

# Development and Evolution of the Amniote Integument: Current Landscape and Future Horizon

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**ABSTRACT** This special issue on the development and evolution of the amniote integument begins with a discussion of the adaptations to terrestrial conditions, the acquisition of water-impermeability by the reptilian integument, and the initial formation of filamentous integumentary appendages that pave the way towards avian flight. Recent feather fossils are reviewed and a definition of feathers is developed. Hierarchical models are proposed for the formation of complex structures, such as feathers. Molecular signals that alter the phenotype of integumentary appendages at different levels of the hierarchy are presented. Tissue interactions and the roles of keratins in evolution are discussed and linked to their bio-mechanical properties. The role of mechanical forces on patterning is explored. Elaborate extant feather variants are introduced. The regeneration/gene mis-expression protocol for the chicken feather is established as a testable model for the study of biological structures. The adaptations of the mammalian distal limb end organs to terrestrial, arboreal and aquatic conditions are discussed. The development and cycling of hair are reviewed from a molecular perspective. These contributions reveal that the structure and function of diverse integumentary appendages are variations superimposed on a common theme, and that their formation is modular, hierarchical, and cyclical. They further reveal that these mechanisms can be understood at the molecular level, and that an integrative and organismal approach to studying integumentary appendages is needed. We propose that future research should foster interdisciplinary approaches, pursue understanding at the cellular and molecular level, analyze interactions between the environment and genome, and recognize the contributions of variation in morphogenesis and evolution. *J. Exp. Zool. (Mol. Dev. Evol.) 298B:1-11, 2003.*

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## INTRODUCTION

The integument mediates between an organism and its environment and is accordingly diverse. It includes not only the epidermis and dermis, but also integumentary derivatives, namely glands and appendages, such as scales, hairs, feathers, claws, and teeth. Various associated structures, such as dermal and subcutaneous muscles, connective tissue, nerves, and blood vessels link the integument to the rest of the organism. The rich biology of vertebrate integuments was reviewed by Bereiter-Hahn ('86 and references therein). In this special issue, we focus on recent progresses in amniotes, i.e., the reptiles, birds, and mammals (Fig. 1).

The function of the integument is as diverse as its structure. As amniotes adapted to land conditions, early reptiles acquired protection against excessive evaporative water loss through the body

surface by lipidization and cornification of their epidermis. Protection against mechanical trauma was achieved by the formation of cornified scales that are separated by a less cornified epidermis to provide a tough, yet flexible body surface. Later, amniotes evolved filamentous integumentary appendages, such as feathers or hairs, which expanded the possible roles of the integument. By forming a contiguous feather coat or pelage, these integumentary appendages are able to protect against heat loss, as well as against water loss and mechanical trauma. The interfollicular

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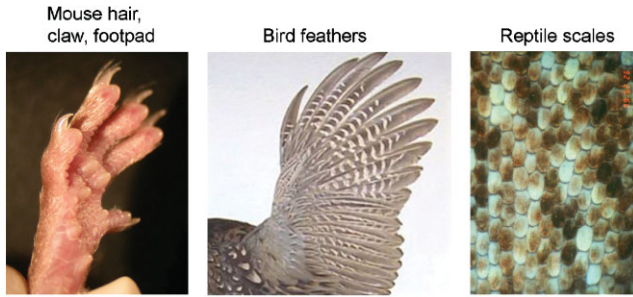


Fig. 1. Representative amniote integuments. Mouse paw shows hair, claw, and footpad. Pheasant wing shows remiges and covert feathers. Lizard trunk shows scales.

epidermis between these integumentary appendages could, therefore, reduce its cornification and become pliant and elastic as part of the hydraulic skeleto-muscular apparatus that is needed to move and stabilize the integumentary appendages (Homberger and de Silva, 2002). Integumentary appendages can also serve intra- and interspecific communication (e.g., the tail of peacocks, the raising of hairs), tactile functions (e.g., vibrissae, filoplumes), and defense (e.g., quills of porcupines, spines of hedgehogs). The skin can also synthesize chemical compounds with vitamin, neuro-endocrine or immunological functions (Chuong et al., 2002 and references therein).

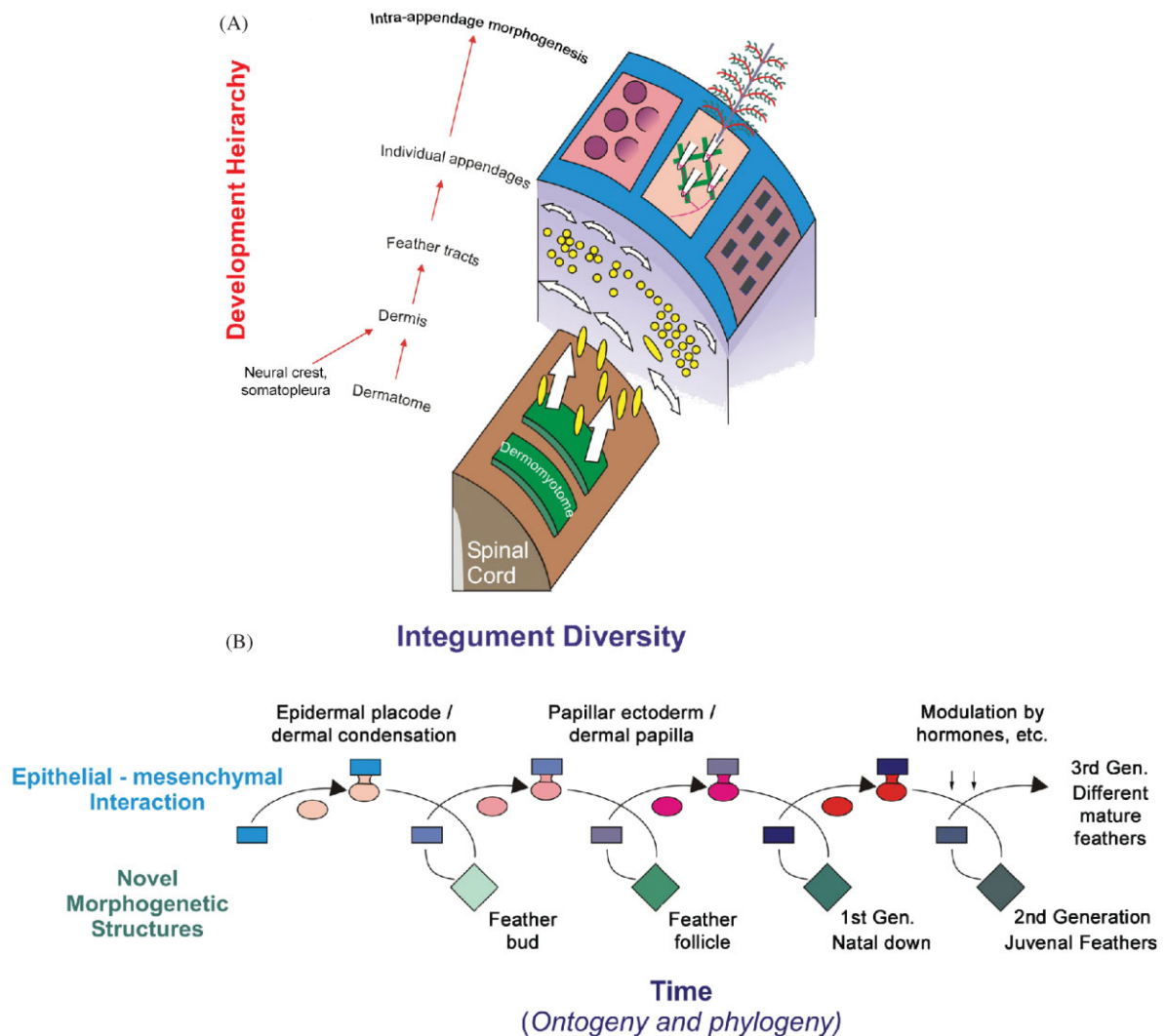


Fig. 2. Modular, hierarchical, and cyclical morphogenesis of the integumentary appendages. Panel A shows different levels of skin development. Panel B shows how the induction between epidermis and mesenchyme occurs in feather buds, feather follicles, and the subsequent molting cycles. At each level or cycle of morphogenesis, signaling molecule pathway

modules are recruited to regulate specific cellular events (e.g., proliferation, migration, apoptosis). The same pathway may perform different tasks in different contexts. Not all integumentary appendages have evolved to include all the levels or cycles. Through these opportunities for modulation, a great diversity of integumentary appendages can be achieved.

TABLE 1. Major interesting issues in the biology of integuments

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- Structure, differentiation characteristics and functional morphology.
  - Pattern formation.
  - Topological organization (size, axis, symmetry) of tissues/organs thorough cell proliferation, migration, death and differentiation.
  - Determination of phenotypes (hair, glands, scale, feathers, etc.)
  - Control of cycling and regeneration. Regulation of stem cell progression.
  - Pigmentation patterning, chemical and structural colors.
  - Hormone regulation, sexual dimorphism.
  - Neuro-muscular control for locomotion.
  - Biomechanical analyses.
  - Appendages as sensory organs.
  - Genetic analysis or integument phenotype variations.
  - Evolutionary origin, Verifying Evo-Devo models.
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While the influence of natural selection on the course of evolutionary modifications is understood in principle, the molecular and developmental processes that give rise to them are less well understood (Wagner, 2000; Wilkins, 2001). The purpose of this special issue is to identify some fundamental biological questions to which research on the integument as a model can contribute (Table 1). This issue will present examples of the great complexity of the integument and explore how these structures are created in development and evolution. Easily accessible models, as well as extreme examples, will be used to reveal common principles of integumentary organ formation, to detect modulatory mechanisms to create diverse structures, and to trace the evolutionary history of new integumentary organs.

The history of research on the integuments of reptiles, mammals, and birds has taken rather different paths (Bereiter-Hahn, '86, Maderson and Homberger, 2000; references therein). The integument of reptiles has been widely used for systematic purposes, but relatively little is known about its microanatomy, especially in comparison to the microanatomy of the integument of birds and mammals. At the molecular level, the evolutionary significance of beta keratins in *sauropsids* was studied (Sawyer et al., 2003), but much more work remains to be done in this area. Research on the mammalian integument is mainly driven by clinical (human and mouse hairs) and economic considerations (sheep wool and horse hooves), although special modifications of hairs (e.g., vibrissae, spines) have been conducted, and the

morphology and coloration of hairs have been used for systematic purposes. The advent of molecular biology and genetically engineered mice has transformed the mammalian integument into a major model for studying molecular pathways and stem cell regulation (Koster et al., 2002; Fuchs and Raghavan, 2002). The integument of birds occupies a special place among the amniote integuments because its structure, function, biology, physiology, development, and evolution are relatively well known. Landmark publications on the development and morphology of the avian integument are those by Lucas and Stettenheim ('72) and Sengel ('76).

### A MEETING TO SURVEY THE CURRENT RESEARCH LANDSCAPE

Late in 1999, J. Matthias Starck, the Secretary-General of the Sixth International Congress of Vertebrate Morphology (ICVM-6) (Starck, 2001), and the Chair of the Scientific Program Committee, had the vision to suggest that Cheng-Ming Chuong and Dominique Homberger organize a symposium on the development and evolution of feathers. By that time, feathers had gradually emerged as a hot topic due to the spectacular discoveries of feathered fossils in China (see Maderson and Homberger, 2000). From another end, progress had been made at the molecular level, trying to identify molecular basis of tissue interactions as described by Sengel ('76) (Chuong, '93; Chuong et al., 2000a, and references therein).

Both Chuong and Homberger had started to work on the development and functional morphology of the avian integument, respectively, in the mid-1980s (Chuong and Edelman, '85; Homberger and Brush, '86) and had recently organized symposia and edited proceedings related to the development and evolution of feathers (Chuong, '98; Maderson and Homberger, 2000). The idea to organize a symposium in which their expertise could be integrated appealed to both. They met in person at the 5th Symposium of the Society of Avian Paleontology and Evolution (SAPE) in Beijing, China, June 1–4, 2000. At that meeting, they also met Dr. Günter Wagner, editor of *Journal of Experimental Zoology Part B (Molecular Development and Evolution)*, who agreed to publish the proceedings of the ICVM-symposium in a special issue.

The symposium “*Development and Evolution of the Amniote Integument and its Accessory Structures*” was held in Jena, Germany, July 21–26,

2001 (Bragulla and Hirschberg, 2001; Chuong et al., 2001; Chuong and Homberger, 2001; Homberger, 2001a; Hou, 2001; Krättli, 2001; Prum and Dyck, 2001). After the symposium, Chuong and Homberger discussed with Gunter Wagner plans to expand the planned special issue to provide a broader context.

With the adaptation of amniote vertebrates to land conditions, the integument assumed new roles to ensure a successful interaction between the organism and the terrestrial environment: (1) protection against excessive water loss by evaporation through the body surface; (2) protection against mechanical trauma; and (3) locomotion through interactions with the environment, such as land and vegetation surfaces, air, and water. The actual evolutionary history that led from an aquatic to a terrestrial integument, and subsequently to the transformation of a reptilian integument to either an avian or a mammalian integument, has to be reconstructed from indirect evidence that can model the evolutionary processes and events that may have taken place in the evolutionary past. As discussed in this special issue, the lipidization and cornification of the epidermis of terrestrial reptiles is indicative of the major transformation of the integument of aquatic ancestors to that of reptiles as a protection against excessive water loss (Alibardi, 2003).

Fossil feathers and other integumentary appendages provide a yardstick against which to measure the conjectures and hypotheses that are developed based on indirect and experimental evidence (Chuong et al., 2003). Similarities in the developmental and molecular biology of the integument and, especially, the “beta-keratins” of alligators (as representatives of the archosaurs) and birds illuminate the evolutionary origin of feathers (Sawyer and Knapp, 2003). A theoretical model provides a conceptual framework for the hierarchical and modular construction of the complex feathers and their developmental and evolutionary variations (Prum and Dyck, 2003). The diversity of structures in domesticated birds provides an estimate of the breadth of genetic variability, which may be greater under the relaxed selective regime of captive conditions than that observed in natural populations (Bartels, 2003). The molecular processes underlying the morphogenetic pathways of developing chicken feathers serve as models for the molecular evolution of feathers (Yu et al., 2002; Widelitz et al., 2003).

Functional morphological studies can gauge the selective regime that may have been responsible

for driving the evolutionary modification of a particular structure (Bartels, 2003; Bragulla and Hirschberg, 2003; Hamrick, 2003; Homberger and de Silva, 2003). The direct mechanical interactions between the integument and its environment (i.e., land surfaces, air, and water) make locomotion possible. To move on a surface, the integument and its accessory organs need to sustain impacts and generate sufficient friction (see e.g., Bragulla and Hirschberg, 2003; Hamrick, 2003). On land, the appendicular end organs of the limbs (e.g., hooves, foot pads, finger tips, claws) have diversified to facilitate locomotion on various surfaces and need to be especially resistant to mechanical abrasion and injury (see Bragulla and Hirschberg, 2003; Hamrick, 2003). Movable imbricate scales are instrumental for the locomotion of limbless vertebrates, such as snakes. Skin folds in gliding reptiles and mammals (e.g., geckoes, sugar gliders) and flying mammals (e.g., bats) facilitate aerial locomotion. Feathers are responsible for the aerodynamically streamlined body and the airfoils of the wings and tail that enable birds to fly. Furthermore, to move through air, the integument needs to ensure a laminar airflow over the body surface (e.g., see Homberger and de Silva 2003). The various physical interactions between the body surface and the environment are complex and dynamic, and they create powerful selective regimes that are responsible for a significant part of the structural diversity of the vertebrate integument (see Bragulla and Hirschberg, 2003; Hamrick, 2003; Homberger and de Silva, 2003).

Experimental studies on hair development and hair cycling has emancipated mammalian skin into a model for analyzing tissue regeneration, epidermal stem cell regulation, and temporal cycling of organs (Botchkarev and Paus, 2003).

Although the present selection of contributions represents only a fraction of the actual diversity of recent and ongoing studies that relate to the vertebrate integument, we hope that these papers represent a good survey of what the landscape of integument research looks like at the beginning of the 21st century. We hope this special issue will stimulate, and be followed by, new syntheses in the future.

## AN EMERGING CONSENSUS

At this point in time, a consensus is emerging on the fundamental principles in integument morphogenesis.



### ***A common theme and its superimposed elaborations***

Despite their diversity, the integumentary appendages of the various amniotes (Fig. 1) show many commonalities early in their development. They are composed of epithelial and mesenchymal cells and result from interactions (i.e., signal exchange) between these two tissues. In most cases, epithelial cells need to become competent to differentiate further, while mesenchymal cells provide the information for the specific differentiation of the integumentary appendages. If this process is perturbed, such as in genetic mutants, congenital anomalies, or through experimental manipulations, the phenotypes of the integumentary appendages can be converted from one type to another (Hardy and Bellows, '78; Dhouailly et al., '80; Fisher et al., '88; Chuong et al., 2003). Hence, the specific elaborations of the integumentary appendages are considered as variations of a common theme (Chuong, '98).

The various integumentary appendages pass through a series of developmental stages: induction, morphogenesis, differentiation, and molting or regeneration (Widelitz et al., 2003; Fig. 2). Individual appendage primordia are first induced in certain arrangement patterns on the surface of an organism's integument. Epithelial cells within the primordia subsequently respond by morphogenetic movements; they may protrude above the skin surface, invaginate, branch, or form local thickenings. Once the anlage of an epithelial organ is formed, it starts to differentiate structurally (e.g., rachis of prospective feather, claw) or chemically (e.g., secretion of substances). Not all integumentary appendages undergo molting cycles, but many have the capacity to regenerate following injury.

The epidermal portion of the integument and its appendages consist of keratinocytes that assume specific arrangements in different integumentary appendages. These keratinocytes receive signals from both the mesenchymal and epithelial cells. They traverse temporally and spatially specific cellular events, including cell proliferation, migration, adhesion, differentiation, and apoptosis, to achieve different configurations of epithelial organs. Some of the same fundamental processes can be observed during the development of very different organs. For example, the periodic and elaborate invaginations of the epidermis into the dermis occur in both feathers and horse hooves (Bragulla and Hirschberg, 2003).

### ***The hierarchical, modular, and cyclical formation of integumentary appendages***

Integumentary organs can reach great complexity because they develop in a modular fashion through hierarchical levels and cyclical progression (Fig. 2). At each point of their development, the outcome of morphogenesis can be modulated by replacing modules that stimulate different cellular events at different times and locations (von Dassow and Munro, '99; Prum and Dyck, 2003).

During the development of the skin, the dermis has to form beneath the embryonic ectoderm (Fig. 2A). The mesenchyme of the presumptive dermis originates from the dermatome, somatopleura, or cephalic neural crest (Sengel, '76). The mesenchymal cells migrate toward the body surface, where they meet the epithelia of the prospective epidermis, including part of the oral mucosa. When the mesenchymal cells reach their final destination, they stimulate the epidermis to form skin and integumentary appendages. Within each body region, the skin and integumentary appendages assume locally specific shapes and structures, such as feathers versus scales, flight feathers versus contour feathers, or teeth versus oral mucosa. The shaping of the individual integumentary appendages occurs in several hierarchical steps (Prum and Dyck, 2003). Layer upon layer, molecular modules can be called upon and used in different contexts to build an increasingly complicated structure (Harris et al., 2002). Furthermore, the individual integumentary appendages need to be integrated within the organism (Homberger and de Silva, 2003).

Postnatally, certain integumentary appendages undergo cyclical replacement, or molting (Fig. 2B). By retaining a few keratinocytes with stem cell properties, integumentary appendages, such as hairs and feathers, have the capacity to regenerate completely. During the growth phase of the replacement cycle, the integumentary appendages are induced through an interaction between the dermal papilla of the integumentary appendage and the keratinocyte stem cells. These stem cells remain apart during the other phases of the replacement cycle and the morphogenetic and differentiation stages of the integumentary appendages. The replacement process is best understood for the cycling of hair and its anagen, catagen, exogen and telogen phases (Botchkarev and Paus, 2003). Each replacement cycle provides new options for the developmental program of

the integumentary appendage to be reset and to modify the shape, size, and structure of the integumentary appendages. This phenomenon, for example, can be observed in the hormonal modulation of integumentary appendages (e.g., bird feathers that change during breeding seasons). The length of the integumentary appendage can also be modulated by varying the length of the growth phase. In Angora mice, for example, the long hairs are the result of an anagen phase that is a couple of days longer than normal (Sundberg et al., 1997). Similar changes may occur in the pelage of some hares that have longer hairs in winter, but shorter ones in summer.

### ***The development of integumentary appendages in molecular terms***

Over the past decade, several molecular pathways underlying the morphogenesis of integumentary appendages were identified. This discovery was catalyzed by *Drosophila* genetics, in which mutants with modified numbers of bristles (e.g., smoothed, hedgehog) or modified types of appendages (e.g., antennopodia, in which a leg replaces an antenna) have led to the discovery of a series of signaling molecules that are involved in morphogenesis (reviewed in Wilkins, 2001 and references therein). Homologues of these molecules were identified and found to be also involved in the morphogenesis of the vertebrate integument (Table 2, references therein). In birds, during the formation of the skin (Fig. 2A), Wnt 1 is required for the formation of the dermis from the dermatome (Olivera-Martinez et al., 2001).

In the induction of feather buds, beta-catenin is essential for epithelial competence. The appearance of periodic patterning results from the interaction of extracellular activators (e.g., FGF, SHH) and inhibitors (e.g., BMP) in a mechanism that involves reaction diffusion. Subsequently, Wnt 7a and Notch are involved in setting up the antero-posterior axis of feather buds (Widelitz et al., 2003). In feather filament morphogenesis, BMP and SHH are involved in barb formation (Yu et al., 2002; Harris et al., 2002). In addition, appropriate keratin differentiation is required in scales, feathers, and other appendages (Sawyer and Knapp, 2003).

In mammals, the activity of the Wnt/beta-catenin pathway is critical for early hair germ induction (Millar, 2003; Fuchs and Raghavan, 2002), and the level of activity may specify the fate of hairs, sebaceous glands, and the epidermis. In mice, there are primary and secondary hair follicles. The formation of the primary hair follicles is based on the activity of the Eda pathway, while that of the secondary hair follicles is dependent on noggin (a physiological antagonist of BMP) activity. After birth, the progression of hair cycles between anagen/catagen, telogen/anagen, etc., are also regulated by different molecular pathways. Some molecules promote the progression, while others inhibit it. Therefore, the length of each phase is the result of a balance of molecular activity (Botchkarev and Paus, 2003). In the case of Angora mice, the mutation affects FGF5, which is normally required to end the anagen phase, and leads to an extended anagen phase and longer hairs (Hebert et al., '94). Although not covered in this issue, there is also

TABLE 2. Major morphogenesis related signaling molecular pathways

Ligands		Signaling Pathway	Functions	References
Agonist	Antagonist			
FGFs	Sprouty	Tyr phosphorylation	Proliferation	Wilkie et al., 2002
BMPs	Noggin Gremlin, etc.	SMADs, Ser phosphorylation	Differentiation, Apoptosis	Botchkarev, 2003 Hogan, 1999
SHH	Hip	Ptc, Smo, Gli	Signaling Center	Oro and Higgins, 2003 Chuong et al., 2000b
Notch	Hairless	Delta, Serrate, Su(H)	Fate Specification, Growth control	Lin et al., 2000; Lowell et al., 2000
Wnts $\beta$ -catenin	DKK Sfrz, etc.	$\beta$ -catenin (canonical) PKC	Stem cell property, Cell rearrangement, Proliferation	Millar, 2003 Widelitz et al., 2000,
Eda		Tumor necrosis receptor like	Fate Specification, Morphogenesis, Apoptosis	Headon et al., 2001

\*The functions and pathway members listed here are for exemplary purposes and are not comprehensive. References are selected when those related to the integument are available. They are listed only as guides for integument biologists who like to learn more about these pathways.

new information about the evolutionary development of mouse teeth (Keränen et al., '98; Jernvall et al., 2000). An understanding of the molecular biology of other integumentary appendages of mammals is less comprehensive, but has begun to be elucidated. For example, the formation of distinct foot pads, papillary ridges, or sebaceous glands may be regulated by homeobox genes (Hamrick, 2003).

Hence, similar molecular signaling modules can be called upon to do different jobs at different stages of morphogenesis. By analyzing morphogenetic processes of integumentary appendages at the molecular level, their commonality is even more noticeable (Table 2). It is the spatial and temporal regulation of the activities of these molecular pathways that modulates cell behaviors at different hierarchical levels and in each cycle. With so many opportunities for modulation, evolutionary novelties can be generated by duplication and modification of molecular pathways.

### ***The need for an integrative and comparative approach***

To acquire a comprehensive understanding of its evolutionary development, the integument has to be considered as a part of a whole, mechanically coherent organism, because the various structural elements of an organism exert a multitude of influences on the integument. For example, integumentary appendages are connected to and affected by muscles, nerves, blood vessels, etc. and integumentary patterns may be induced and maintained by regimens of mechanical forces acting on, or generated by, the organism (Bragulla and Hirschberg, 2003; Homberger and de Silva, 2003).

In addition, natural selection, through the interaction of an adult organism with its environment, exerts yet another level of coordinating control over the development and final phenotype of the integument. This level of integration can be understood only if the various organ systems of an organism have been analyzed and integrated into a mechanically coherent model of the organism. For example, the loss of teeth and the formation of a cornified beak in birds was driven by a selective regime favoring aerodynamically streamlined body contours, which are characteristic of avian flight (Homberger, '99, 2002; Homberger and de Silva, 2000). With the development of teeth and integumentary appendages being under similar molecular control, it is plausible to imagine that

the overall regimen of mechanical forces arising from the interactions between a bird and its environment during flight may impose coordinating constraints on the expression of genes.

## **FUTURE ISSUES**

### ***Multi-disciplinary integration***

Scientists of different disciplines usually specialize on different issues. Systematists may focus on the diversity of certain integumentary traits. Evolutionary biologists may be interested in the emergence and disappearance of species and the reasons for the selective survival of certain phenotypes. Paleontologists may want to discover fossils that represent missing links. Developmental biologists may investigate how certain shapes and structures are made during morphogenesis. Cell biologists may focus on molecular pathways. Theoretical biologists may be fascinated by regular or irregular patterns. Dermatologists may study the clinical and physiological aspects of the skin and apply medical technologies. Plastic surgeons may be interested in the healing process of the skin. Tissue engineers may want to isolate stem cells and try to generate skin in vitro. Therefore, interdisciplinary collaboration, drawing from different backgrounds, perspectives and approaches, has the greatest potential to bring together all the knowledge and tools that are needed for a comprehensive understanding of the biology of the integument.

### ***Identification of the molecular basis of developmental processes***

How are the one-dimensional DNA codes translated into the three-dimensional structures and modified through evolution? Some of these processes are understood at the molecular level, and it turns out that many developmental genes are similar among animals (Table 2). The redundant genes provide vertebrates with more options to generate variations. In research, one approach is to study the roles of these candidate molecules. The other approach is to use genetics to identify genes in human or mouse mutants that affect the integument (e.g., Ahmad et al., '98). Yet a third, new approach is through genomics as the genomes of several organisms have been sequenced to date and allow comparative studies to analyze molecular mechanisms that are responsible for variations.

### ***Correlating the structural, physical, chemical and molecular properties of integumentary appendages***

Differences in the physical properties of integumentary organs and appendages, such as rigidity, flexibility, resistance to abrasion, etc., depend on the interplay between the material properties of epidermal tissues on the one side and the internal configuration and external shape of the integumentary structures on the other side. Different types of feathers exhibit different degrees of strengths and softness (Bonser, '96a, b, 2001) and different parts of the skin have different mechanical properties (Shadwick et al., '91; Bauer et al., '93a, b). Since integumentary appendages are cornified structures, differences in their physical properties also reflect differences in the structure of keratin molecules (Homberger and Brush, '86). Hence, a more detailed knowledge of the chemical composition and genetic control of keratins will elucidate the correlation between molecular structure and physical property. Along this line, there is evidence for gene duplication in the evolution of various types of alpha and beta keratins (Sawyer and Knapp, 2003). The degree of differences among the keratins could, hence, be used as an indirect measure of genetic and evolutionary distances.

### ***Interactions between the genome and the environment***

Recent experimental assays in the developmental biology of the integument have focused on the roles of molecules and their genetic basis. However, evidence is mounting that environmental factors can influence gene expression, either in physiological conditions or during development. For example, oxygen concentration during development can influence the level of tracheal tube branching (Jarecki et al., '99). Mechanical forces may be instrumental in modulating and even inducing the timing, distribution, and local activity of gene expression and signaling molecules (Homberger and de Silva, 2003). Research on human pathological conditions (e.g., atherosclerosis and osteoporosis) has shown that mechanical forces may affect gene expression through integrin and, subsequently, the enhancer region of certain genes. In the chick embryo, the primary row of feather primordia appears along the dorsal midline where growth, probably through cell proliferation, is faster (Sengel, '76). It is not unreasonable to speculate that the formation of

this primary row occurs in reaction to the strains arising from this growth (Wessels and Evans, '68). Future experimental assays will need to take into account the possible effects of mechanical and other physical forces on the expression, actions and distribution patterns of signaling molecules. In addition, the enhancer control of gene expression will need to be examined.

### ***Deciphering the rules of morphogenesis***

Progress in understanding the rules of morphogenesis has been made at the molecular and organismal levels, but not as much at the tissue and organ levels. Evolutionary novelties that add new axes to organs, or new levels of organization based on molecular pathway modules, have been proposed (Chuong et al., 2000a; Harris et al., 2002). Analyses of the horse hoof and avian feather showed remarkable branching patterns at the level of epithelio-mesenchymal interactions that may be based on similar morphogenetic principles (Homberger, 2001b; Bragulla and Hirschberg, 2003). Heterochrony may explain the evolutionary origin of feather evolution (Sawyer and Knapp, 2003). The hierarchical model of feather morphogenesis creates a conceptual framework from which testable hypotheses can be derived. (Prum and Dyck, 2003, Fig. 2A). However, the common rules, which underlie the morphogenesis of the diverse integumentary appendages, still need to be identified.

Although genes control the sequence of steps that are involved in the morphogenesis and distribution patterns of integumentary appendages, it would be unrealistic to assume that they control the exact shapes and precise positions of each individual integumentary appendage. Because there are micro-variations in local conditions within the overall design of an organism, it is more realistic to assume that integumentary patterns are the result of an equilibrated balance of developmental rules and environmental influences, than to assume that it is the simple readout of a genetically coded blueprint (Jiang et al., '99). Therefore, integumentary patterns are similar but not identical among different individuals. In human finger prints, for example, the width of the ridges is constant because it follows developmental rules. The final pattern and branching are the result of randomness, so that fingerprints can be used to identify individuals. In another example, contour feathers are oriented and shaped specifically and individually to ensure



that they contribute to a coherent and contiguous feather coat across the body surface. In other words, the morphogenetic program of contour feathers must be flexible enough to allow local variations to modulate the final shape and size of feathers so that aerodynamically streamlined body contours can be created. Because the strains and stresses acting on the integument are highly variable in each region and at every point of the skin surface, it is biologically realistic to conjecture that regimens of mechanical forces may affect the integrated development of the integument (Homberger and de Silva, 2003). Hence, we need to learn more about the roles of local factors in morphogenesis.

### ***Laboratory models and verification through diverse animals***

Laboratory models provide controls over the variables in an experimental setting for the study of the molecular and/or physical principles governing morphogenetic processes and to test hypotheses. The concentration in developmental biology on only a few model organisms under controlled conditions (e.g., mice, human, chicken, *Drosophila*, *C. elegans*) has provided us with a deep, though not yet complete, understanding of developmental processes (summarized in Wilkins, 2001). We are ready now to apply this knowledge to a greater diversity of organisms, such as domesticated birds with extreme feather phenotypes (see Bartels, 2003), and other animals in nature with unusual adaptations.

### **CONCLUSIONS**

All integuments and their appendages are derived from epithelial stem cells. To form different tissues and structures, they simply are organized in different ways. What needs to be learned is how these stem cells are guided by different molecular pathways to form different integumentary appendages. If we consider the analogy that the human genome project is like compiling the dictionary for the language of life, then each species, extant or extinct, is like a chapter in the book of nature. The task of evo-devo research is to understand the grammar of this language. This present special issue may have deciphered some aspects and fundamental principles of this grammar. But, on the basis of the above survey of the current research landscape, we also tried to present some key issues that we consider to be relevant for future research agendas.

We hope that the insights provided by the various contributions in this special issue will catalyze new understanding, foster new collaborations, inspire new approaches, and take us to a new level of understanding of the vertebrate integument.

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### **LITERATURE CITED**

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