

## S06-1 Follicles and the origin of feathers

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**Abstract** Feathers evolved in the mid- to late-Jurassic and first appeared in the fossil record on *Archaeopteryx* (150 MYA). All feathers are constructed from a family of closely related phi-keratin proteins. The structural genes for these proteins exist as a moderately repeated, tandemly repeated, subset of beta-keratin genes. The mechanisms underlying the novel appearance of feathers are based in their development. A phylogeny for feathers based on morphology and molecular information indicates that the most likely common ancestor was a simple, tubular epidermal filament derived from a conical tubercle. The formation of the follicle followed. The developmental programming underlying the rapid appearance and diversification of feathers was modular and involved genetic and morphological duplication. These mechanisms account for the appearance and function of the follicle, the most basic product of which would be an unbranched, tubular structure of phi-keratin. Further duplication events and regulation of growth rates led to the branched structure of feathers and to their rapid diversification. While ontogeny does not recapitulate evolution, it can account for both the basic morphological constraints and phenotypic variation of feathers. Because this model is sufficient to account for all features of the feather character it is not necessary to invoke a functional interpretation for feather evolution.

**Key words** Feather, Evolution, Phylogeny, Morphology

### 1 Introduction

Extensive evidence now supports the hypothesis that modern birds are derived from a sister group that includes theropod dinosaurs (Padian and Chiappe, 1998). The presence of feathers is an important element of this argument (Prum and Brush, 2002). There is evidence for derived feathers, formally the quintessential character of birds, in advanced maniraptorian dinosaurs (Prum, 2002). Feathers have their origin deep within theropod dinosaurs and a primitive feather structure occurs in the even more basal coelurosaurians (Chen et al., 1998). Undoubtedly feathers evolved in dinosaurs prior to the appearance of *Archaeopteryx*, the oldest bird in the fossil record (150 MYA), and the presence of feathers in these primitive taxa provides essential clues to feather origins. The sudden appearance of such a novel feature also poses questions regarding mechanisms (Brush, 1993, 1996). In this essay I therefore focus on the earliest patterns in ontogeny, especially that of the presumptive feather: my intent is to relate early ontogenetic processes to evolutionary events.

### 2 Background factors underlying feather evolution

Feathers are a morphologically diverse group of epidermal appendages. The modern contour feather with pennaceous, symmetrical vanes, a hollow keratinous rachis, and branching, hollow barbs and barbules is structurally complex; but it is not the only feather type evident on fossil dinosaurs and only one of many alternative phenotypes on

modern birds. Such complexity and structural diversity implies functional diversity as well. Phenotypic diversity originates in the earliest phases of ontogeny and may be responsible for the emergence of evolutionary novelties (West-Eberhard, 1989; Brush, 1993). Regardless of their morphology, all feathers are produced in a follicle. This singular structure is capable of generating the entire spectrum of feather phenotypes present over the lifetime of a single bird.

Feathers are not simply transformed reptilian scales. That is, an archaeosaurian scale was not transformed over time into contour feathers to enable flight as has been proposed (Feduccia, 1995, 1996; see also Padian and Homer, 2002). Rather, the earliest ontological processes were modified through variation and selection to produce a unique structural element (Prum, 1999). Specifically, a simple tubular, unbranched filament, composed of  $\phi$ -keratins (=feather  $\phi$ -keratin) and produced in an epidermal follicle, would qualify as a feather. Structures that meet all these criteria exist in many forms on modern birds and, as far as is known, describe the filaments on *Sinosauropteryx*, a coelurosaurian dinosaur (Chen et al., 1998; Brush, 2000). Simple filaments and more complex pennaceous feathers have been found on various dromaeosaurs (e.g. Xu et al., 2001) and, of course, were present on *Archaeopteryx*. The processes of development and growth are plastic enough to account for a range of functions and to generate the vast structural complexity of feathers observed in living birds (Prum and Brush, 2002).

The evolution of feathers and their attendant mor-

phological diversity is linked closely to early ontogenetic events in the epidermis. The properties of symmetry, structural and genetic redundancy, and timing played major roles. Clearly, the changes in organization had a high potential to produce diverse morphological phenotypes (Wagner and Schwenk, 2000). The new organization generated numerous phenotypes, as evidenced by both the diversity in the fossil record and the plumage of living birds. Duplicated genes support rapid changes in patterns of protein synthesis, fine-tuning of structure, and ultimate adaptive potential (Brush, 1993, 2001). An expanded “genetic repertoire” that involves multiple gene families generates variation leading to new functions. The processes impinge on the squamous layers of the early epidermis and all occur widely in vertebrate development.

What changed is geometry and control, for example tissue folding (Oster and Albrecht, 1982). The basic, but static, features of epidermal morphology, histology and cytology are known. The interactions among signaling and synthetic pathways, and the timing and effects of messages among cells and between tissue layers, are essential to the processes that regulate differentiation and tissue specialization. These are, only now, becoming understood (Brush, 2000; Prum, 2000). Further, evolutionary changes are not entirely predictable from the descriptive information derived in classical embryology. Development of a follicle was key to the origin of feathers, and what occurs in the tissue before there is visible morphological evidence for change can be crucial. It is not coincidental that the earliest fossil feather matches the simplest feather phenotype on extant birds.

### 3 Feather ontogeny

The presumptive feather begins as a flat sheet of cytologically uniform cells. The distribution of the placode primordia is determined by the action of a series of cell adhesion molecules such as Cell Adhesion Molecules (CAM) and  $\beta$ -Caldesmon (Chuong et al., 2001). These molecules originate in the underlying mesenchymal cells. All feathers, regardless of their location or morphology, begin as a placode. Placode cells undergo rapid multiplication and increase in size. This hypoplasia produces morphological changes, such as tissue folding of the cell sheet, that are produced by a combination of internal physical forces driven in part by the cytoskeletal proteins, apoptosis and additional external signals. Harris et al. (2002) have demonstrated that Sonic hedgehog (*Shh*), a member of the TGF $\beta$  family of growth factors, and Bone Morphogenetic Protein 2 (*Bmp 2*) are active at the early stages and are important in timing events that establish the polarity of the placode. Minimally, they appear to signal the proliferation and differentiation of placode cells. Further morphogenesis is also influenced by factors such as  $\beta$ -catenin. Simultaneously, cellular differentiation begins with the production of  $\Phi$ -keratin mRNA.

Early ontogeny of the feather follicle is essentially similar in all avian species (Starck, 1982). It is independent

of ultimate feather structure and whether the species is precocial or altricial. The derived condition is one in which contour feathers group in geometric arrays, the pterygiae. The first primordia appear in the presumptive dorsal feather tract, followed quickly by all pterygiae. The early follicle has features important to subsequent growth and form. Papilla display radial symmetry and internal redundancy. Consequently, a combination of cellular differentiation and apoptosis leads to regionalization into ramogenetic zones, the areas that produce the barb (Prum, 1999). The long axis of the barbs is generated under the control of the *Hox* genes, various growth factors (e.g., TGF- $\beta$ , FGF) and BMP which act as switches in the protocols, promote apoptosis, and control a variety of genes related to different cell types. Together this activity sculpts the overall tissues. Specialized feather  $\beta$ -keratin protein synthesis is initiated at this point. The resulting spectrum of forms are generated by the internal physical properties of cell aggregates and controlling morphogenetic molecules (i.e.,  $\beta$ -catenin). The papilla then elongates, the dermal pulp forms and the collar invaginates (Prum, 1999).

At this stage the presumptive feather has undergone compartmentalization. A module has formed that is essentially separated from the surrounding tissue by protein sheaths that contain alpha- and beta-keratins other than  $\phi$ -keratin. The follicle has become a functional unit with self-contained structure and activity. It is symmetric and relies on the existence of reiterated structural units, new patterns of genetic regulation, and operational molecular gradients. Within the module, changes in molecular level regulatory events are added, deleted, or altered as the result of genomic activity. Modular organization permits enormous latitude in the developmental routines that will, in turn, strongly influence phenotype. Its uniqueness encourages selection to act on nonlethal phenotypic variation. This is apparent in even the first generation of feathers that differ according to location on the body surface.

### 4 Factors affecting feather ontogeny

Experimental manipulation has shown that the phenotypes of skin appendages are dependent on messages from the mesenchyme (reviewed in Sawyer et al., 2000). Structures at some stage may be interconvertible. Once cell proliferation is initiated the tissue is committed to producing an appendage. Levels of retinoic acid, which can convert presumptive scale to feather, can influence the outcome, however. Molecules such as N-cadherin can influence symmetry of the placode or follicle and subsequent morphology. Differential patterns of protein synthesis are associated with different phenotypes both within and between feathers. This, too, can be under direct or indirect genomic control.

Changes in gene expression and timing, the emergence of a module in the epidermis, redundancy and symmetry, all modify ontogenetic protocols and influence the phenotype. Rather than manipulate the transformation of a pre-existing scale, they provide the variation necessary

in the earliest stages of development to create alternative pathways for basic morphological change (Carroll et al., 2001). Here a branched, tubular structure rather than a flat plate holds the potential for production of phenotypic variety. The earliest stage of the follicle, with the complex folding and invasion of mesodermal tissue, is sufficient to produce a single filament. This matches the filaments on coelurosaurian dinosaurs (e.g., *Sinosauropteryx*), and is presumably the most primitive feather. Diversification in shape, size, and degree of branching followed.

## 5 Conclusions

The appearance of the follicle involved tweaking existing morphogenetic processes and employing an obscure family of genes for proteins with the ability to form filaments. The proteins were related chemically to the larger group of beta-keratin proteins and, along with sequences and conformational differences, occurred as multiple-tandem repeats. Changes in both time and space of gene activity accommodated the appearance of a newly organized appendage. The mechanisms that produce and break symmetry are tied to other groups of genes which may have variable functions associated with different functional pathways. Metaphorically, the placode generates the blueprint for the production of feathers; and the follicle provides the machinery of fabrication and selection for morphological diversity.

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