found in hairs that house stem cells. We recently identified feather stem cells located as a concentric ring sitting at the bottom of the feather follicle (Yue et al., 2005). Interestingly, in radially symmetric feathers the stem cell ring is placed horizontally. In bilaterally symmetric feathers, the stem cell ring tilts toward the anterior rachis position. This topological difference led us to propose that there would be a break of symmetry in the ramogenic plane where feather branches start to form (Fig. 4). Indeed in bilaterally symmetric feathers, we found a Wnt 3a gradient from anterior to posterior positions which does not exist in radially symmetric feathers. Flattening the Wnt 3a gradient using RCAS retrovirus mediated gene mis-expression converted bilaterally symmetric feathers to radially symmetric feathers (Yue et al., 2006). A local Wnt 3a gradient released from a bead causes the forming barb ridge branches to swirl toward the Wnt 3a source. Swapping dermal papillae between radial and bilaterally symmetric feathers shows that the dermal papilla determines the gradient configuration and feather symmetry, while stem cells can respond to the morphogenetic gradient to make different forms of feathers. This is an excellent example showing how a microgradient within a feather follicle can set the organ shape.

Patterns That Can Be Changed in the Adult

Traveling Wave: Hair follicles go through regenerative cycles: they cycle through growth (anagen), regression (catagen), hair shaft shedding (exogen) and resting phases (telogen) (Paus and Cotsarelis, 1999; Paus and Foitzik 2004; Stenn & Paus 2001). Visualizing hair waves is facilitated by hair cycle dependent changes in integument pigmentation and timed hair loss in nude mice (Militzer, 2001; Suzuki et al., 2003), *Msx2* null mice (Ma et al., 2003), calcineurin B1 (Mammucari et al., 2005), etc. As a result, we observe skin regions cycling through discrete stages, each representing a different "status" of hair follicle (Fig. 1S, T). The regions can appear as waves of dramatic pattern changes across the adult mouse skin.

Militzer (2001) analyzed more than 400 nude mice on albino (NMRI, *foxn1*^{nu}) and pigmented (C57BL/6, *foxn1*^{nu}) backgrounds for more than one year. Pink skin turns dark when hairs enter anagen III and returns to pink when anagen is completed. Skin pigmentation changes travel in a wave-like fashion on the skin surface of these mice. When mice are young, all hair cycles synchronize initially, but with increasing age the hair cycles over different regions desynchronize. Thus, the skin pigment pattern breaks into distinct stripes and patches. As mice age, the stripes and patches become narrower / smaller and eventually appear random.

Dramatic traveling hair waves occur in the *Foxn1*^{nu} strain of nude mice on the C57BL/6 background (Suzuki et al., 2003). These mutant mice have a defect in the *Foxn1* gene that results in the precocious termination of the hair cycle. It also has faster hair cycling. Thus the dynamic pigmentation pattern changes described above progress faster than those observed in classical nude mice. In the young mice, the pigmentation oscillation takes place synchronously throughout the mouse skin. The wider pigmented stripes progress to become narrower bands as the mice age. Some mice (usually >7 months) show narrow, roughly evenly spaced pigmented stripes that travel along the trunk; however, many mice show irregular, fragmented or very wide stripes (Fig. 5).

The pattern can become more complex. Ma et al., (2003) reported "cyclic alopecia" in *Msx2* knockout mice. The phenotype is due to the fact that hair fibers are defective and are dislodged specifically during catagen phase. The skin of these mice during anagen is black and hairy, but during telogen is bald and non-pigmented. As the hairs cycle, the alopecic regions re-enter anagen and regain pigmentation in a progressive order. Long-term observation of hairy and bald skin regions revealed a "cyclic alopecia" phenomenon. Hairs within one skin domain cycle in waves but not with hairs in neighboring domains (which also cycle in waves, but with an independent rhythm). In essence, the “traveling stripes” of the *Foxn1*^{nu} mice are the manifestation of the same phenomenon. Notch 1 activation in keratinocytes can go through the RBP or calcineurin B1 pathways. Recently, mice with a calcineurin B1 deletion also showed a cyclic alopecia phenotype (Mammucari et al., 2005). In humans, each hair follicle cycles independently, so there are no wave-like patterns.

Hormone Based Changes of Appendage Pattern: Since hair or feather follicles can go through regenerative cycles, a completely new type of skin appendage can re-form with a new shape and size through a regenerative cycling mechanism. This is most obvious in sex-hormone dependent changes (Mayer et al., 2004). Upon puberty, skin appendages in specific regions are transformed when sex hormone (estrogen and androgen) pathways are activated. Sex steroids can also affect the melanogenic activity of epidermal melanocytes, giving rise to hormonally based changes of skin and skin appendage patterns as evident in birds (Fig. 2A). This is most apparent in tail feathers of hens / roosters and peahens / peacocks. Sexual dimorphism characteristics are also observed in mammals including humans (Wheeler, 1991). In the human beard, axilla and genital regions, hair follicles are transformed from the vellus to the terminal state. With increasing age, the reverse tends to occur, leading to androgenic alopecia. Vellus hairs also can be transformed into unwanted terminal hairs (e.g. on the upper lip and lower legs) when properly stimulated by androgens, leading to hirsutism. Here, terminal hairs in the frontal and parietal scalp are affected but not those in the occipital region. As a result, the type of hairs that form and the region of hair growth (hairline) change at different ages. The mechanism controlling how scalp and occipital hairs respond to sex hormones is not known but appears to be mediated by differences in dermal papillae which exhibit varying response to stimulation with androgens or estrogens (Inui et al. 2002, Randall et al., 2001; Conrad et al. 2005). Thus, hormonally based skin lesion patterns are also the consequence of region-specific developmental programming.

Environmental Based Pattern Changes: Change in light/dark cycles produced by the seasonal lengthening and shortening of days or changes in temperature can alter the type or coloring of skin appendages. This can be seen in the seasonal (summer/winter) hair coat variation of horses, snow shoe rabbits, etc. 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 (Rose et al. 1987). Now that we know that human and rodent skin and hair follicles are extra-pituitary sites of melatonin synthesis (Slominski et al. 2002, 2003; Kobayashi et al. 2005), 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.

Patterns Due to Skin Lesions.

Since the skin covers the surface of an individual, patterns on the skin are the most recognizable. They have been used as diagnostic clues to the dermatologist (Bolognia et al. 2003). In addition to the developmental and physiological causes, patterns on the skin that develop can be due to pathological or artifactual causes. A multi-authored review featuring several view points focusing on skin lesion patterns was recently edited by Dr. Ralf Paus for Experimental Dermatology (Chuong et al., 2006). Here we will briefly summarize those discussions.

Lineage, genetically based: Molecular expression within cells is changed genetically or epigenetically during development. The changes can be transmitted to daughter cells because they involve somatic mutations in DNA or are mediated by epigenetic mechanisms such as X chromosome inactivation, DNA methylation, etc. This collection of different patterns has mainly been studied in human diseases. The offspring of the mutated cells share a similar abnormality. The distinct phenotypes of these cells then manifest themselves in the skin. These ectopic changes are named Naevus (Happle, 1995 and see glossary). There are several striking examples in which lesions are limited to the left or right side of the body, regional segments, checkerboard patterns or linear distributions (Happle, 1993, 1995, 2004; Fig. 6A). The most striking example in the epidermal lineage is the Blaschko lines (Jackson, 1976). A recent case of linearly distributed acne turns out to be due to a somatic mutation in the FGF receptor in one epidermal cell lineage (Munro and Wilkie, 1998). The mechanism leading to the Blaschko lines is fundamental and not limited to humans. When early chicken embryo epidermal cells (embryonic day 2) were labeled along the dorsal midline with replication defective virus expressing beta-galactosidase, their cellular descendents showed multiple parallel blue lines radiating from the midline across the dorsal skin of late chicken embryos resembling Christmas tree branches (Chuong et al., 1998; Fig. 6B, C). However, analyses of these patterns show that formation of feather primordia or feather filaments are not based on lineage, but on the local environment at the time of formation (Fig. 6D).

There are many different types of ectodermal organs on the integument. Many of them share morphogenetic signaling pathway. Perturbation of one pathway can lead to changes in multiple organ (Plikus et al., 2004). In human, when a molecule, such as EDA, that is fundamental to these processes is mutated, it can lead to ectodermal dysplasia which affect multiple epithelial organs (Bolognia et al. 2003).

Anatomically or physiologically based: Distinct regions such as skin appendages, skin ridges, cutaneous nerves, blood vessels, etc. can contribute to patterns of skin lesions. When skin lesions develop, they may follow these obvious anatomical borders or follow "hidden" latent patterns based on physiological differences. Through various pathogenetic mechanisms, these different skin regions may result in different susceptibility to diseases. It is upon this dynamic landscape that skin lesions develop, become distributed and shaped.

Artificial: Human behaviors can also cause patterned lesions. For example, chronic sun exposure can lead to the characteristic UV-light-induced patterns corresponding to unclothed skin regions. Tattoos, skin paintings, hair dyes, cosmetic surgery, etc. can lead to further visible patterned changes on the skin.

Conclusion

The skin is an excitable medium. In development, it conducts reactions among signaling molecules that determine the formation of skin appendages or the distribution of active melanocytes. In the adult, the regenerative hair cycling provides a rich opportunity for the skin to renew itself based on hormonal and environmental changes. Patterns of skin lesions provide diagnostic clues to skin or systematic diseases. The convergence of genetic, epigenetic and regenerative events to generate complex patterns on this very visible organ also provides a great experimental opportunity to study the many unknown mechanisms of biological pattern formation.

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References

Ackerman AB. 1997. Histologic Diagnosis of Inflammatory Skin Diseases: An Algorhythmic Method Based on Pattern Analysis, 2nd ed., Baltimore, Wilkins & Wilkins.

Ashe HL, Briscoe J. 2006. The interpretation of morphogen gradients. Development. 133:385-394.

Ball P. 1999. The self-made tapestry: pattern formation in nature. Oxford: Oxford Univ. Press.

Bolognia JL, Jorizzo JL, Rapini RP. (eds.) 2003. *Dermatology*, Mosby: London.

Braunwald E, Fauci A, Kasper D, et al. 2004. Harrison's Principles of Internal Medicine. 16th edition. New York: McGraw-Hill.

Camazine S, Deneubourg JL, Franks NR, et al. 2003. Self-Organization in Biological Systems. Princeton: Princeton University Press.

Candille SI, Van Raamsdonk CD, Chen C, et al. 2004. Dorsoventral patterning of the mouse coat by Tbx15. PLoS Biol. 2:E3.

Chang HY, Chi JT, Dudoit S, et al. 2002. Diversity, topographic differentiation, and positional memory in human fibroblasts. Proc Natl Acad Sci 99:12877-12882.

Chuong CM, 1993. The making of a feather: Homeoproteins, retinoids and adhesion molecules. BioEssays 15: 513-521.

Chuong CM, Jung HS, Noden D, et al. 1998. Lineage and pluripotentiality of epithelial precursor cells in developing chicken skin. Cell Biol Biochem 76:1069-1077.

Chuong CM, Wu P, Plikus MV, et al. 2006. Engineering stem cells into organs: Topobiological transformations demonstrated by beak, feather and other ectodermal organ morphogenesis. Curr Top Dev Biol 72:237-274.

Chuong CM, Dhouailly D, Gilmore S, et al. 2006. What is the biological basis of pattern formation of skin lesions? Expt Dermatology 15:547-564.

Conrad F, Ohnemus U, Bodo E, et al. 2005. Substantial sex-dependent differences in the response of human scalp hair follicles to estrogen stimulation in vitro advocate gender-tailored management of female versus male pattern balding. J Investig Dermatol Symp Proc. 10:243-246.

Deutsch A, Dormann S, Maini PK. 2004. Cellular Automaton Modeling of Biological Pattern Formation. Boston: Birkhauser.

Duboule D. 1998. Hox is in the hair: a break in colinearity? Genes Dev. 12:1-4.

Dhouailly D, Hardy MH, Sengel P. 1980. Formation of feathers on chick foot scales: a stage-dependent morphogenetic response to retinoic acid. J Embryol Exp Morphol 58:63-78.

Dhouailly D, Olivera-Martinez I, Fliniaux I, Missier S, Viallet JP, Thelu J. 2004. Skin field formation: morphogenetic events. Int J Dev Biol. 48:85-91.

Fliniaux I, Viallet JP, Dhouailly D. 2004. Ventral vs. dorsal chick dermal progenitor specification. Int J Dev Biol 48:103-106.

Forgacs G, Newman SA. 2005. Biological Physics of the Developing Embryo. New York: Cambridge University Press.

Gierer A, Meinhardt H. 1972. A theory of biological pattern formation. Kybernetik 12: 30-39.

Guha U, Mecklenburg L, Cowin P, et al. 2004. BMP signaling regulates postnatal hair follicle differentiation and cycling. Am J Pathol 165:729-740.

Guo N, Hawkins C, Nathans J. 2004. Frizzled6 controls hair patterning in mice. Proc Natl Acad Sci USA 101:9277-9281.

Gworys B, Domagala Z. 2003. The typology of the human fetal lanugo on the thorax. Ann Anat. 185:383-386.

Happle R. 1993. Mosaicism in human skin. Understanding the patterns and mechanisms. Arch Dermatol 120: 1460-1470.

Happle R. 1995. Epidermal nevus syndromes. Semin Dermatol 14:111-21.

Happle R. 2004. Patterns on the skin. New aspects of their embryologic and genetic causes. Hautarzt. 55:960-961, 964-968.

Inui S, Fukuzato Y, Nakajima T, et al. 2002. Androgen-inducible TGF-beta1 from balding dermal papilla cells inhibits epithelial cell growth: a clue to understand paradoxical effects of androgen on human hair growth. FASEB J 16:1967-1969

Jackson R. 1976. The line of Blaschko: a review and reconsideration: observations of the cause of certain unusual linear conditions of the skin. Br J Dermatol 95:349-360.

Jain AK, Prabhakar S, Pankanti S. 2002. On the similarity of identical twin fingerprints. Pattern Recognition 35:2653-2663.

Jiang TX, Jung HS, Widelitz RB, et al. 1999. Self-organization of periodic patterns by dissociated feather mesenchymal cells and the regulation of size, number and spacing of primordia. Development. 126:4997-5009.

Jiang TX, Wideltz RB, Shen WM, et al. 2004. Integument pattern formation involves genetic and epigenetic controls operated at different levels: Feather arrays simulated by a digital hormone model. Int J Dev Biol 48:117-136.

Jung HS, Francis-West PH, Widelitz RB, et al., 1998. Local Inhibitory Action of BMPs and Their Relationships with Activators in Feather Formation: Implications for Periodic Patterning. Dev Biol 196:11-23.

Kanzler B, Prin F, Thelu J, et al. 1997. CHOXC-8 and CHOXD-13 expression in embryonic chick skin and cutaneous appendage specification. Dev Dyn. 210:274-287.

Kobayashi H, Kromminga A, Dunlop TW, et al. 2005. A role of melatonin in neuroectodermal-mesodermal interactions: the hair follicle synthesizes melatonin and expresses functional melatonin receptors. FASEB J. 19:1710-1712.

Loomis CA, Harris E, Michaud J, et al. 1996. The mouse Engrailed-1 gene and ventral limb patterning. Nature 382:360-363.

Ma L, Liu J, Wu T, et al. 2003. Cyclic alopecia in Msx2 mutants: Defects in hair cycling and hair shaft differentiation. Development 130:379-389.

Mammucari C, Tommasi di Vignano A, Sharov AA, et al. 2005. Integration of Notch 1 and calcineurin/NFAT signaling pathways in keratinocyte growth and differentiation control. Dev Cell. 8:665-676.

Mayer JA, Chuong CM, Widelitz R. 2004. Rooster feathering, androgenic alopecia, and hormone dependent tumor growth: What is in common? Differentiation 72:474-488.

McGlinn E, Tabin CJ. 2006. Mechanistic insight into how Shh patterns the vertebrate limb. Curr Opin Genet Dev. 16:426-432.

Meinhardt H. 1982. Models of biological pattern formation. Academic Press, New York.

Meinhardt H, Gierer A. 2000. Pattern formation by local self-activation and lateral inhibition. Bioessays. 22:753-760.

Militzer K. 2001. Hair growth pattern in nude mice. Cells Tissues Organs 168:285-294.

Moore GP, Jackson N, Isaacs K, et al. 1998. Pattern and morphogenesis in skin. J Theor Biol. 191:87-94.

Monsoro-Burq AH, Le Douarin N. 1999, Molecular aspects of vertebral chondrogenesis. J Soc Biol. 193:263-268.

Munro CS, Wilkie AO, 1998. Epidermal mosaicism producing localised acne: somatic mutation in FGFR2. Lancet 352:704-705.

Murray JD. 1993. Mathematical Biology. New York: Springer-Verlag.

Murray JD. 2003. Mathematical Biology II: Spatial Models and Biomedical Applications. New York: Springer-Verlag.

Murray JD, Oster GF. 1984. Generation of biological pattern and form. IMA J Math Appl Med Biol. 1:51-75.

Nagorcka BN, Mooney JR. 1985. The role of a reaction-diffusion system in the initiation of primary hair follicles. J Theor Biol. 114:243-272.

Nagorcka BN, Mooney JR. 1992. From stripes to spots: Prepatterns which can be produced in the skin by a reaction-diffusion system. Math Med Biol. 9:249-267.

Newman SA, Comper WD. 1990. Generic physical mechanisms of morphogenesis and pattern formation. Development 110: 1-18.

Newman SA, Forgacs G, Muller GB. 2006. Before programs: The physical origination of multicellular forms. Int. J. Dev. Biol. 50: 289-299.

Paine ML, Paine CT, Machin GA. 2004. Hair whorls and monozygosity. J Invest Dermatol 122:1057-1058.

Paus R, Cotsarelis G. 1999. The biology of hair follicles. N Engl J Med 341:491-497.

Paus R, Foitzik K. 2004. In search of the “hair cycle clock”: a guided tour. Diffrentiation 72:489-511

Pearton DJ, Ferraris C, Dhouailly D. 2004. Transdifferentiation of corneal epithelium: evidence for a linkage between the segregation of epidermal stem cells and the induction of hair follicles during embryogenesis. Int J Dev Biol 48:197-201.

Plikus M, Wang WP, Liu J, et al. 2004. Morpho-regulation of ectodermal organs: Integument pathology and phenotypic variations in K14-noggin engineered mice through modulation of BMP pathway. Am J Pathology 164:1099-1114.

Plikus M, Chuong CM. 2004. Making waves with hairs. J Invest Dermatol 122:VII-IX.

Pourquié O. 2003. The Segmentation Clock: Converting Embryonic Time into Spatial Pattern. Science 301:328 – 330.

Prin F, Dhouailly D. 2004. 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. 48:137-148.

Prum RO, Williamson S. 2002. Reaction-diffusion models of within-feather pigmentation patterning. Proc Biol Sci. 269:781-792.

Randall VA, Hibberts NA, Thornton MJ, et al. 2001. Do androgens influence hair growth by altering the paracrine factors secreted by dermal papilla cells? Eur J Dermatol. 11:315-320.

Reid AI, Gaunt SJ. 2002. Colinearity and non-colinearity in the expression of Hox genes in developing chick skin. Int J Dev Biol. 46:209-215.
Robinson ME, Gibbins AM, Hardy MH. 1990. Persistence of added retinoids in organ culture media during induction of mucous metaplasia and glandular morphogenesis in hamster cheek pouches. Experientia 46:513-517.

Rodriguez-Esteban C, Tsukui T, Yonei S, et al. 1999. The T-box genes Tbx4 and Tbx5 regulate limb outgrowth and identity. Nature 398:814-818.

Rose J, Oldfield J, Stormshak F. 1987. Apparent role of melatonin and prolactin in initiating winter fur growth in mink. Gen Comp Endocrinol 65:212-215.

Sengel P, 1976. *Morphogenesis of Skin*. Cambridge University Press. Cambridge. Pp 218-251.

Sengel P, 1990. Pattern formation in skin development. Int. J. Dev. Biol. 34:33-50, 1990.

Shen WM, Will P, Galstyan A, Chuong CM. 2004. Hormone-inspired self-organization and distributed control of robotic swarms. Autonomous Robots 17:93-105.

Slominski A, Pisarchik A, Semak I, et al. 2002. Serotoninergic and melatoninergic systems are fully expressed in human skin. FASEB J 16:896-898.

Slominski A, Pisarchik A, Zbytek B, et al. 2003. Functional activity of serotoninergic and melatoninergic systems expressed in the skin. J Cell Physiol. 196:144-153.

Small S, Levine M. 1991. The initiation of pair-rule stripes in the Drosophila blastoderm. Curr Opin Genet Dev. 1:255-260.

Steinberg MS. 1996. Adhesion in development: an historical overview. Dev Biol. 180:377-388.

Stelnicki EJ, Komuves LG, Kwong AO, et al. 1998. HOX homeobox genes exhibit spatial and temporal changes in expression during human skin development. J Invest Dermatol 110:110-115.

Stenn KS, Paus R. 2001. Controls of hair follicle cycling. Physiol Rev. 81:449-494.

Suzuki N, Hirata M, Kondo S. 2003. Traveling stripes on the skin of a mutant mouse. Proc Natl Acad Sci USA 100:9680-9685.

Turing A. 1952. The chemical basis of morphogenesis. Philos. Trans. R. Soc. London B 237:37-72

Wheeler MD, 1991. Physical changes of puberty. Endocrinol Metab Clin North Am 20:1-14.

Widelitz RB, Jiang TX, Lu J, et al. 2000. Beta catenin in epithelial morphogenesis: Conversion of part of avian foot scales into feather buds with a mutated beta catenin. Dev Biol 219:98-114.

Wolfram S, 2002. A New Kind of Science. Wolfram Media. Champaign, IL.

Wolpert LJ, 1969. Positional information and the spatial pattern of cellular differentiation. Theor Biol. 25:1-47.

Yamaguchi Y, Itami S, Watabe H, et al. 2004. Mesenchymal-epithelial interactions in the skin: increased expression of dickkopf1 by palmoplantar fibroblasts inhibits melanocyte growth and differentiation. J Cell Biol 165:275-285.

Yue Z, Jiang TX, Widelitz RB, et al., 2005. Mapping stem cell activities in the feather follicle. Nature 438:1026-1029.

Yue Z, Jiang TX, Widelitz RB, et al., 2006. Wnt 3a gradient converts radial to bilateral feather symmetry via topological arrangement of epithelia. Proc Natl Acad Sci (USA) 103:951-955.

Glossary Box

Cellular automata: a method of modeling a biological system. The developing field is divided into a number of sites, each in one of a finite number of discrete states. The evolution of each site is determined according to a set of rules which take into account the state of the site and those of its neighbors.

Cyclic alopecia: The cyclic appearance of bald and hairy states in the same skin region. It appears when 1) the hair follicle can regenerate and 2) the hair filament of this follicle is lost at a specific time during the hair cycle.

Hair cycle: the change of the states of hair follicles from growth (anagen), regression (catagen), to resting phases (telogen). An exogen phase can be added to highlight the shedding of hair shafts.

Oscillation: the periodic variation, typically in time, of some measure. A wide variety of biological systems display oscillations, consider for example, the circadian clock and the hair cycle, and the period of oscillation can range from fractions of a second to a year.

Pattern: a recognizable shape or arrangement of objects within a field.

Reaction diffusion mechanism: a process via which patterns are generated from an initially homogenous field, due to diffusion of the reacting constituents. Interactions between the components and unequal diffusion rates lead to the amplification of small fluctuations into a regular pattern.

Wave: a disturbance which propagates across a field. Typically thought to carry information and / or energy, but can also be thought of as the movement of objects, or different states of an organ, across a field.

Figure legends

Fig. 1. Basics on pattern formation. A-E, schematic drawings showing basic patterns. F-T, possible mechanisms that can lead to pattern changes. U-Z, additional factors that can influence biological pattern formatting due to growth. Panel E is from Yue et al., 2005. Panel M is from Jiang et al., 2004. Panels U-X represent the trunk with the midline on the top. There are three possible ways new cells can be added, which are indicated by the green color. Panels Y, Z indicate the changes of field shape which can represent the growth of limb bud, tail bud or feather bud. Please see text for further explanation.

Fig. 2. Patterns on avian skin and skin appendages and hierarchical morphogenesis. A) Male and female pheasants show regionally specific skin appendages and sexual dimorphism. Also note the thick pigment stripes and dots in the tail feather. Prum and Williamsons (2002) proposed a reaction diffusion model for feather pigment patterning. B) Different developmental stages of skin appendage morphogenesis. Note the different types of skin appendages, including the schematic radially and bilaterally symmetric feathers. Modified from Wu et al., 2004.

Fig. 3. Temporal wave. A. Shh in situ hybridization of embryonic chicken skin. Midline is indicated by an arrow. Feather bud formation starts from the midline, and then lateral buds appear sequentially. From the lateral edge to the midline are regions of no feather primordia, feather placodes, short buds, long buds and feather filaments with branch formation. B. Beta catenin in situ hybridization. The feather field first homogenously expresses beta catenin at a moderate level in the morphogenetic zone. Then the periodically arranged buds emerge gradually expressing high levels of beta catenin, while the lateral inhibitory zone does not express beta catenin. From Widelitz et al., 2000.

Fig. 4. Morphogenetic gradient. Left column: an idealized radially symmetric feather. Right column: a bilaterally symmetric feather. A, E, The proximal follicle shows ordered compartments of stem cells (orange color), TA cells and differentiating cells (ramogenic zone) (Yue et al., 2005). In radially symmetric feathers, the ring is horizontal. In the bilaterally symmetric feathers, the ring is tilted from zero to about 45^o. The molecular gradient in the ramogenic zone is shown in shades of blue. B, C, In an open follicle preparation, the feather filament cylinder is opened to form a plane. In radially symmetric feathers, all new barb ridges form at the same time and in parallel. In bilaterally symmetric feathers, the tilting of the stem cell ring results in a discrepancy of maturation due to the fact that the TA cells have to travel (or are displaced) different distances before they reach the ramogenic zone (m1 and m2). On the anterior side, cells are more mature. The shift in cell position is represented by vectors AB, AC and AD. D, According to this model, there should be a molecular gradient along the A-P axis. Indeed we found a Wnt 3a gradient. Flattening the gradient converted feathers from bilateral to radial symmetry (Yue et al., 2006).

Fig. 5. Traveling stripes. A. In the adult mouse, hair follicles go through regenerative cycling. They appear as black in the anagen period. In this mutant nude mouse, hair filaments are lost in the telogen period and appear white. This helps us visualize the

changing states of hair follicles, which appear as traveling waves (after Suzuki et al., 2003; Plikus and Chuong, 2004). Arrows describe the direction of wave propagation. B. Msx2 null mice show cyclic alopecia in which hair shafts are dislodged at a specific time of hair cycle but can also regenerate. As a result, patches of hairy domains (black) and bald domains (white) are formed. These domains alternate between growth and resting phases, and give the impression of traveling stripes. The shape and size of these domains, their relative configuration changes over time, and situation in A is a special example of this phenomenon. Based on Ma et al., 2003.

Fig. 6. Genetic mosaicism on the skin. A. Lines of Blaschko. Through x chromosome inactivation, the lineage of epithelia cells can be seen to be distributed in lines horizontal to the body A-P axis. Several examples of checkerboard or patch patterns on human skin are seen in several human diseases (Happle, 1995, 2004). After Happle's viewpoint 2 in Chuong et al., 2006. B, Equivalent lines of Blaschko in embryonic chicken. Embryos are injected with non-replicative virus carrying beta-galactosidase. C, line drawing of B. D, Different cell lineages are represented by different colors. Analyses show that individual feather buds or individual barb ridges are made of cells from different lineages, not from a single lineage. Therefore, the local environment at the time of feather morphogenesis is more important than lineage. B-D, from Chuong et al., 1998.