

# Adaptation to the Sky: Defining The Feather With Integument Fossils From Mesozoic China and Experimental Evidence From Molecular Laboratories

CHENG-MING CHUONG<sup>1\*</sup>, PING WU<sup>1</sup>, FU-CHENG ZHANG<sup>2</sup>, XING XU<sup>2</sup>,  
MINKE YU<sup>1</sup>, RANDALL B. WIDELITZ<sup>1</sup>, TING-XIN JIANG<sup>1</sup>, AND LIANHAI HOU<sup>2</sup>

<sup>1</sup>*Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, California*

<sup>2</sup>*Institute of Vertebrate Paleontology & Paleoanthropology, Chinese Academy of Sciences, Beijing, Peoples Republic of China*

**ABSTRACT** In this special issue on the Evo-Devo of amniote integuments, Alibardi has discussed the adaptation of the integument to the land. Here we will discuss the adaptation to the sky. We first review a series of fossil discoveries representing intermediate forms of feathers or feather-like appendages from dinosaurs and Mesozoic birds from the Jehol Biota of China. We then discuss the molecular and developmental biological experiments using chicken integuments as the model. Feather forms can be modulated using retrovirus mediated gene mis-expression that mimics those found in nature today and in the evolutionary past. The molecular conversions among different types of integument appendages (feather, scale, tooth) are discussed. From this evidence, we recognize that not all organisms with feathers are birds, and that not all skin appendages with hierarchical branches are feathers. We develop a set of criteria for true avian feathers: 1) possessing actively proliferating cells in the proximal follicle for proximo-distal growth mode; 2) forming hierarchical branches of rachis, barbs, and barbules, with barbs formed by differential cell death and bilaterally or radially symmetric; 3) having a follicle structure, with mesenchyme core during development; 4) when mature, consisting of epithelia without mesenchyme core and with two sides of the vane facing the previous basal and supra-basal layers, respectively; and 5) having stem cells and dermal papilla in the follicle and hence the ability to molt and regenerate. A model of feather evolution from feather bud → barbs → barbules → rachis is presented, which is opposite to the old view of scale plate → rachis → barbs → barbules (Regal, '75; *Q Rev Biol* 50:35). *J. Exp. Zool. (Mol. Dev. Evol.)* 298B:42–56, 2003. © 2003 Wiley-Liss, Inc.

## INTRODUCTION

Among all organisms, birds have one of the most complex forms and physical structures. It allows them to live in water, land, and air. Birds today share fundamental features but show enormous diversity in order to adapt to different ecological environments (Lucas and Stettenheim, '72; Bereiter-Hahn, '86; Gill, '94). While adaptation to these different environments required the diverse morphological features of feathers, the basic functions of feathers are considered to be insulation, communication, and flight (Chiappe, '95; Chatterjee, '97; Feduccia, '99). In this issue, Alibardi's article discusses the essential adaptation of the reptilian integument to land. In this article, we will discuss

the adaptation of avian integuments to the sky. When did this evolutionary transformation begin? How did the feather evolve? Did feather-like appendages evolve only once, or more than once, in history? (also see Prum and Brush, 2002; Homberger, 2003; Sawyer and Knapp, 2003). To

---

Grant sponsor: NIH; Grant numbers: RO1AR42177, RO1AR47364; Grant sponsor: NCI; Grant number: R21CA094392; Grant sponsor: NSF; Grant sponsor: IBN9808874 to C.M.C.; Grant sponsor: NCI; Grant number: R01CA83716 to R.B.W.; Grant sponsor: NSP co-sponsored project "Study of Jehol Biota"; Grant number: 4982020; Grant sponsor: Institute of Vertebrate Paleontology and Paleoanthropology to F.-C. Z., X. X., L. H.

\*Correspondence to: Cheng-Ming Chuong, Department of Pathology, University of Southern California, 2011 Zonal Ave, HMR 315B, Los Angeles, CA 90033. E-mail: chuong@pathfinder.usc.edu

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.b.00025

support flight, what feather forms and accessory structures had to evolve before birds were adapted to the sky (Homberger and de Silva, 2002, 2003)?

It is generally thought that birds evolved from reptiles, although when they evolved and from which lineage remains controversial (Feduccia, '99; Chiappe and Witmer, 2002). The reptile integument is made mainly of scales (Landmann, '84; Alibardi, 2003). In birds, there are scales on the foot; most of the rest of the body is feathered (Lucas and Stettenheim, '72). A long held view is that avian feathers evolved from reptile scales; first through elongation of reptile scales, later through etching of the elongated scales to produce the branched feather vanes, and finally the interwoven pennaceous feather barbs became plumulaceous (Regal, '75). Two major events in the last decade have shaken this classical view and catalyzed a new understanding of the evolution of feathers. One comes from a series of discoveries of many intermediate forms of feather-like appendages from the Jehol Biota of China (also see Sawyer and Knapp, this issue). The other comes from progress in molecular developmental biology and the ability to change one appendage form into another using today's chickens as an experimental model (see Widelitz et al. in this issue).

Special conditions in the Jehol Biota in China allowed for the excellent preservation of soft tissues, hence many integuments of Mesozoic creatures evolving from reptiles to birds about 120 million years ago were well preserved in fossils (Chen et al., '98; Zhou et al., 2003, Fig. 1). The most remarkable discoveries are the various dinosaur skeletons bearing diverse feather-like appendages. While birds and feathers used to be considered equivalent, the statement "All feathered animals are birds" is no longer true. The statement "All branched skin appendages are feathers" is also challenged. When should a skin appendage be called a branched scale, a proto-

feather (Chen et al., '98), a non-avian feather (Jones et al., 2000), or a real feather (Xu et al., 2001)? Here we review the integuments of major feathered dinosaur and Mesozoic bird fossils from China, but also include related fossils from other regions. We will evaluate the early integument appendages of these creatures and compare them with the characteristics of today's feathers (Table 1).

All vertebrate skin appendages are made of epidermis and dermis, and are the result of epithelial mesenchymal interactions. They all go through induction, morphogenesis, and differentiation stages to achieve different phenotypes (Chuong, '98; Widelitz and Chuong, '99). Tissue interactions of feathers and scales have been analyzed in classical experiments that showed the reciprocal interactions between epidermis and dermis and their respective roles (reviewed in Sengel, '76; Dhouailly and Sawyer, '84; Zeltinger and Sawyer, '91; Song and Sawyer, '96). Since the 1990s, RCAS retrovirus mediated gene mis-expression has allowed direct molecular analysis of early events of feather morphogenesis (reviewed in Chuong et al., 2000; Widelitz et al., this issue; Fig. 3A, B). With the advent of Evo-Devo research, developmental models have been proposed (Prum, '99; Chuong et al., 2000; Alibardi and Sawyer 2002; Harris et al., 2002; Sawyer et al., 2003a). However, it has been difficult to do molecular analyses of later events in feather morphogenesis. The novel plucking/regeneration/molecular mis-expression feather model developed by Yu et al. (2002; and Fig. 3C, D) has opened a new way to analyze the forms of adult feathers. Through these experiments, we now are able to tackle the molecular pathways underlying feather morphogenesis and alter feather forms (Fig. 5B). We can assume that some of these changes may reflect what happened in evolution. We will present several examples

TABLE 1. *Developing a definition for feathers\**

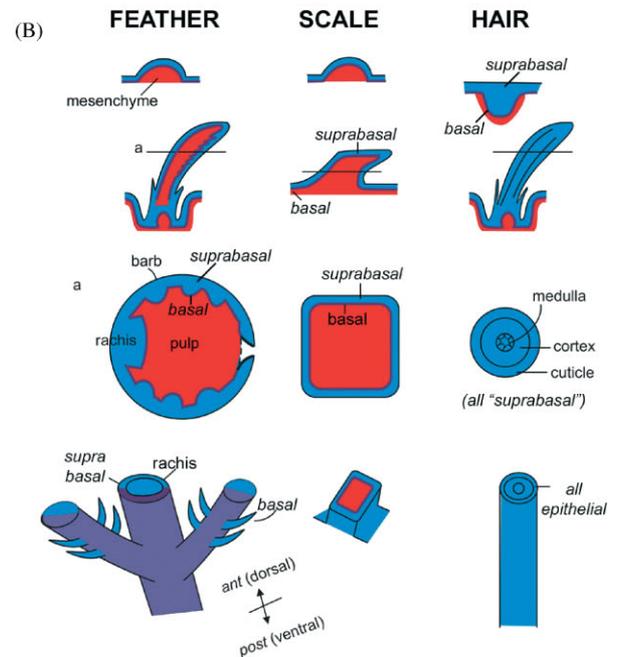
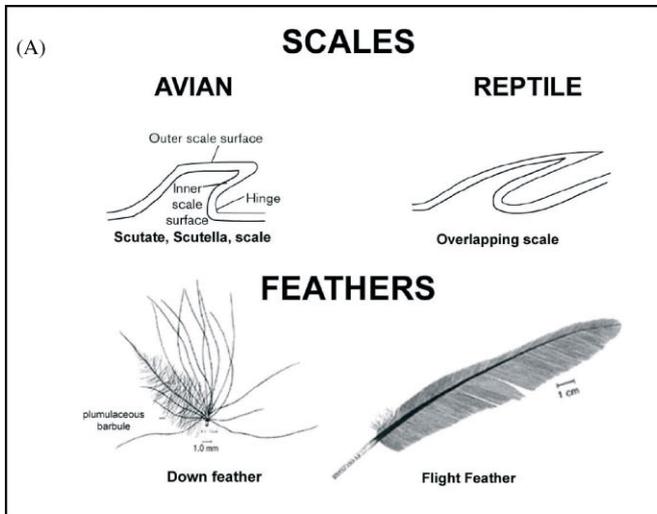
- 
1. Has localized zones of proliferating cells positioned proximally, with a proximal–distal growth mode.
  2. Forms hierarchical levels of branches of rachis, barbs and barbules. Barbs form by differential cell death, and can be bilaterally or radially symmetric.
  3. Has a follicle structure, with mesenchymal core wrapped inside during development, forming the pulp.
  4. When matures, the two sides of the feather vane face the previous basal and supra-basal layer respectively. The pulp is gone.
  5. Has stem cells and dermal papilla in the follicle, hence the ability to go through molting cycle physiologically and to regenerate after plucking.
- 

\*Here we mean true avian feathers today have all these characteristics. The numbers do not indicate the order of appearance or pre-requisite for subsequent characters. For those branched appendages that only have some, but not all of the characteristics, the research community will have to decide to call them branched skin appendages, feather-like appendages, proto-feathers, non-avian feathers, or else.



Fig. 1. 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*. g-l, close up view of feathers. g, tail feathers of Protopteryx show dominant rachis (Zhang and Zhou, 2000) as suggested by

Regal, 1975 (Fig. 5A). j, remiges of Confuciusornis. h, i, k, l, dissociated feathers. Size bar from panel g-l, 1 cm. Panels b-d reprinted with permission from National Geographic (Ackerman et al., 1998). Panel e is from Xu et al., 2003. Panel f is from Hou et al., 2000. Panel h-l is from Hou 1997, pp 61-64.



and discuss how they have influenced our thinking on feather evolution.

***Jehol Biota, a Mesozoic landscape that preserved the integuments of intermediate species during the reptile – bird transition***

The Jehol Biota spreads across the northern part of China and contains fossils of organisms that lived 120–145 million years ago (mya) as determined by isotope and plant dating methods. The Jehol Biota occupies a wide region including the Yixian Formation, Jiufotang Formation, and other regions (Fig. 1a, Chen et al., '98). It is a geological layer, in some parts 50–100 m thick, that represents the transition between the late-Jurassic and early Cretaceous times. The landscape used to be rich in freshwater lakes with active volcanoes nearby and, therefore, contains many well-preserved biological specimens, including their soft tissues. Recent excavations and research on its plants, invertebrates, fishes, amphibians, reptiles, birds, and mammals, as well as on its geology and climate, have established the Jehol Biota as one of the best-preserved ecosystems (reviewed in Zhou et al., 2003) in the world. The Jehol Biota provides a valuable window to the biological diversity of the Mesozoic period. Each fossil specimen offers an opportunity to examine how the animal interacted with its environment and with other species.

Birds were still evolving from reptiles during this period, and their forms underwent intensive “re-engineering” in order to be adapted to the sky. They transformed from a primarily land-dwelling tetrapod form, to a smaller, winged bipedal animal, which lived mainly in the trees and traveled through the air. The Jehol Biota in China provided a unique record in which the different integuments in transition were extraordinarily well-preserved. This is particularly valuable for the analysis of skin appendage evolution from dinosaurs to birds. It provides a rich source of new information for studying the origin and

evolution of feathers and other integument related structures.

***Feathered dinosaurs?***

The major events during the evolution from reptiles to birds have been reviewed (Chiappe, '95; Chatterjee, '97; Feduccia, '99; Chiappe and Witmer, 2002). The discovery of several fossils, including *Archaeopteryx* (145 mya) in Germany, led to the compelling dinosaur-bird hypothesis, suggesting that birds evolved from the dinosaur (Ostrom, '74). Dinosaurs began flourishing in the Late Triassic, about 215 mya, and dominated the earth for the next 150 million years (Sereni, '99). During the Triassic, the dinosaur clade split into two groups, the Ornithischia and the Saurischia. The latter gave rise to the hypothesized bird ancestors, the theropods. Before the Triassic ended, theropods had branched into the tetanurans from which the coelurosaurs arose. Coelurosaurs eventually evolved into the maniraptorans, which, in the Late Jurassic, gave rise to Aves, the bird clade. Later, Aves gave rise to the Ornithothoraces that then branched off into the Enantiornithes (“opposite birds”) and Euornithes (“true birds”). The latter group radiated in the Late Cretaceous and gave rise to the Neornithes, modern birds.

There are some objections to the dinosaur-bird hypothesis based on differences in skeletal structures. These authors consider that the bird and dinosaur share common ancestors, such as the basal archosaur, an ancient reptile, and the “feathers” found on dinosaurs resulted from later convergent evolution (Chatterjee, '97; Feduccia, '99). In either case, one has to agree that feathers evolved from the reptile integument, and that there is a gradual transformation from the simple scale to the advanced forms of feathers (Prum, '99; Maderson and Homberger, 2000; Chuong et al., 2000; Sawyer et al., 2003a, b), or through heterochrony of appendage morphogenetic events during embryogenesis (Sawyer and Knapp, this issue).

Fig. 2. Morphology and topological organizations of feathers, scales, and hairs. A. Avian foot scales and reptiles are very similar in morphology. However, their homology in evolution remains to be determined. Note the radial and bilateral symmetry of downy and flight feathers respectively. Also see Fig. 1 of Prum and Dyck in this issue for a contour feather. Panel A is modified from Lucas and Stettenheim ('72) and Chuong et al. (2000). B. Comparison of epithelium and mesenchyme composition of feathers, scales and hairs. Mature feathers are made of supra-basal epithelia only. The two sides

of feather vanes originally face the suprabasal and basal side respectively. The mesenchyme core only exists transiently during feather morphogenesis. Mature scales still have a mesenchymal core. All the scale surfaces are covered by the suprabasal layer. The mature hairs are made of suprabasal epidermal cells. Blue, epithelium, or suprbasal epithelia; purple, basal side of the epithelium (note in mature feather, the basal layer is gone, but still shown here to illustrate the topology of feather follicles); red, mesenchyme.

The skeleton of *Archeopteryx* suggests that it is an intermediate form between reptiles and birds. It already has different types of feathers over the body (e.g., tracts have already evolved), toothed jaws, claws on the wing, and a bony tail. The wing flight feathers have a closed pennaceous vane and are asymmetric, suggesting that it can fly (Feduccia and Tordoff, '79), although it may not have been an excellent flyer. Therefore in the spectrum of reptile-bird transition, *Archeopteryx* is closer to birds than to reptiles. For over a hundred years, scientists have been hoping to find new fossils representing the earlier forms that are closer to the reptiles and have a glimpse of the process of feather evolution.

New findings from several sites in the Jehol Biota in China brought us new and exciting information. Theropods were a group of carnivorous, bipedal, terrestrial dinosaurs with small forelimbs and special predatory features, such as long hands with three digits for scratching and/or grasping prey (Padian and Chiappe, '98; Sereno, '99). Some non-avian theropod dinosaurs displayed a variety of skin appendage types, from simple filament-like proto-feathers to complex modern asymmetric feathers. *Sinosauropteryx* was among the first found to have well-preserved skin appendages (Chen et al., '98; Fig. 1b; about 120 mya). It has "fuzz fibers" on the body, especially along the dorsal midline. These filamentous "protofeathers" are about 20 mm long, ranging from 5 – 40 mm. They appear to be rather homogeneous over the body, without apparent signs of regional tract specificity. The filaments resemble down feathers, lacking aerodynamic properties. They may be hollow. They appear to have a short shaft with "barb" branches, but no further branching "barbules" were identified. These filaments may represent "proto-feathers" or some early branching skin appendages that may have provided insulation (Chen et al., '98).

Two theropods, *Beipiaosaurus* and *Sinornithosaurus*, had large patches of filament-like integumentary structures preserved on the forelimbs, hindlimbs, and body (Xu et al., '99a, b). These filaments are arranged in parallel to each other and almost perpendicular to the bone. Some of the filaments seem to have branching distal ends. These primitive filaments appeared to be hollow, resembling the cylindrical feather filament. Further analyses (Xu et al., 2001) showed that skin appendages on *Sinornithosaurus* (Fig. 1c) have compound structures containing multiple filaments that are joined together. There are two

types of branched structures identified. One is similar to avian downy feathers, and another one similar to avian pennaceous feathers, but lacking identifiable barbules. Another dinosaur, the smallest known non-avian theropod dinosaur, *Micro-raptor zhaoianus* (Xu et al., 2000), displayed a more advanced filament pattern near the femur. The filaments are long and contain a rachis. The fossil suggests that true feather structures may have already existed in these dinosaurs.

Feathers similar in shape to modern feathers were first found in *Caudipteryx* and *Protarchaeopteryx* (Ji et al., '98). *Caudipteryx* (Fig. 1d) evolved different types of feathers over different body regions, indicating the establishment of feather tracts as an evolutionary novelty. This allowed the development of different types of feathers on different parts of the body; specialized functions for each body part could evolve and enrich integument function. *Caudipteryx* formed spectacular pennaceous feathers in both the wing (remiges) and tail (retrices) with tapering shafts. The bilaterally symmetric pennaceous structures in *Caudipteryx* and *Protarchaeopteryx* have been accepted as vaned feathers (Prum and Brush, 2002). However, the vanes lacked the asymmetric vane required for flight and were probably used for display to either attract or frighten others. It is unknown if asymmetric feathers had yet to evolve, or if the asymmetry was lost in adapting to life on land. There were plumulaceous feathers covering the body, most notably at the hips and the proximal region of the tail. *Protarchaeopteryx* also had bilaterally symmetric pennaceous feathers. The tail retrice feathers of *Protarchaeopteryx* were plumulaceous in the proximal part and pennaceous above the mid-shaft region (Ji et al., '98). The vaned *Protarchaeopteryx* feathers appeared to be structurally transitional between the proto-feather-like structures of *Sinosauropteryx* and the modern feathers of *Archaeopteryx*.

Modern feathers were also detected in other non-avian theropod dinosaurs. Ji et al. (2001) reported a Dromaeosauridae covered with filamentous feather-like structures over its entire body. Three types of filamentous structures were identified in this specimen. The first type had single fibers. The second type had long plumulaceous fibers. The third type had symmetric pennaceous feathers, which may have had barbules. This type of pennaceous feathers with a rachis and symmetric barbs were also found in a different species of Dromaeosauridae (Norell et al., 2002).

The most interesting discovery to date among the feathered dinosaurs is the four-winged dinosaur recently reported by Xu et al. (2003), *Microraptor gui* of Dromaeosauridae (Fig. 1e). Both fore and hind limbs were covered with pennaceous feathers arranged in a similar pattern. Feathers at the distal limb portion had asymmetric vanes. The remiges were preserved with the primary remiges longer than the secondary remiges. This arrangement may have been for improved aerodynamics as similar patterns are observed in modern birds. The body was covered with plumulaceous feathers. The “flight feathers” in the hind limb are not well designed for active flight. The creature may have adopted a gliding behavior in the flourishing Mesozoic jungles, gliding from one tree to another as seen today in some mammals.

Skin appendages in non-avian theropod dinosaurs displayed a spectrum of integument appendages, from non-branched filaments to branched filaments to symmetric pennaceous vanes to asymmetric pennaceous vanes. Many of these skin appendages are considered possible homologues of avian feathers. The dinosaur integument coverings were probably very complex, including structures in addition to scales and feathers. The Mesozoic landscapes likely shaped many unusual appendages: some with branches and some not. Conceivably, there were many different occasions when having branched appendages was an adaptive advantage. Branched skin appendages may have formed independently in different ways. One should not take for granted that all branching appendages are feathers.

Let us look at some examples. A “non-avian feather” was reported for the Triassic archosaur, *Longisquama* (Jones et al., 2000). In the dorsal midline, *Longisquama* has a series of paired elongated integumentary appendages that form branches. However, the branches are perpendicular to the main axis and look like branched scales depicted by Regal ('75 [Fig. 5B]). The branches are atypical for those of feathers and have few resemblances to today's avian feathers. Integument appendages that existed and vanished through evolutionary history are given names by modern scientists. Given our current understanding of skin appendage evolution is it appropriate to call them “non-avian feathers,” or would a different category of names be more appropriate?

Recently, a bristle-like, non-branched integumentary structure was found in the non-theropod dinosaur (Mayr et al., 2002). They are in the tail of

the horned dinosaur (parrot-beaked dinosaur), *Psittacosaurus*. These bristle-like structures are much longer and thicker than the proto-feathers in *Sinosauropteryx* and *Sinornithosaurus*, and were interpreted as cylindrical and possibly tubular epidermal structures. As it is cylindrical, a characteristic considered very important in the first step of feather evolution (Prum and Brush, 2002), should we consider it as an early proto-feather, or as a modified feather, like bristles on modern birds, or similar to the bristles of the beard in wild turkeys (Sawyer et al., 2003b)? On the other hand, they may not be homologous to those integument appendages on the theropods.

A tentative set of criteria is proposed in Table 1. Should we name only those that fit all proposed criteria as true avian feathers? More research and revision is necessary to develop a consensus.

Another example of a potentially non-homologous skin appendage can be compared by analyzing integument appendages from an animal similar to those we are considering here: the turkey. Turkey beard bristles are structures distinct from feathers, although they express feather-type beta keratin and show simple branching patterns (Sawyer et al., 2003b). These appendages are hollow at the distal end, and the branching may be due to partial separation (Lucas and Stettenheim, '72). They lack follicles, yet grow continuously. Could these bristles, which resemble those found in *Sinornithosaurus*, be homologues of dinosaur filamentous integument appendages, or are they simply modified from modern avian feathers?

Paleontological research has shed new light on feather evolution. With the advent of EvoDevo research, the application of modern biochemical methods has begun to further enhance our understanding of this field. Using antibodies to beta keratin, Schweitzer et al. ('99) showed immunological cross reactivity with feather-like structures of the alvarezsaurid dinosaur, *Shuvuuia deserti*. Together with mass spectrometric data, they suggested that beta keratin existed in these dinosaurs. This type of molecular approach, when established, would be a revolutionary link between paleontology researches and molecular developmental researches.

### ***Feathers of Mesozoic birds***

The continuous reptile-bird transition led to the formation of Mesozoic birds together with *Archaeopteryx*, described above, as the prototype.

It had already evolved different types of feathers including down feathers, tail feathers arranged along the long bony tail, and asymmetric flight feathers. The crow sized *Confucisornis* from late Jurassic-early Cretaceous also had both down and flight feathers (Fig. 1f). They had evolved beaks with no teeth (Hou et al., '95, '96). The asymmetric flight feathers and toothless beak suggest it was a reasonably good flyer (Feduccia, '99; Homberger, 2002). The fossils even show sexually dimorphic tail feathers and these *confucisornis* birds appear to have lived in groups (Fig. 1F; Hou et al., 1996).

Enantiornithes is the dominant group of Mesozoic birds (Chiappe and Witmer, 2002; Hou et al., 2003). The skeletal characteristics suggest that they are different from modern birds. In China the major species of this branch include *Eoenantiornis*, *Cathayornis*, *Sinornis*, *Otogornis*, *Longipteryx*, etc. The other branch, Ornithurine, represents the ancestors of the modern birds. Major members found in Mesozoic China include *Liaoningornis*, *Chaoyangia*, *Gansus*, etc. Most of these interesting and unusual Mesozoic birds already have true feathers, although they seem to have reached different levels of streamlined body shape for adaptation to the sky. One may speculate that the neuromuscular control; the coordinated motions in the wing, tail, and trunk; and the different flight behaviors must also have been evolving (see Homberger, 2002; Homberger and de Silva, 2003). The Mesozoic birds in China are introduced in books by Hou ('97).

While Mesozoic feathers fit many criteria in Table 1, examination of feathers from Mesozoic birds show some unusual characteristics that are not seen in modern birds. For example, a primitive enantiornithine bird, *Protopteryx fengningensis*, has a thick, wide, and flat rachis in the elongate central retrices (Zhang and Zhou, 2000, Fig. 1g). The retrices of *Confucisornis* also have a similarly dominant rachis, although not to the same extent (Fig. 1b). What is the purpose of this spectacular tail feather? Was it used for display, similar to peacock tail feathers, and evolved due to sexual selection? Was it used as a defensive weapon, like in some dinosaurs, to fend off predators? Presumably both *Confucisornis* and *Protopteryx* lived an arboreal life, so these tail feathers, which appear to be clumsy, may have been used to maintain balance while climbing trees, as is true of modern squirrels. As the edge has branched barbs and the feathers look very much like those described in Regal ('75; Fig. 1g and 5A), this

feather supports the theory that feathers evolved from the elongated scales. However, Prum and Brush (2002) indicated that this evidence could not stand because the enlarged rachis was more likely to be formed later by the pronounced fusion of barbs. Also, its presence should have been more widespread (e.g., all over the body) to be considered as a prototype of evolving feathers. In view of its existence on only the tail, it appears that these are specialized feathers and the giant shaft is more likely to be secondarily derived for specialized functions as discussed above. Recent molecular data (Yu et al., 2002) also offer an explanation through a developmental mechanism that such a giant shaft can be produced by over-production of BMP. Some dissociated feathers with typical or atypical feather structures are also found (Fig. 1i-l). They may belong to either Mesozoic birds or dinosaurs.

### *Developmental biology of the feather*

These fossils show a sampling of the diversity and complexity of skin appendages found in the Jehol Biota in the Mesozoic landscape. Now let us look into their developmental biology to find a common theme and variations among different integument appendages. Reptilian integuments have scales that are periodic epithelial infoldings (see Alibardi, 2003). Their primary functions are to contain water and to provide protection. Most scales are short (Fig. 2A), although some reptiles can also grow long appendages as seen in today's iguana. Chickens have three major types of scales: the reticulate, scutate, and scutella scales (Sawyer et al., '86). The radially symmetric reticulate scales are on the footpad and express only alpha keratin. Scutate and scutella scales have anterior-posterior polarity, with an outer surface composed of beta keratin and an inner surface and a hinge region composed of alpha keratin (Sawyer and Knapp, 2003).

A typical feather in today's birds consists of a shaft (rachis) from which barb branches are inserted. The barbs themselves are composed of a shaft (ramus) and numerous minute branches (barbules) (Lucas and Stettenheim, '72; also see Prum and Dyck, 2003). Down feathers are mainly radially-symmetric (the rachis is absent or very short). Most contour feathers have bilateral symmetry (against the rachis axis). Flight feathers are bilaterally symmetric or asymmetric. Another level of complexity is within the barb. The barbules on the barbs can be bilaterally symmetric

(across the ramus) and are therefore fluffy (plumulaceous). The distal and proximal barbules can have different shapes. Distal barbules can form hooklets, enabling them to interlock with the proximal barbule of the next (more distal) barb in a Velcro-like mechanism to form a vane structure (pennaceous). In the base, feathers form follicles that protect the epithelial stem cells and dermal papilla and provide mechanical structures for muscle attachment and coordinated movement. New cell proliferation at the follicle base pushes the more differentiated portions of the feather to the distal end.

How do scales compare with feathers and hair? Let us consider the basic configuration of these different skin appendages. They are each made from epithelial-mesenchymal interactions resulting in the formation of an epithelial placode and an underlying dermal condensation. Structurally, there is a base membrane between the epithelium and mesenchyme, and the basal layer of the epidermis is on top of the base membrane. However, there are some fundamental differences in the developmental processes that ensue. Scales do not form follicular structures. Proliferation is more diffuse (Tanaka and Kato, '83), so the scales thicken and only elongate slightly. One major difference between scales and feathers is that the mature scales are made of an epithelial shell and a mesenchymal core. The outside of the epithelial shell is the suprabasal layer. This is a different topological organization in comparison to feathers (Fig. 2).

The structures of avian foot scales and reptile scales are similar, although it is unclear whether avian foot scales are homologous to reptile scales or whether they are secondarily derived (see more discussion in Sawyer and Knapp, 2003). If the four-winged dinosaur (Fig. 1E) is the prototype of an early dino-bird transition, not a special adaptation, one may say that avian foot scales are secondarily derived.

Feathers, on the other hand, initially start to proliferate from the tip of feather buds. Therefore, feather buds protrude out first (rather than hair pegs that invaginate first, see Botchkarev and Paus, 2003). As the buds elongate, the localized proliferation zone gradually shifts through the shaft and localizes proximally to the base of the feather (Chondankar et al., 2003). In the meantime, epidermis surrounding the feathers starts to invaginate into the dermis to form a follicular wall. The dermal papilla is situated at the base of the follicle, inducing the epithelial collar above to

continue proliferation. This allows for continual growth, and facilitates feather cycling, as stem cells would be protected in the follicle. In adult feather morphogenesis, the feather filament is a cylindrical structure with the pulp inside (Fig. 2), facilitating branching formation (Prum and Brush, 2002). However, during development, feather branching can initiate in feather buds before the formation of follicles or feather filament cylinders (Sawyer and Knapp, 2003). The pulp contains loose mesenchyme made of blood vessels and nerves to support the growth of developing feathers. Toward the distal end, epithelial cells in the ramogenic zone start to differentiate into barb and rachial ridges, which form the feather backbone (rachis) and branches (barbs and barbules). Marginal plate cells between barb ridges, pulp epithelium, and pulp in the filament center later degenerate to allow the opening of the feather filament cylinder into a two dimensional vane. This vane is made of epithelial cells only, with one side toward the original basal layer and the other side toward the supra-basal layer. However, the mature keratinized structure is made of only the supra-basal cells, as the basal layer has become the marginal plate and the pulp epithelium, both disappear when the feather vane opens. Also see Prum and Dyck, and Bragulla and Hirschberg (2003) in this issue for more detailed discussion.

For comparison, we also examine the development of hairs. Hair placodes invaginate to form a hair peg. They then elongate with the dermal papilla (Fig. 2). Epithelial cells above the dermal papilla become the matrix, the localized growth zone of the hair. Epithelial cells between the matrix and inter-follicular epidermis become the outer root sheaths. Together with the dermal sheath, they form the follicular wall. Part of the distal follicle wall enlarges and forms two swellings. The upper swelling becomes the sebaceous gland, while the lower swelling forms the follicle bulge, the presumptive site housing stem cells (Cotsarelis et al., '90). Above the matrix, epithelial cells differentiate into the hair filaments and inner root sheath. The most unique distinction between hair and feather is the lack of a mesenchymal component within the hair filament. Above the dermal papilla, it is all made of epithelial cells. For more complete discussion of this process and the molecules involved, please see Botchkarev and Paus, this issue.

With this developmental understanding, we should know that not all epithelial appendages

that form branches are feathers. The term “epithelial appendage” is a much broader name that includes all special derivatives of epithelial structures (Chuong, '98). However, in terms of feathers, we may have to limit to those that share similar growth modes, most of the developmental processes, and many of the biochemical properties. Here we try to develop a set of criteria for feathers (Table 1). We try to include the major points discussed above, but there are also many other important characteristics of feathers we did not include at this stage. The numbers in the table do not indicate temporal order in evolution, or a prerequisite for the subsequent criteria. Each component can be exaggerated or reduced to a minimum, thus allowing more shape possibilities (Bartels, 2003). Some feather variants today may not have barbules (e.g., some feathers in the lyre bird and egret), or have poorly developed barbs (e.g., penguins). Biochemically, they must contain beta keratin and share fundamental molecular pathways (Shames and Sawyer, '87; Shames et al., '91).

Fossils offer information that is helpful in the search for the evolutionary origin of feathers. Unfortunately, some criteria in Table 1 are defined functionally and cannot be measured in fossils. Based on morphological evidence, we can still do our best to look for tube-like follicles, analyze the arrangement of branches, and evaluate the whole configuration of the appendages. For example, the branched scales of *Longisquama* do not fit this definition of true avian feathers and were named “non-avian feathers” (Jones et al., 2000). We can also evaluate unusual integument appendages today with these criteria. For example, turkey beard bristles grow continuously from finger-like outgrowths but do not assume the localized growth mode from the proximal to the distal end seen in feathers, even though they showed simple branching and expressed feather-type beta keratins (Sawyer et al., 2003b). Using the criteria in Table 1, turkey beard bristles would not be classified as feathers.

### ***Evolution of other integumentary appendages: tooth, beak, and others***

For the bird to adapt to a life in the sky, coordinated changes in structures other than feathers are also required. The loss of teeth and formation of a cornified beak have been shown to have been driven by a selective regime favoring aerodynamically streamlined body contours, a fundamental characteristic for avian flight

(Homberger, 2002). The non-avian theropod dinosaurs in the Jehol Biota display a variety of tooth types. *Sinosauropteryx* has unserrated premaxillary, but serrated maxillary teeth (Chen et al., '98). There are four serrated premaxillary teeth preserved in the *Protarchaeopteryx*. *Caudipteryx* also had four preserved elongated, hooked premaxillary teeth (Ji et al., '98). The smallest feathered dinosaur, *Microraptor Zhaoianus*, has developed posterior teeth that have a less compressed crown and a constriction “waist-like morphology” beneath the crown. The heterodont dentition pattern represents the transition from the non-avian theropod type of dentition to that of Mesozoic birds (Xu et al., 2000). Interestingly, an Oviraptorosaurian, *Incisivosaurus*, had more diverse tooth types than other feathered dinosaurs: it had rodent-like, incisor-like premaxillary teeth and tapered cheek teeth (Xu et al., 2002).

*Archeopteryx* had many teeth remaining in its snout. A long-tailed, large, basal bird, *Jeholornis*, had no teeth in its maxilla, and only three small conical teeth on its mandible (Zhou and Zhang, 2002). *Confucisornis* had a true beak and no teeth (Hou et al., '95). While some of these Mesozoic birds had no teeth, others had different numbers of teeth remaining with the beak, suggesting a balance between the loss of teeth (facilitating flight) and tooth maintenance (facilitating catching prey), and that the loss of teeth was a later event compared to the evolution of flight. The fossil finds suggest that from feathered dinosaurs to Mesozoic birds, the trend is toward a reduction in number of teeth. Modern birds do not have teeth.

The gradual diversification of integument appendages (beak, teeth, feathers, different types of claws) has allowed different trophisms to develop (Zweers et al., '97). It allowed Mesozoic birds to reach different niches that were only possible after an adaptation toward flight, and contributed to the bio-diversity of the Mesozoic world. These are vividly introduced in Hou ('97) and Hou et al. (2003) (also Fig. 2).

### ***Molecular conversion of one appendage phenotype into another***

Changes of these integument appendages have to be based on available developmental pathways. “Evolutionary novelties” are added when new developmental pathways are produced. The evolution of novel developmental mechanisms is usually based on the alteration of homologous

RCAS retrovirus mediated viral transduction

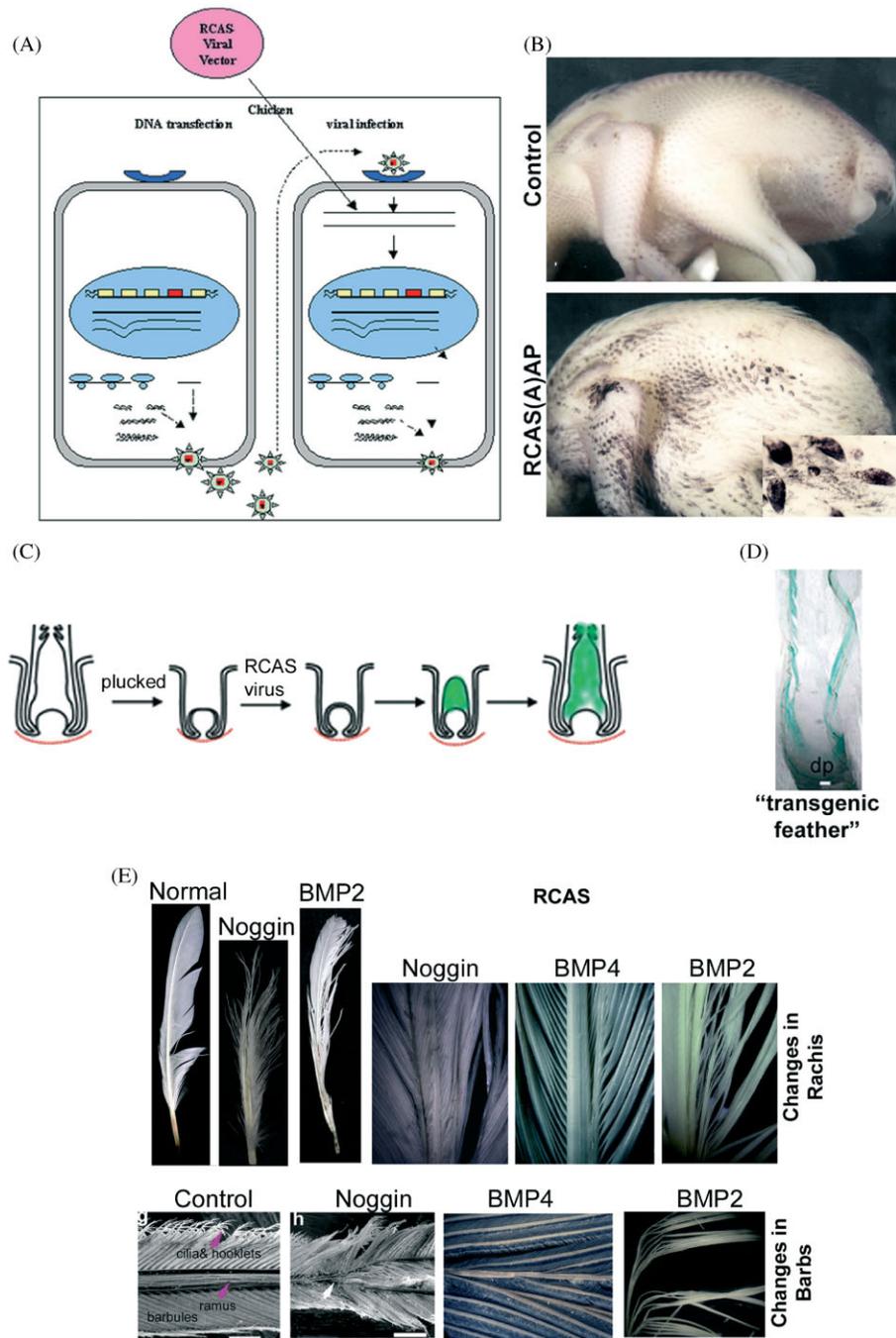


Fig. 3. Molecular biology technology used to mis-express genes and convert developmental pathways of skin appendages. A. RCAS retroviral vectors used to mis-express genes in chicken. B. Demonstration of mis-expressed genes (in this case alkaline phosphatase, AP) in chicken embryos (The head is toward the left and not shown). Note the patch staining. The insert in lower right corner shows high expression of AP genes in the elongating feather buds. C. Strategies to mis-express genes in feather follicles of hatched chickens. After feather plucking, stem cells regenerate a new feather. This is

the opportunity to transduce exogenous genes to these regenerating epithelial stem cells (gray color, and panel D). E. The balance of molecular pathways is perturbed and the forms of feathers are altered. Here we show noggin, a BMP antagonist, can split rachis into multiples and enhance barbs to branch more. In contrast, BMP2 and BMP4 can produce giant rachis and enhance barb fusion. Therefore, noggin favors barb formation while BMP favors rachis formation. Modified from Yu et al. (2002).

molecular modules that allow the connection or disconnection of existing molecular pathways (Chuong, '98; Chuong and Noveen, '99; von Dassow and Munro, '99).

Can we explore the molecular basis underlying these changes through laboratory research? Many laboratories have investigated different molecular pathways to discern their expression patterns and roles in the complex developmental processes underlying epithelial appendage development. In general, the order of appearance of these molecules is FGF4, BMP4 → SHH, Wnt-7a → Notch-1, Serrate-1 and Delta-1 → Msx-1, -2 → Hox, NCAM (Chuong and Edelman, '85; Chuong et al., '90; Noveen et al., '95; Song et al., '96; Ting-Berreth et al., '96; Chen et al., '97; Jung et al., '98; Widelitz and Chuong, '99). These pathways affect feather induction, mesenchymal condensation, localized cell proliferation, etc. Our lab has used modern chickens as a research model (Fig. 3A-D). Here we will discuss some recent findings. The first example is the changing balance between barb and rachis formation (Fig. 3E). The second example represents the gain of a pathway: growing feathers from scale epidermis (Fig. 4A). The third example represents the reactivation of a lost pathway: growing teeth from the chicken oral mucosa (Fig. 4B).

To investigate the molecules involved in feather branching, we looked for genes with expression patterns suggesting involvement in this process. BMP4 was first expressed in the dermal papilla and overlying pulp, but later switched to the barb ridges in the ramogenic zone. BMP2 was expressed in the marginal plate but also moved to the barb plate. Noggin, a BMP antagonist, was expressed as a gradient in the pulp, with highest expression levels found at the ramogenic zone. To further explore the role of this pathway in branching morphogenesis, we used the RCAS retrovirus to deliver noggin to the regenerating feather follicles of modern chickens (Yu et al., 2002). The resulting rachidial ridges were severely fragmented. Retroviral mediated expression of BMP4 to the regenerating follicles produced feathers that lacked branches. The rachidial ridges were often fused. These data suggest that the BMP pathway is involved in feather branching (Fig. 3E; Yu et al., 2002).

Beta-catenin was first found to interact with APC in the colon (Rubinfeld et al., '96). APC deletions or mutations of APC led to increased accumulation of beta-catenin and to the formation of colon polyps, which are epithelial growths.

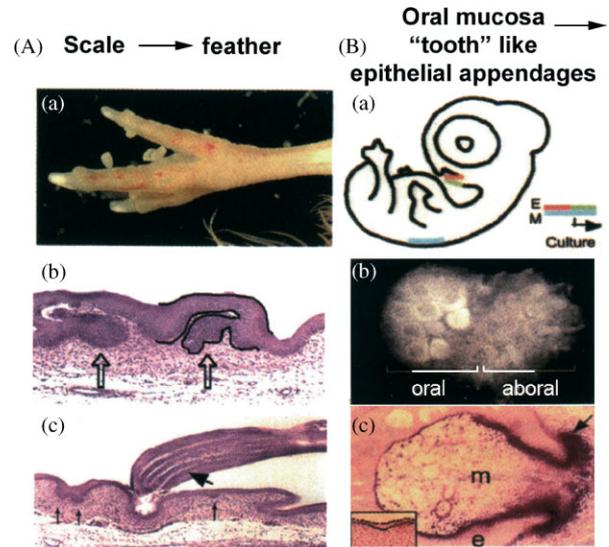


Fig. 4. Molecular conversion of feathers/scales and tooth/oral mucosa. A. When RCAS beta catenin was used to infect embryonic chicken hind limb, scales are converted into feathers. a. feathers growing out from scale region. b. Follicles are seen to form from part of the scutate scale surface. c. Barb ridges form in these induced feathers. The newly induced feathers do have follicular structures and form barb ridges. Note it is part of the scale surface that is converted into feathers, not the transformation of the whole scales into feathers. It appears that some appendage stem cells may have remained in scale epidermis and are activated by beta catenin to form a feather. Modified from Widelitz et al. (2000). B. Embryonic epithelia containing oral mucosa and chin epidermis were recombined with feather mesenchyma from the dorsal skin (a). The explant is developed in culture. Chin regions form feather buds while the oral mucosa region forms many tooth-like appendages (left part of the explant, panel b), while the right part forms feather buds. Sections show follicular structures (panel c). Modified from Chen et al. (2000).

Thus, accumulation and activation of beta-catenin is able to stimulate epithelial cells towards growth. Transgenic mice expressing exogenous beta-catenin had new hairs and formed hair tumors (Gat et al., '98). We characterized the expression of beta-catenin in chicken skin and found that it was first expressed throughout a feather tract and then, as the feather buds began to form, was up-regulated within the bud primordia and down-regulated in the interbud regions. To test its role in feather bud development, we mis-expressed a truncated, constitutively active form of beta-catenin from the replication competent avian sarcoma virus (RCAS). Increased beta-catenin expression in the scale primordia induced formation of feathers from the leading edge of the scales (Fig. 4A; Widelitz et al., 2000). Manipulating other

pathways also led to an induction of feathers from scale forming regions. Activation of the delta pathway and suppression of the BMP pathway in scales also induced some feathered scales (Zhou and Niswander, '96; Crowe and Niswander, '98; Viallet et al., '98). These molecular pathways are likely to intersect and work in concert during the conversion of scales to feathers. We believe that similar, but not necessarily identical, molecular processes may have occurred during avian evolution to initiate the formation of ancestral feathers.

Mesozoic birds had teeth that were lost in the evolution of modern beaks. In the third example, we will examine whether latent molecular signals specifying tooth development were retained by modern birds but not expressed. The fact that the oral mucosa of modern chickens still forms a dental lamina, which soon degenerates, suggested that the ancestral molecular mechanisms might still exist. Classical experiments using recombination to form a chimera of mouse dental mesenchyme with chicken oral mucosa led to the expression of a "chicken enamel gene" (Kollar and Fisher, '80), and to the formation of characteristic dental mesenchymal structures (Wang et al., '98). What are the molecular signals involved? Some of these latent signals were revealed by in situ hybridization, which indicated that the chicken oral mucosa expressed *Pitx2*, *Pax9*, and *FGF8*, but not *Bmp4*, *Msx1*, and *Msx2*. All of these genes are expressed in the mouse oral mucosa and are considered to be essential for tooth formation. In fact, epithelial signaling to the mesenchyme involves a  $BMP4 \rightarrow Msx1 \rightarrow BMP4$  pathway (Chen et al., '96). Knockout mice lacking *Msx-1* and *Msx-2* fail to grow teeth. It is possible

that during beak evolution a defect in the  $BMP4 \rightarrow Msx1 \rightarrow BMP4$  pathway developed, which led to the loss of teeth from modern birds. To test this theory, we tried to rescue tooth odontogenesis from the chicken oral mucosa by releasing BMP4 from beads. BMP4 did induce the expression of

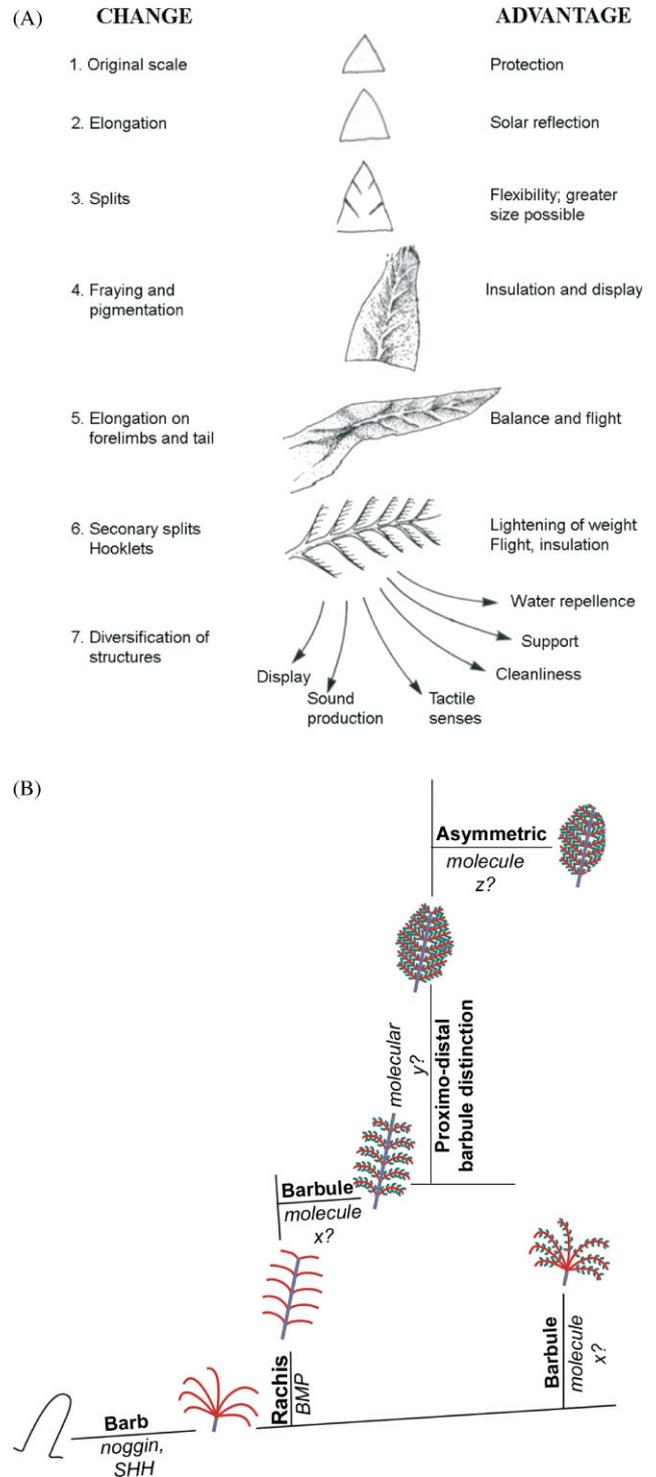


Fig. 5. Models of scale/feather transformation. Panel A is from Regal ('75) that suggested the order for transformation is as follows: the scale like planes → partial pennaceous vanes with emerging rachis → bilaterally symmetric feather → plumulaceous barbs → radially symmetric downy feathers. We propose the order in panel B, favoring the order being cylindrical feather filaments splitting to form primitive barbs without barbules → radially symmetric downy feathers with plumulaceous barbs → bilaterally symmetric plumulaceous feathers → bilaterally symmetric pennaceous vanes → bilaterally asymmetric vanes. Some of the molecular pathways known to be involved under each new evolution process are indicated. Compare with Prum, 1999 that has combined several events here into stages. There are likely to be some lineages not depicted here. Some lineages may have been selected out during Mesozoic time and became extinct while some lineages persisted and flourished till today. The model we showed here is for the avian feather today. We acknowledge some other branched appendages may have evolved through different mechanisms.

*Msx1* and *Msx2* from the chicken oral mesenchyme. FGF released from beads in a similarly designed experiment had an even greater effect. The effect was greater still when applied to dorsal skin feather producing mesenchyme (Fig. 4B; Chen et al., 2000). It is difficult to be sure that these skin appendages were truly teeth since there is no chicken tooth marker. However, these experiments clearly show that the dental lamina has the ability to form follicular structures for integument appendages. Recently, chicken oral mucosa has shown the ability to corroborate with mouse neural crest to form tooth-like appendages in chimeric embryos (Mitsiadis et al., 2003). This is consistent with the above thesis that some signaling modules were lost in the chicken oral mucosa mesenchyme, and that it is possible to rescue, at least partially, the tooth-forming pathway.

### ***Models of feather evolution and conclusion***

The feather is the centerpiece of bird flight and its origin and evolutionary history have long puzzled scientists. A long-held view is that the feather evolved from an elongation of protective scales. It was then subdivided over time to form pennaceous and then plumulaceous feather types (Regal, '75). Therefore, the order of formation is from the scale-like plates → partial pennaceous vanes with emerging rachis → bilaterally symmetric feather → plumulaceous barbs → radially symmetric downy feathers (Fig. 5A). Two major advances in the last decade have shaken this classical view: (1) a series of fossil discoveries representing intermediate forms of feathers or feather-like appendages from the Jehol Biota of China, and (2) molecular and developmental biological experiments using chickens as a model organism. Feather forms can be modulated using retrovirus mediated gene mis-expression that mimics those found in nature today and in the evolutionary past. Together the results favor an evolutionary sequence of feather filaments splitting to form primitive barbs without barbules → radially symmetric downy feathers with plumulaceous barbs → bilaterally symmetric plumulaceous feathers → bilaterally symmetric pennaceous vanes → bilaterally asymmetric vanes (Fig. 5B).

This order occurs in development, and we feel that it should have occurred in evolution too, in a broad sense of ontogeny repeating phylogeny. Each arrow probably represents one evolutionary novelty (Prum, '99; Prum and Brush, 2002). Work

in the molecular biology laboratories has allowed us to start to identify molecular pathways involved in each of these "evolutionary novelty" processes (Fig. 5B; Yu et al., 2002; Harris et al., 2002).

Integument and integument appendages are all made of ectodermal cells. They share common appendage stem cells. Indeed, all the diverse appendages can be viewed as variations on top of a common theme (Chuong, '98). With experimental manipulation of molecular pathways, we now can modulate feather forms from one form to another, and we can also convert appendage phenotypes from one type to another. In the context of Evo-Devo, feather morphogenesis presents an excellent paradigm with rich fossil evidences, theoretical models (Prum and Dyck, 2003) and experimental possibilities (Sawyer and Knapp, 2003; Widelitz et al., 2003). We are positioned to identify more molecular bases of evolutionary novelties that eventually adapt the birds to the sky.

### **ACKNOWLEDGMENT**

We thank Ms. Marijane Ramos for help in the manuscript preparation, and Drs. G. P. Wagner, D. Homberger, R. H. Sawyer, and L. W. Knapp for very helpful comments on the manuscript.

### **LITERATURE CITED**

- Alibardi L. 2003. Adaptation to land: the skin of reptiles in comparison to that of amphibians and endotherm amniotes. *J Exp Zool (Mol Dev Evol)* 298B:12–41.
- Alibardi L, Sawyer RH. 2002. Immunocytochemical analysis of beta keratins in the epidermis of chelonians, lepidosaurians, and archosaurians. *J Exp Zool* 293:27–38.
- Bartels T. 2003. Variations in the morphology, distribution and arrangement of feathers in domesticated birds. *J Exp Zool (Mol Dev Evol)* 298B:91–108.
- Botchkarev V, Ralf P. 2003. Molecular biology of hair morphogenesis: Development and cycling. *J Exp Zool (Mol Dev Evol)* 298B:164–180.
- Bragulla H, Hirschberg RM. 2003. Horse hooves and bird feathers: Two model systems for studying the structure and development of highly adapted integumentary accessory organs-The role of the dermo-epidermal interface for the micro-architecture of complex epidermal structures. *J Exp Zool (Mol Dev Evol)* 298B:140–151.
- Bereiter-Hahn J. 1986. *Biology of the integument*. New York: Springer Verlag.
- Chatterjee S. 1997. *The Rise of Birds*. Baltimore, MD: John Hopkins University Press.
- Chen P, Dong Z, Zhen S. 1998. An exceptionally well-preserved theropod dinosaur from the Yixian Formation of China. *Nature* 391:147–152.
- Chen Y, Bei M, Woo I, Satokata I, Maas R. *Msx1* controls inductive signaling in mammalian tooth morphogenesis. *Development*. 1996. 122:3035–3044.

- Chen CWJ, Jung HS, Jiang TX, Chuong CM. 1997. Asymmetric expression of Notch, Serrate and Delta is associated with the anterior posterior axis of feather buds. *Dev Biol* 188:181–187.
- Chen YP, Zhang Y, Jiang TX, Barlow A, Amand TR, Hu Y, Heaney S, Francis-West P, Chuong CM, Maas R. 2000. Conservation of early odontogenic signaling pathway in Aves. *Proc Natl Acad Sci* 97:10044–10049.
- Chiappe LM. 1995. The First 85 million years of Avian Evolution. *Nature* 378:349–355.
- Chiappe LM, Witmer LM, editors. 2002. Mesozoic birds: above the heads of dinosaurs. University of California Press Berkeley, CA.
- Chodankar R, Chang CH, Yue Z, Jiang TX, Suksaweang S, Burrus L, Chuong CM, Widelitz R. 2003. Shift of localized growth zones contributes to skin appendage morphogenesis: role of the Wnt/beta-catenin pathway. *J Invest Dermatol* 120:20–26.
- Chuong CM, Edelman GM. 1985. Expression of cell-adhesion molecules in embryonic induction. I. Morphogenesis of nestling feathers. *J Cell Biol* 101:1009–1026.
- Chuong CM, Oliver G, Ting SA, Jegalian BG, Chen HM, De Robertis EM. 1990. Gradients of homeoproteins in developing feather buds. *Development* 110:1021–1030.
- Chuong CM, editor. 1998. Molecular Basis of Epithelial Appendage Morphogenesis. Austin, TX: Landes Bioscience.
- Chuong CM, Noveen A. 1999. Phenotypic determination of epithelial appendages: Genes, developmental pathways and evolution. *J Invest Dermatol Sym Proc* 4:307–311.
- Chuong CM, Chodankar R, Widelitz RB, Jiang T-X. 2000. Evo-Devo of Feathers and Scales: Building complex epithelial appendages. *Curr Opin Dev Gene* 10:449–456.
- Crowe R, Niswander. 1998. Disruption of scale development by Delta-1 mis-expression. *Dev Biol* 195:70–74.
- Cotsarelis G, Sun TT, Lavker RM. 1990. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* 61:1329–1337.
- Dhouailly D, Sawyer RH. 1984. Avian scale development. XI. Initial appearance of the dermal defect in scaleless skin. *Dev Biol* 105:343–350.
- Feduccia A, Tordoff HB. 1979. Feathers of Archaeopteryx: Asymmetric Vanes indicate Aerodynamic Function. *Science* 203:1021–1022.
- Feduccia A. 1999. The Origin and Evolution of Birds. 2nd Edition. New Haven, CT: Yale University Press.
- Gat U, DasGupta R, Degenstein L, Fuchs E. 1998. De novo hair follicle morphogenesis and hair tumors in mice expressing a truncated  $\beta$ -catenin. *Cell* 95:605–614.
- Gill FB. 1994. Ornithology, 2nd Edition. New York, NY: Freeman.
- Harris MP, Fallon JF, Prum RO. 2002. Shh-Bmp2 signaling module and the evolutionary origin and diversification of feathers. *J Exp Zool (Mol Dev Evol)* 294:160–176.
- Homberger DG. 2002. The aerodynamically streamlined body shape of birds: Implications for the evolution of birds, feathers, and avian flight. In: Zhou Z, Zhang F, editors. Proceedings of the 5th Symposium of the Society of Avian Paleontology and Evolution, Beijing, 1–4 June 2000. Beijing, China: Science Press. p 227–252.
- Homberger DG. 2003. Avian origins revisited. *J Biosci* 28: 135–138.
- Homberger DG, de Silva KN. 2000. Functional microanatomy of the feather-bearing avian integument: Implications for the evolution of birds and avian flight. *Amer Zool* 40: 553–574.
- Homberger DG, de Silva KN. 2003. The role of mechanical forces on the patterning of the avian feather-bearing skin: A biomechanical analysis of the integumentary musculature in birds. *J Exp Zool (Mol Dev Evol)*. 298B: 123–139.
- Hou LH. 1997. Mesozoic Birds of China. In Chinese. Mutual Culture Co. Taiwan. (English edition in prep)
- Hou LH, Zhou ZH, Martin LD, Feduccia A. 1995. A beaked bird from the Jurassic of China. *Nature* 377:616–618.
- Hou LH, Martin LD, Zhou ZH, Feduccia A. 1996. Early Adaptive Radiation of Birds: Evidence from Fossils from Northeastern China. *Science* 274:1164–1167.
- Ji Q, Currie PJ, Norell MA, Ji SA. 1998. Two feathered dinosaurs from northeastern China. *Nature* 393:753–761.
- Ji Q, Norell MA, Gao KQ, Ji SA, Ren D. 2001. The distribution of integumentary structures in a feathered dinosaur. *Nature* 410:1084–1088.
- Jones TD, Ruben JA, Martin LD, Kurochkin EN, Feduccia A, Maderson PFA, Hillenius WJ, Geist NR, Alifanov V. 2000. Non-avian feathers in a late Triassic Archosaur. *Science* 288:2202–2205.
- Jung HS, Francis-West F, Widelitz RB, Jiang TX, Ting S, Tickle C, Wolpert L, Chuong CM. 1998. Local inhibitory action of BMPs and their relationships with activators in feather formation: Implications for periodic patterning. *Dev Biol* 196:11–23.
- Kollar EJ, Fisher C. 1980. Tooth induction in chick epithelium: expression of quiescent genes for enamel synthesis. *Science* 207:993–995.
- Landmann L. 1984. The Skin of Reptiles: Epidermis and Dermis. In: Bereiter-Hahn J, Matoltsy AG, Richards S, editors. Biology of the Integument. New York: Springer-Verlag. p 150–187.
- Lucas AM, Stettenheim PR. 1972. Avian Anatomy Integument. In: Agriculture Handbook 362. Agricultural Research Services. Washington, DC: US Department of Agriculture.
- Maderson PFA, Homberger DG. 2000. The evolutionary origin of feathers: A problem demanding interdisciplinary communication. *Amer Zool* 40:455–460.
- Mayr G, Peters DS, Plodowski G, Vogel O. 2002. Bristle-like integumentary structures at the tail of the horned dinosaur Psittacosaurus. *Naturwissenschaften* 89:361–365.
- Mitsiadis TA, Cheraud Y, Sharpe P, Fontaine-Perus J. 2003. Development of teeth in chick embryos after mouse neural crest transplantation. *Proc Natl Acad Sci* 100:6541–6545.
- Norell M, Ji Q, Gao K, Yuan C, Zhao Y, Wang L. 2002. ‘Modern’ feathers on a non-avian dinosaur. *Nature* 416: 36–37.
- Noveen A, Jiang TX, Ting-Berret SA, Chuong CM. 1995. Homeobox genes Msx-1 and Msx-2 are associated with induction and growth of skin appendages. *J Invest Dermatol* 104:711–719.
- Ostrom JH. 1974. Archaeopteryx and the Origin of Flight. *Q Rev Biol* 49:27–47.
- Padian K, Chiappe LM. 1998. The Origin of Birds and their Flight. *Scientific American* February: p 38–47.
- Prum RO. 1999. Development and Evolutionary Origin of Feathers. *J Exp Biol (Mol Dev Evol)* 285:291–306.
- Prum RO, Brush AH. 2002. The evolutionary origin and diversification of feathers. *Quart Rev Biol* 77:261–295.

- Prum RO, Dyck J. 2003. A hierarchical model of plumage: Morphology, Development, and Evolution. *J Exp Zool (Mol Dev Evol)* 298B:73–90.
- Regal PJ. 1975. The Evolutionary Origin of Feathers. *Q Rev Biol* 50:35–66.
- Rubinfeld B, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P. 1996. Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. *Science* 272:1023–1026.
- Sawyer RH, Knapp LW. 2003. Avian skin development and the evolutionary origin of feathers. *J Exp Zool (Mol Dev Evol)* 298B:57–72.
- Sawyer RH, Knapp LW, O'Guin WM. 1986. The skin of birds: epidermis, dermis and appendages. In: Bereiter-Hahn J, editor. *Biology of the integument*. Vol. 2. Vertebrates. New York: Springer Verlag. p 194.
- Sawyer RH, Salvatore BA, Potylicki TT, French JO, Glenn TC, Knapp LW. 2003a. Origin of feathers: Feather beta (beta) keratins are expressed in discrete epidermal cell populations of embryonic scutate scales. *J Exp Zool (Mol Dev Evol)* 295:12–24.
- Sawyer RH, Washington LD, Salvatore BA, Glenn TC, Knapp LW. 2003b. Origin of archosaurian integumentary appendages: The bristles of the wild turkey beard express feather-type beta keratins. *J Exp Zool* 297B (Mol Dev Evol):27–34.
- Schweitzer MH, Watt J, Avci AR, Knapp L, Chiappe L, Norell M, Marshall M. 1999. Beta-keratin specific immunological reactivity in feather-like structures of the Cretaceous Alvarezsaurid, *Shuvuuia deserti*. *J Exp Zool (Mol Dev Evol)* 285:146–157.
- Sengel P. 1976. *Morphogenesis of skin*. Cambridge: Cambridge University Press.
- Sereno PC. 1999. The Evolution of Dinosaurs. *Science* 284:2137–2147.
- Shames RB, Sawyer RH. 1987. Expression of beta-keratin genes during development of avian skin appendages. *Curr Top Dev Biol* 22:235–253.
- Shames RB, Jennings AG, Sawyer RH. 1991. Expression of the cell adhesion molecules, L-CAM and N-CAM during avian scale development. *J Exp Zool* 257:195–207.
- Song HK, Sawyer RH. 1996. Dorsal dermis of the scaleless (sc/sc) embryo directs normal feather pattern formation until day 8 of development. *Dev Dyn* 205:82–91.
- Song H, Wang Y, Goetinck PF. 1996. Fibroblast growth factor 2 can replace ectodermal signaling for feather development. *Proc Natl Acad Sci U S A* 93:10246–10249.
- Tanaka S, Kato Y. 1983. Epigenesis in developing avian scales. II. Cell proliferation in relation to morphogenesis and differentiation in the epidermis. *J Exp Zool* 225: 271–283.
- Ting-Berreth SA, Chuong CM. 1996. Sonic Hedgehog in feather morphogenesis: induction of mesenchymal condensation and association with cell death. *Dev Dyn* 207: 157–170.
- Viallet JP, Prin F, Olivera-Martinez I, Hirsinger E, Pourquie O, Dhouailly D. 1998. Chick Delta-1 gene expression and the formation of the feather primordia. *Mech Dev* 72: 159–168.
- von Dassow G, Munro E. 1999. Modularity in animal development and evolution: elements of a conceptual framework for EvoDevo. *J Exp Zool (Mol Dev Evol)* 285:307–325.
- Wang YH, Upholt WB, Sharpe PT, Kollar EJ, Mina M. 1998. Odontogenic epithelium induces similar molecular responses in chick and mouse mandibular mesenchyme. *Dev Dyn*. 213:386–397.
- Widelitz RB, Chuong CM. 1999. Early events in skin appendage formation: induction of epithelial placodes and condensation of dermal mesenchymal cells. *J Invest Dermatol* 4:302–306.
- Widelitz RB, Jiang TX, Lu J, Chuong CM. 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.
- Widelitz RB, Jiang TX, Yu M, Shen T, Shen J-Y, Wu P, Yu Z, Chuong C-M. 2003. Molecular biology of feather morphogenesis: A testable model for evo-devo research. *J Exp Zool (Mol Dev Evol)* 298B:109–122.
- Xu X, Tang ZJ, Wang XJ. 1999a. A therizinosauroid dinosaur with integumentary structures from China. *Nature* 399:350–354.
- Xu X, Wang XL, Wu XC. 1999b. A dromaeosaurid dinosaur with a filamentous integument from the Yixian Formation of China. *Nature* 401:262–266.
- Xu X, Zhou Z, Wang X. 2000. The smallest known non-avian theropod dinosaur. *Nature*. 408:705–708.
- Xu X, Zhou Z, Prum RO. 2001. Branched integumental structures in *Sinornithosaurus* and the origin of feathers. *Nature* 410:200–204.
- Xu X, Cheng YN, Wang XL, Chang CH 2002. An unusual oviraptorosaurian dinosaur from China. *Nature*. 419: 291–293.
- Xu X, Zhou Z, Wang X, Kuang X, Zhang F, Du X. 2003. Four-winged dinosaurs from China. *Nature* 421:335–340.
- Yu M, Wu P, Widelitz RB, Chuong CM. 2002. The morphogenesis of feathers. *Nature*. 420:308–312.
- Zeltinger J, Sawyer RH. 1991. Avian scale development. XIII. Epidermal germinative cells are committed to appendage-specific differentiation and respond to patterned cues in the dermis. *Dev Biol* 144:335–352.
- Zhang F, Zhou Z. 2000. A primitive enantiornithine bird and the origin of feathers. *Science* 290:1955–1959.
- Zhou Z, Zhang F. 2002. A long-tailed, seed-eating bird from the Early Cretaceous of China. *Nature*. 418:405–409.
- Zhou Z, Barrett PM, Hilton J. 2003. An exceptionally preserved Lower Cretaceous ecosystem. *Nature* 421: 807–814.
- Zou H, Niswander L. 1996. Requirement for BMP Signaling in Interdigital Apoptosis and Scale Formation. *Science* 272:738–741.
- Zweers GA, Vanden Berge JC, Berkhoudt H. 1997. Evolutionary pattern of avian trophic diversification. *Zoology* 100:25–57.