# Chapter 9
# Molding and Carving Cell Surfaces: The Joke of a Fold and the Origin and Evolution of Feathers

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## 9.1 Cells with Different Corneification Form Microornamentation at Their Interface

Cornification of cells in the epidermis of amniotes is different from that of derivatives such as scales, feathers, hairs, horns, nails, claws etc. (Maderson, 1985; Wu et al., 2004). Eventually epidermal layers detach (shedding or molting) from the remaining epidermis. Molting occurs along intra epidermal regions made by cells with different types of keratinization, and this interface often produces microornamentation of variable shapes.

In reptilian scales, layers of epidermis containing beta-keratin alternate with those containing alpha-keratin. A specific layer termed oberhautchen produces micro ornamentation that interdigitates with those of the upper layer, termed clear layer, and forms the shedding complex. Remarkable micro ornamentation is formed in the climbing lamellae of geckos and anolid lizards, a type of specialized scales that allow the lizard to climb vertical surfaces (Maderson, 1970; Hiller, 1972; Alibardi, 1997; see Fig. 9.1). In these special scales, the 0.5–1.5 μm thick spinulae of the oberhautchen layer, that normally are 2–4 μm in length, grow into bristles or setae that can reach over 100 μm in length. Setae grow inside the cytoplasm of clear cells that form a cytoskeletal belt around the growing setae that probably molds their shape (Fig. 9.1C, D, and E). At the beginning of setae formation the cytoplasm of both setae and clear cells is soft but progressively becomes corneous. Setae are mainly composed of beta-keratin of 12–18 kDa while the cytoskeleton of clear cells is made of other types of proteins, including cytokeratins (Alibardi and Toni, 2006; Rizzo et al., 2006). Partial primary sequence of some proteins of setae hasshown that they share a common amino acid sequence with chick scale and feather keratin (Alibardi and Toni, 2006; Dalla Valle et al., unpublished observations). This central region, made of twenty, amino acids has a typical beta-strand secondary conformation, and is likely involved in the formation of long keratin filaments.

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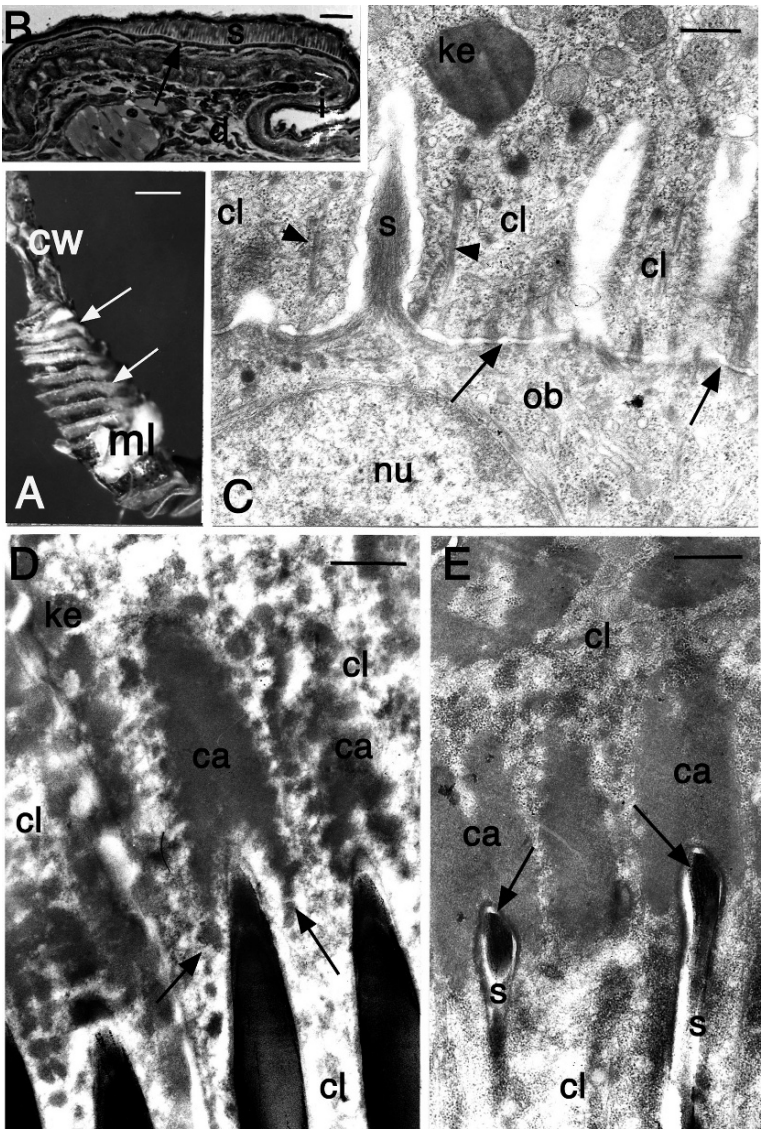


**Fig. 9.1** Micrographs illustrating the main cytological process responsible for the formation of setae in the iguanid lizard *Anolis lineatopus*. **A**, detailed view of digit lamellae (*arrows*). Bar, 0.5 mm. **B**, longitudinal section of a lamella with forming setae from the thin oberhautchen layer (*arrow*). Bar, 10 μm. **C**, ultrastructural detail of early differentiating oberhautchen cell which is still joined (*arrows*) with a clear cell. Early differentiating setae are surrounded by the cytoskeletal fibrils (*arrowheads*) of the cytoplasm of clear cells. Bar, 0.5 μm. **D**, detail of growing setae surrounded (*arrows*) by dense corneous material produced in clear cells. The distal part of setae is capped by a dense keratohyaline-like material. Bar, 0.5 μm. **E**, apical part (*arrows*) of elongated setae surrounded by corneous cap produced by a condensing cytoplasm of clear layer cells. bar, 0.5 μm. Legend: ca, corneous cap; cl, cytoplasm of clear cell; cw, claw; d, dermis; ke, keratinohyaline-like granules; i, inner scale surface; ml, molting epidermis; nu, nucleus; ob, cytoplasm of oberhautchen cell; s, setae

In feather cells, bundles of keratin are made of various proteins among which feather keratins and histidine-rich proteins predominate, used for cell elongation and hardening, (Gregg and Rogers, 1986; Brush, 1993; Sawyer et al., 2000, 2005). Cornified, elongated cells possess the high resistance of beta-keratins coupled with the deformation necessary to sustain climbing, gliding, or flying.

Finally, in growing hairs, the cuticle has a different composition with respect to that of the adjacent cells of the inner root sheath (Rogers, 2004). Immature, cuticle cells of hairs are joined by cell junctions to cuticle cells of the surrounding inner root sheath. During the beginning of hair differentiation the two layers form a slightly serrated interfaced surface. Progressing in differentiation, the cytoplasm of hair cuticle cells becomes cornified and produces a serrated interface with cornified cuticle cells of the inner root sheath. The latter is degraded when hairs exit on the epidermal surface but the hair cuticle remains scaled.

Epidermal layers of developing feathers are heterogenous in their modality of cornification, and determine the most complex and unique type of micro ornamentation present in skin derivatives of vertebrates, the feather (Fig. 9.2). The specific and unique characteristics of feathers is the more or less branched micro-structure that consists in a ramified syncitium of barb and barbule cells formed by a resistant form of keratin, feather keratin (Gregg and Rogers, 1986; Brush, 1993; Sawyer et al., 2000). Feathers are formed from a complex network of barb and barbule cells (Lucas and Stettenheim, 1972; Sengel, 1975; Chuong and Widelitz, 1999; Prum, 1999). These long cells are made of corneocytes containing feather keratin (Gregg and Rogers, 1986; Brush, 1993; Sawyer et al., 2000). Recent ultrastructural studies have clarified many details on the process of formation of barb and barbules (Alibardi, 2005a, b, 2006a, b). The origin of such a complex micro ornamentation derives from the presence of a special process of epidermal morphogenesis (the barb ridge), and the interaction between feather keratin-producing cells (barbules and barbs) with supportive cells (barb vane ridge cells and marginal plate cells).

Feathers are born without a follicle but acquire one later in development, which serves for both holding feathers in the skin and for their regeneration (Yue et al., 2005). No other appendage in the skin of vertebrates possess the refined, dicotomic branching pattern of feathers. Neither mammalian nor most of reptilian proteins have the small dimension and chemical-physical properties of feather keratin to form thin and resistant filaments. During development, feathers derive from specific layers of the embryonic epidermis.

## 9.2 Embryonic Layers in Archosaurian Epidermis

During development in all extant archosaurians (crocodilians and birds) the first 3–10 epidermal layers are transient and unique, and are shed during hatching so that the embryonic epidermis is replaced by the definitive epidermis (Alibardi and Thompson, 2001, 2002; Alibardi, 2003; Sawyer and Knapp, 2003; Sawyer et al., 2003, 2004; Alibardi et al., 2006; Fig. 9.2). Embryonic epidermis comprises the subperiderm layer that, aside from alpha-keratin, also contains feather keratin in

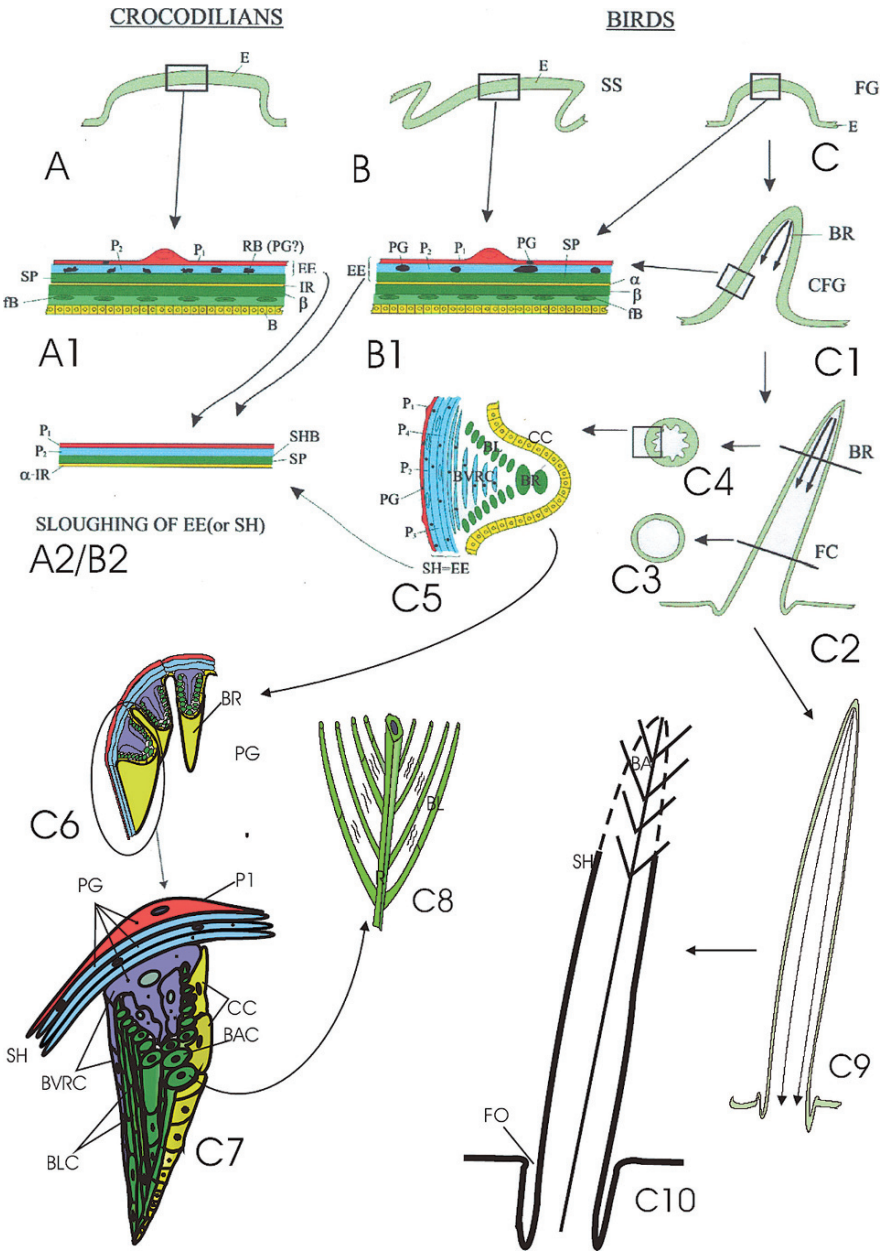


**Fig. 9.2** Schematic drawing illustrating epidermal layers in the alligator (crocodilians, **A–A2)** and birds (**B–B2**), the outline of the barb ridge (**C7**, in a schematic three-dimensional representation to show the relationshi ps and shapes of the different cells), and the formation of the basic ramification of feathers (**C–C9)** (see text for further details). Legends: α,) alpha-layer; **B**, basal layer of the epidermis; β, beta-layer; BA, barbs; BL, barbules; BLC, barbule cells; BR, barb ridges (the curved arrows indicate the apical-basal progression of barb ridges differentiation); BVRC, barb vane ridge

both alligator and avian embryonic epidermis. The presence of feather-like keratin in embryonic epidermis suggests that these keratins are constitutive for archosaurian epidermis.

After shedding of the embryonic epidermis (Fig. 9.2A2/B2) the remaining epidermal layers contain alpha keratins (in apteric or interfollicular areas), scale keratin (in scales), claw keratin (in claws), and beak keratin (in the beak). The new beta-keratins possess a slightly higher mass (14–16 kDa) then feather keratin (from 10–12 kDa) (Gregg and Rogers, 1986; Brush, 1993; Sawyer, 2003). Only in down-feathers does feather keratin remains in cells of the subperiderm that are transformed into barb and barbule cells during feather morphogenesis (Sawyer et al., 2003, 2004; Sawyer and Knapp, 2003; Alibardi, 2005a, b, 2006a, b; Alibardi and Sawyer, 2006; Fig. 9.2D–D11).

The change in position of feather-keratin positive subperiderm cells (colored in green in Figs. 9.2 and 9.5) is shown at different levels of the growing feather filament. The V-shaped displacement within barb ridges (Fig. 9.2, 9.3, 9.4, and 9.5) forms barbule plates while barb vane ridge cells (red in Fig. 9.5) colonise the axial plate and penetrate the space between barbule cells (Alibardi, 2005a, b).

Despite their common reactivity to feather keratin, subperiderm and barb/barbule cells contain a morphologically different organization of keratin bundles. In fact, while subperiderm cells contain tangled bundles of keratin with no orientation, feather keratin in barb/barbule cells is organized in long bundles with axial orientation within barb and barbule cells (Matulionis, 1970; Kemp et al. 1974; Bowers and Brumbaugh, 1978; Alibardi, 2006b; Alibardi and Sawyer, 2006). The elongation of barb cortical and barbule cells is due to the linear polymerization of feather keratin after cells have fused into a syncitium while supportive cells degenerate (Figs. 9.3, 9.4, and 9.5).

Embryonic feathers or downfeathers in hatchlings derive from the differentiation of the embryonic epidermis within feather germs that elongate into feather filaments (Matulionis, 1970; Chuong et al., 2003) (Fig. 9.2C–C8). In feather filaments barb ridges are formed, and the aggregation of chains of cells form barbules and barbs. The ultrastructural study of barb and barbule cells differentiation, and the three-dimensional organization of these cells have clarified the transformation of subperiderm into barb and barbule cells (Alibardi, 2005a, b, 2006a, b).

Recent studies have indicated that feathers derive from specific cell populations of the embryonic layers of the generalized archosaurian and avian epidermis (Sawyer et al., 2003, 2004; see Fig. 9.2A–B). The embryonic epidermis of birds and of their closest extant relatives, the crocodilians, is made of an external outer

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**Fig. 9.2** (continued) cells (supportive cells); CC, cylinder cells (the special basal layer of barb ridges destined to degenerate); CFG, conical feather germ (feather filament); E, epidermis; EE, embryonic epidermis (sloughed at maturity); fB, forming definitive beta-layer; FC, cylindric epidermis near the base of the feather filament; FG, feather germ; IR, intermediate layer (cells containing feather beta-keratin); P1, outer periderm; P2, inner periderm; P3, third periderm (part of the sheath); P4, fourth periderm layer (part of the sheath); PG, periderm granules; RB, reticulate body; SH, feather sheath; SS, scutate scale; SP, subperiderm layer; Similar colors represent cell layers homology: note in particular the gree hue of subperiderm and barb/barbules

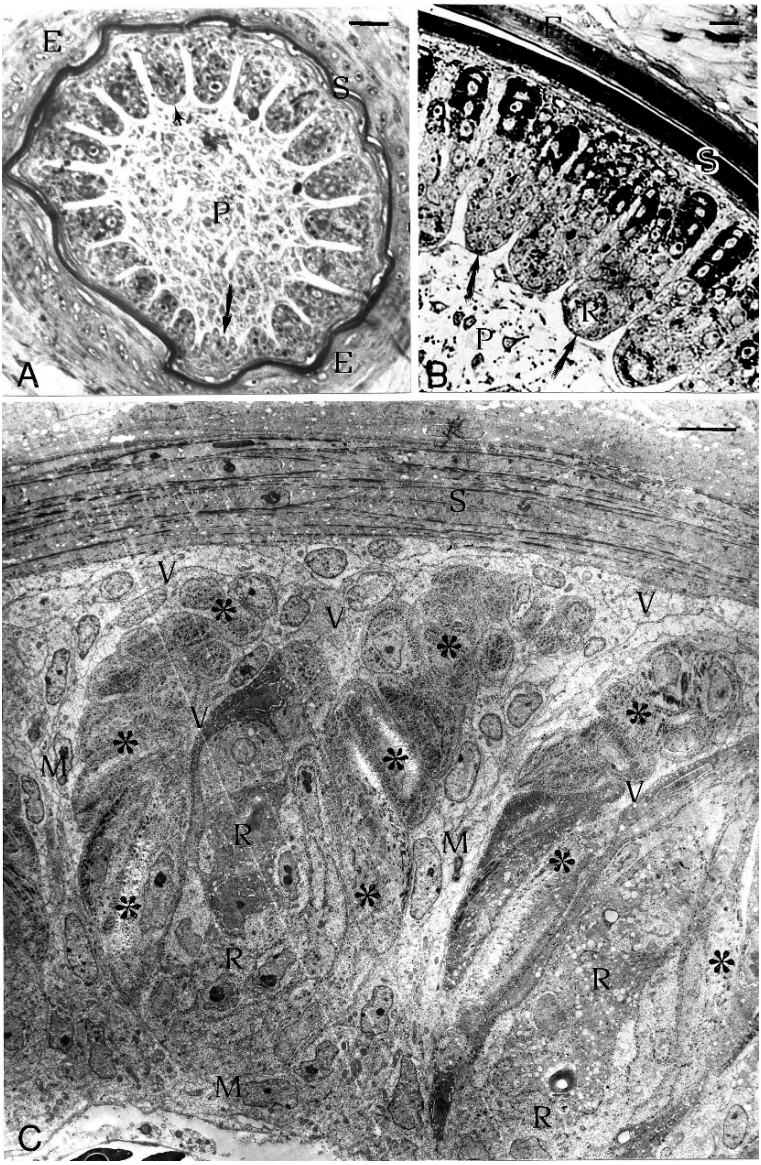


**Fig. 9.3** Cross sections of feather follicle illustrating barb ridges. **A**, cross section of a follicle of a juvenile feather from a zebrafinch (*Taeniatopigia castanotis*) with forming (*arrow*) and long (*arrowhead*) barb ridges. Bar, 20 μm. **B**, detail of barb ridges with pigmented barbule plates (*arrows*) in *T. castanotis*. Bar, 10 μm. **C**, ultrastructural view of barb ridges of chick downfeather with barbule plate (asterisks). Bar, 2.5 μm. Legends: E, epidermis; M, marginal plate cells; P, pulp; R, ramus area; S, sheath; V, barb ridge vane cells

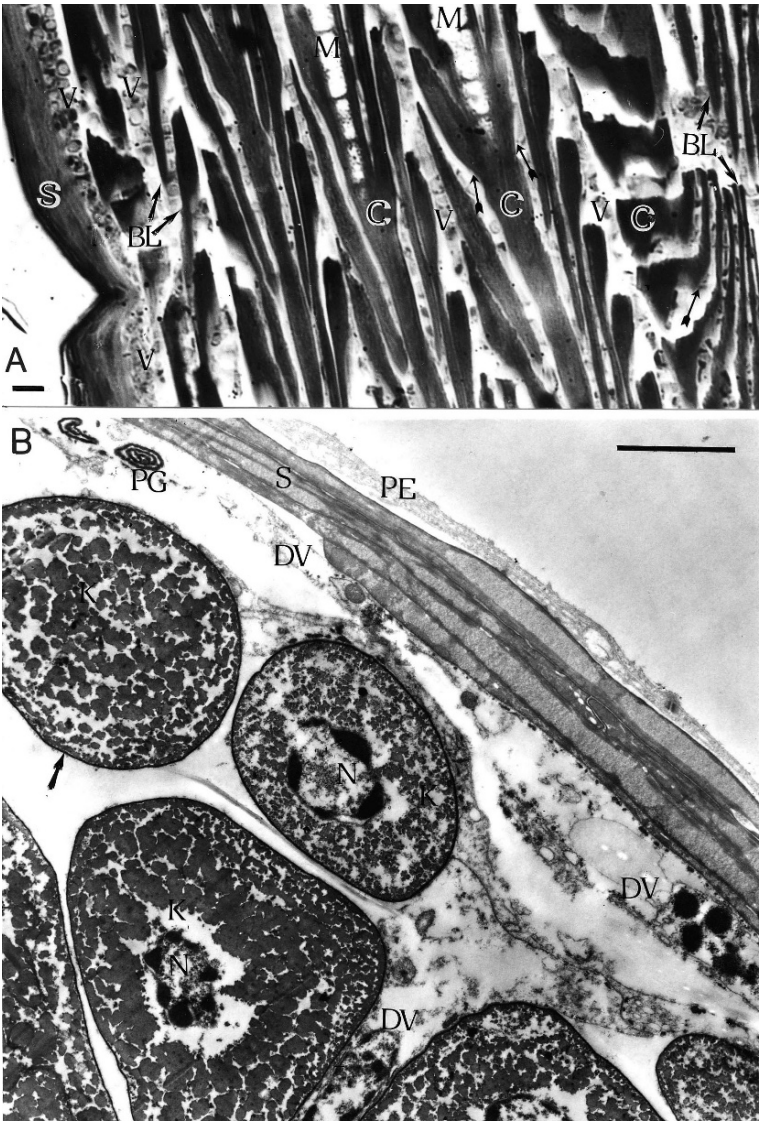


**Fig. 9.4** Longitudinal sections of differentiating feather filaments. **A**, elongated branching of single rami with lateral barbules (*arrows*) among which degenerating barb vane ridge cells are present. Bar, 10 μm; **B**, ultrastructural cross section showing barbule cells (*arrow*) containing keratin bundles among which degenerating barb vane ridge cells are present and form a shedding layer underneath the sheath. Bar, 2.5 μm. Legends: BL, barbule cells; DV, degenerating barb vane ridge cells; C, barb cortical cells of the ramus; K, feather keratin bundles; M, barb medullary cells; N, nuclei; PE, periderm; PG, periderm granule; S, sheath; V, barb vane ridge cells

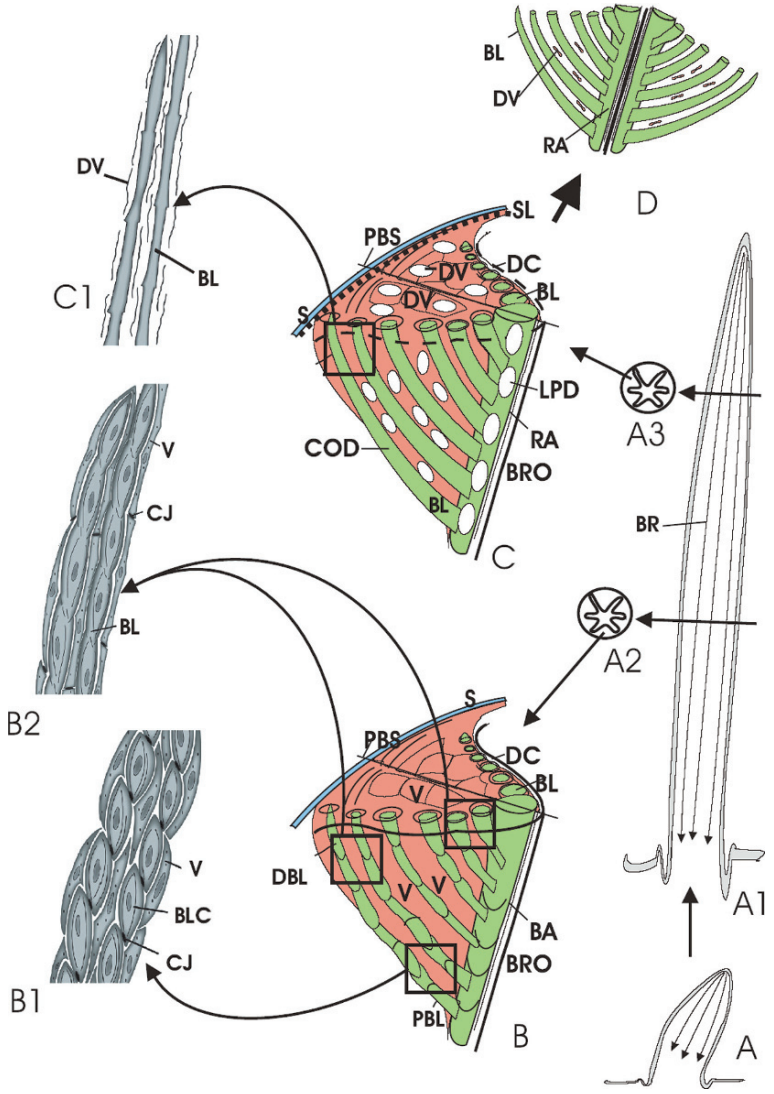


**Fig. 9.5** Drawing illustrating a feather germ **(A)** growing into a feather filament **(A1)** with the cytological details of barbule maturation in barb ridges of different maturity **(A2** and **A3)**. Lower level **(A2, B)** shows a cellular barb ridge with joined barbule cells to form chains separated by barb vane ridge cells (details in squares **B1** and **B2)**. Square in **B2** shows a more differentiating stage than in **B1**, where barbule cells have merged into a syncitium and junctions have disappeared. In the more mature barb ridge at the upper level (**A3**, **C)** barbules have shrunken and nodes have formed where cell boundaries were present (square in **C1)**. Barb ridge cells are degenerating (white areas in **C**) while holes are formed in the ramus (white areas). A section through the plane of symmetry of the barb ridge illustrates the aspect of the mature barb ridge when it opens-up the ramification after sheath shedding (**D**). Legends**:** BA, barb cells; BL, barbules; BLC, barbule cells; BR, barb ridges (the *arrows* indicate the apico-basal direction of formation); BRO, barb ridge outline (*dashes* indicate it is disappearing); CJ, cell junction; COD, corneification of barb/barbule cells where the

periderm, an inner periderm, a subperiderm layer and a germinal layer (Sawyer et al., 2003, 2004; Alibardi, 2002, 2003; Sawyer and Knapp, 2003; Alibardi, 2006). At the end of embryonic development, the embryonic epidermis is lost and the definitive epidermis remains to cover the body, except in feathers (Figs. 9.2, 9.5). In fact, embryonic layers remain with a vertical distribution in feather filaments and eventually produce a downy feather (Fig. 9.2C–C9). The detailed cytological study using the electron microscope has documented the transformation of cells of the embryonic layers into the different types of cells forming downfeathers (Alibardi, 2005a, b; 2006b, c). In particular, the fine morphological study coupled to the use of a specific antibody against feather keratin (Sawyer et al., 2000, 2003) has allowed detecting the presence of this marker protein in the subperiderm and feather cells.

As opposed to scales, interfollicular epidermis, beak, claws, and downfeather are embryonic appendages that persist after hatchings, as they derive from embryonic epidermal layers left in place after hatching (Fig. 9.2C9, 4). Since feathers are believed to derived from scales of pro-avian ancestors (Spearman, 1966; Maderson, 1972; Maderson and Alibardi, 2000; Alibardi, 2005c) it is thought that also feather keratin evolved from a primitive scale keratin (Brush, 1993; Gregg and Rogers, 1986). This molecular change might have occured through a main deletion of a repeated nucleotidic sequence coding for a glycine-rich 52 amino acid sequence localized in the central part of scale keratin. A feather-keratin antibody recognizes a feather-specific epitope in a 10–12 kDa feather keratin which is absent in scales (Sawyer et al., 2000, 2003, Sawyer and Knapp, 2003; Alibardi and Sawyer, 2006; Alibardi et al., 2006). The feather-specific keratin is produced in the subperiderm present in all regions of avian embryonic epidermis, suggesting that the keratin containing this epitope is produced before any scale, beak or claw keratin. This result suggests that the latter keratins might be derived from a primordial feather-like keratin containing the epitope (recognized by the feather-keratin antibody) by the insertion of the 52 amino acid sequence and of the claw and beak specific sequences. However, feather-like keratin in subperiderm cells forms tangled bundles with no orientation, while feather keratin tends to organize in long bundles with axial orientation in barb and barbule cells. This observation suggests that the two types of keratins are not the same protein or that other proteins necessary for the formation of keratin bundles are missing in subperiderm cells. The elongation of barb cortical and barbule cells is due to the linear polymerization of feather keratin (Brush, 1993).

After the synthesis of feather-like keratin in the subperiderm, the morphogenetic program for scale, claw or beak formation activated a gene for the formation of larger keratins that replace the feather-like keratin. After shedding of the embryonic

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**Fig. 9.5** (continued) plasma membranes have disappeared; DBL, distal (apical) barbule cell of barbule chain; DC, degenerating cylindrical cells of marginal plates; DV, degenerating barb vane ridge cells; LPD, lipid degeneration; PBL, proximal (basal) barbule cell of barbule chain; PBS, plane of bilateral symmetry of the barb ridge; RA, ramus; S, sheath; SL, shedding layer; V, barb vane ridge cells

epidermis, only the larger keratins of scale, beak or claw remain in the definitive epidermis of these appendages (Fig. 9.2A–A2, B–B2). As opposed to feathers, the formation of barb ridges determines the differentiation of barb and barbule cells and the conservation of the feather-like keratin (Fig. 9.2C–C9).

## 9.3 The Joke of an Epidermal Fold: Subperiderm Cells Become Barb/Barbules Organized in Feather Branching

Barb ridges are folds with a conical geometry and their lowermost part, merging into the circular collar, determines the branching organization around the ramus (Fig. 9.5). Once the folds is formed, other processes of cell multiplication contribute to the elongation of barb ridges. Changes of the three-dimensional structures of cells within barb ridges, as derived from the fine analysis of cross, oblique and longitudinal sections has allowed understanding the origin of the branched pattern of feathers (Figs. 9.3, 9.4).

Within barb ridges, barb vane ridge cells produce thin cytoplasmic arms that elongate among the chains of barbule cells so determining the separation of successive barbules. Therefore, supportive cells have a spacer function for the emergence of barbules (Fig. 9.5B–D). Possible other roles for supportive cells when they are still viable, like an exchange of metabolites or signalling molecules with barb/barbule cells, are not known. Cell junctions between barbule cells allow them to pile up into cell chains and their successive fusion to form syncitial branches (Alibardi, 2005a; Alibardi and Sawyer, 2006; Fig. 9.5B1, C1). The molecular mechanism of this specific recognition remains to be studied. The basal most (proximal) cells of a barbule cell chain, branches from the same insertion point on the right and on the left of the ramus (Fig. 9.5B–C). This symmetric branching pattern, derived from the initial displacement of subperiderm cells into symmetric barbule plates, determines the bi-planarity of definitive barb and associated barbules (Fig. 9.5D). The loss of marginal plates determines the disappearing of the organization of barb ridges and results in spacing barbs (rami) one from another. However, only after the detachment of the sheath from the remnant of barb ridges, do barbs become independent from each other forming the downfeather (Figs. 9.2C10, 9.4, 9.5C).

Barb vane ridge cells and marginal plate cells may represent the same cell type. In fact, recent ultrastructural studies on regenerating feathers have shown that periderm granules are occasionally found also in marginal plate cells during their degeneration (Alibardi, unpublished observations). The degenerations of both barb vane ridge cells among barbules and cylindrical cells in marginal plates may derive from the retraction of blood vessels (Lucas and Stettenheim, 1972). As a consequence, cells of the apical regions of the feather filaments become anoxic and die by necrosis. Therefore, no complex genomic information is required to target specific cells within the feather filament and carve out feathers. Among dying cells only those keratinized form the ramified syncitium of barbs and barbules while supportive and sheath cells are eventually lost (Figs. 9.4, 9.5C–D). Sheath cells detaches from

feather cells by degeneration of the interposed layer of barb ridge vane cells that act like a sloughing layer (Figs. 9.4B, 9.5C). The genomic control over the process of keratinization in barb/barbule cells versus that of lipidization in supportive cells remains unknown.

The initial displacement of subperiderm cells into symmetric barbule plates determines the bi-planarity of barbs when they open-up following degeneration of the sheath and supportive cells (Fig. 9.5C–D). Barb and barbule cells merge into a ramified syncitium, and are not replaced by other types of cells, since few cells are formed from the basal layer of growing feathers. The latter forms (cylindrical) cells of the marginal plates that later degenerate. With the replacement of downfeathers by juvenile feathers (molt), a new population of feather keratin cells is produced from the follicle. The formation of periderm granules (embryonic organelles, Kuraitis and Bowers, 1978, see Fig. 9.4B) in supportive cells, among the syncitial barbules of regenerating feathers, confirms the retention of stem cells for the production of supportive cells within the follicle. The retention of stem cells in feather follicles resembles that of hairs where stem cells are mainly retained in the bulge and hair matrix cells in follicles (Botcharev and Paus, 2003): feathers and hairs are basically regenerating embryonic appendages.

Cells of the sheath progressively accumulate alpha-keratin bundles with circular orientation that form a resistant belt around the feather filament (Matulionis, 1970; Alibardi, 2005b, 2006a).

The inner epidermis, contacting the softer mesenchyme, produce barb ridges starting from apical regions of the feather filament (Figs. 9.2C1–C2, 9.5A–A3). Dividing (stem) cells remain at the base of the feather filament and retract to form the collar region within the follicle (Sengel, 1975; Chuong and Widelitz, 1999; Sawyer et al., 2003, 2004; Widelitz et al., 2003; Yue et al., 2005).

## 9.4 The Origin of Feathers from Barb Ridges Derives from Interactions Between Barb/Barbules with Supportive Cells

Detailed information on cell organization and terminal differentiation within barb ridges is essential not only to understand the development but also to make reasonable hypothesis on the evolution of feathers. Unlike previous theories, based on a postulated, progressive complication of the branching pattern of feathers (Prum, 1999; Brush, 2000; Chuong et al., 2003; Wu et al., 2004) the following hypothesis on feather evolution is based completely on the alteration of the three dimensional structure of barb ridges. The present study emphasizes the central morphogenetic role of barb ridge formation and allows formulating some hypothesis on the evolution of feathers (Fig. 9.5).

In the fossil record, feather-like or true feathers have been found in theropods and bird remnants (summarized in Prum and Brush, 2002; Wu et al., 2004). These two groups of archosaurians might have evolved the morphogenetic process of forming

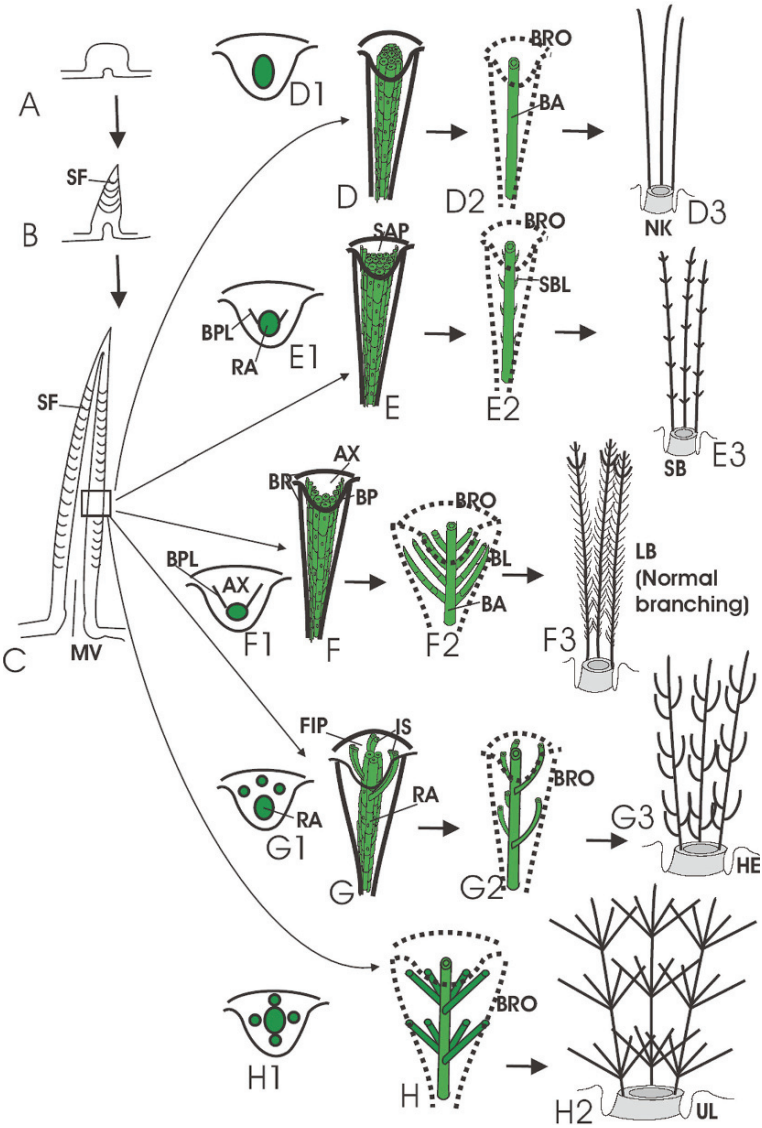


**Fig. 9.6** Schematic drawing of the hypothetical origin of downfeathers (**C**) from tuberculate (**A**) through coniform **(B)** scales. In **D**–**H** five possible modifications of cell displacement within barb ridges (**D1, E1, F1, G1, H1)** are presented (see text). In **D–D3**, where no axial plate is present and cells are aggregated into a single mass, a non-branched barb is derived (**D2**) that forms a naked down (**D3**). In **E–E3**, where the axial plate is shorter, a barb with short branching is) progressively formed (**E2–E3**). In **F–F3**, where a broad axial plate is present, a typical ramified barb is progressively formed (**F2–F3**). In **G–G3** the aggregation of barbule cells into groups that take insertion into the ramus at different levels gives origin to an elicoidal branching downfeather (**G3)**. In **H–H2**, the aggregation of barbule cells into groups and their insertion at intervals on the ramus gives origin to an umbrella or raceme-like branching. The final downfeathers are naked (**D3**), short branched (**E3**), long branched (**F3**), branched with an helical disposition (**G3**), and branched with

barb-ridges inside hairy-like outgrowths, perhaps related to thermical insulation for homeothermy (Fig. 9.6A–C).

Initially, barb ridges remained separated from each other, determining the formation of downy feathers. Cells of the subperiderm layer moved from their original, linear disposition in the embryonic epidermis into a new position in barbule plates. Cell displacement of the subperiderm layer within barb ridges eventually determined the formation of more or less branched barbs in accordance with at least three of the process indicated in Fig. 9.6D–F. In the process still present in modern birds, the bilateral displacement leads to branched barbules (Fig. 9.6F–F3). The progressive fusion of barbule plates with the central ramus area could have produced the partial (Fig. 9.6E–E3) or complete (Fig. 9.6D–D3) disappearance of barbules in downfeathers. The simple branching in primitive feathers found in ancient fossils such as *Synosauropteryx*, *Beipiaosaurus*, *Shuvuuia*, *Sinornithosaurus* (Brush, 2000; Martin and Czerkas, 2000; Prum and Brush, 2002) may be due to this process (compare Fig. 9.5D3 and E3 with primitive feathers presented in Wu et al., 2004).

The displacement of subperiderm/subsheath cells to form isolated groups of barbule cells separated by barb vane ridge cells might have produced barbules with a non-planar, three dimensional organization. For instance, the formation of a main ramus area and three minor barbule areas inserted along the ramus at different points might have produced an irregular, or a helical branching (Fig. 9.6G–G3), or a branched raceme-like structure (Fig. 9.6H–H2). This non-planar branching might have produced feathers mainly useful for thermical insulation and sensory activity, the primary role of feathers.

The passage from a downy to a pennaceous feather (Fig. 9.7A–D) was inherently established in the process of barb ridge formation, and represented an evolutive pre-adaptation. The ordered fusion of long barb ridges to form an axial rachis determined the formation of a planar vane (Fig. 9.7D).

Large feathers derived from the lengthening of barb ridges inside large follicles, and the increase of cells in barbule plates (compare Fig. 9.6B with Fig. 9.7C–C3). Furthermore, the evolution of a process of hooklet formation in regenerating feathers determined the formation of close vanes in feathers. Hooklets seem to be formed in longer barb ridges of regenerating feathers. In fact, the numerous barbule cells that are present in each chain of barb cells allow their incomplete overlapping with the formation of hooklets (Alibardi, 2005b; Fig. 9.7C1–C2). Barb ridges of more

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**Fig. 9.6** (continued) a raceme-like form (**H2**). Legends: AX, axial plate between symmetrically displaced barbule plates; BA, barb; BL, barbules; BPL, barbule plate; BR, barb ridge; BRO, barb ridge outline (disappearing as indicated by *dots*); D1, dorsal view of the lack of cell displacement with absence of barbule plates; **E1**, dorsal view of cell displacement with formation of short barbule plates; **F1**, dorsal view of barb ridge with complete cell displacement with formation of long barbule plates; HEL, helicoidal downfeather; IS, isolated groups of barbule cells; LB, long barbs downfeather; MV, vascular mesenchyme colonizing the whole feather filament; NK, naked down; RA, ramus (barb); SAP, short axial plate; SB, short barbs downfeather; SBL, short barbules; SF, sheath belt filaments (curved lines representing the circular orientation of keratin filaments in sheath cells); UL, umbrella or raceme-like branched downfeather

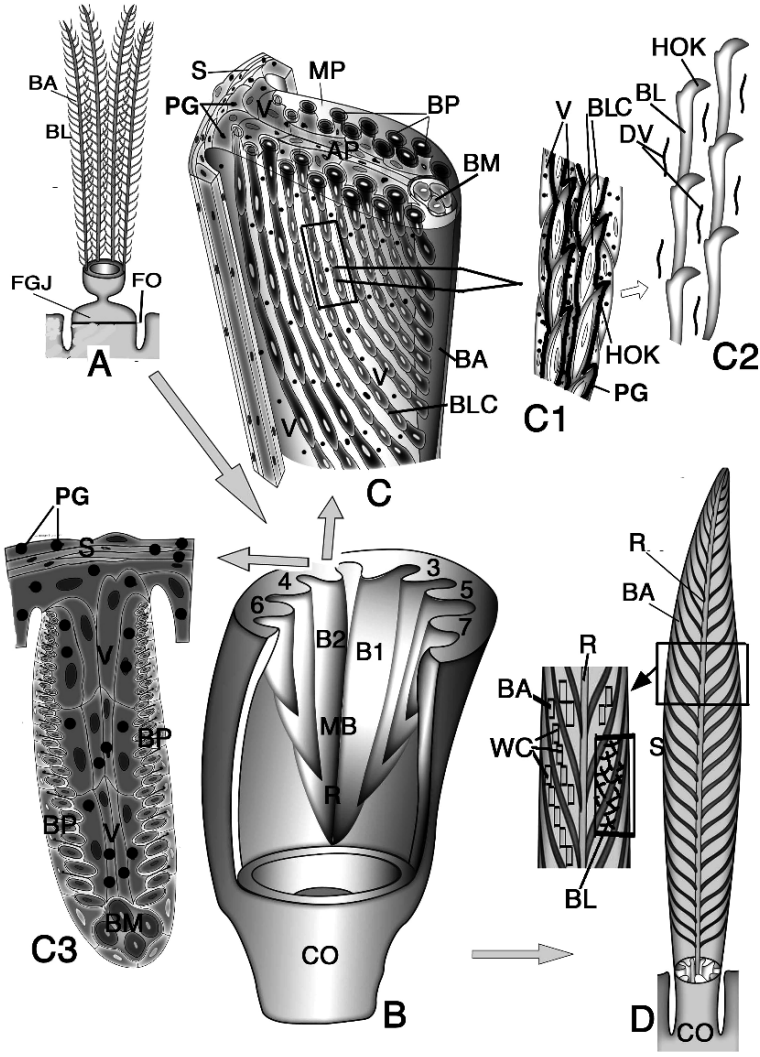


**Fig. 9.7** Schematic drawing of the passage from the downfeather (**A**) to a juvenile feather (**B–D**) (see text for details). A section passing through the enlarged germ of a juvenile feather shows that barb ridges merge into a rachis (**B**). Barb ridges become longer (**C** and **C3**) and often larger with numerous cells (even over 25) to form more numerous and longer barbule plates and barbule chains. Barbule cells often form tile-like overlapping and the exposed (**C1**) tip later differentiates into a hooklet (**C2**). In elongated barbule plates, cells can be ordered in alternating barbules (**C, C3**). The detail of a pennaceous feather (*larger square* in **D**) shows the localization of wedge cells among barbs. The smaller square in the enlargment in **D** shows the thick ramification of barbules. Legends: AP, axial plate; BA, barb (ramus); BL, barbules; BLC, barbule cells (the *square* indicates the enlargment to show chains of barbule cells forming hooklet barbules in **C1** and **C2**); BM, barb medullary cells (ramus); BP, barbule plate; **B1**, barb ridge number 1; **B2**, barb ridge number 2 (numbers 1–7 indicate the following barb ridges that will merge into the rachis); CO, collar; DV, degenerating barb vane ridge cells; FGJ, forming germ of juvenile feather (replacing underneath the downfeather); FO, follicle; HOK, hooklets; MB, merging barb ridges; MP, marginal plate; PG, periderm granules; R, rachis; S, sheath; V, barb vane ridge cells; WC, wedge cells

than 25 cells per barbule plate and with single barbules made by more than 25 cells, are formed in adult, large feathers. In the long chains of barbule cells, cell overlapping is extensive and, as a result of the spacing action of barb vane ridge cells, hooklets of different dimensions are formed (Fig. 9.7C1–C2; Alibardi, 2005b). Recent ultrastructural studies have also indicated that some supportive cells among barbs of pennaceous feathers initially cornify with a different modality than barb and barbule cells (Alibardi, unpublished observations). These cells, indicated as "wedge cells", form long chains of corneous cells localized among barbs and the rachis (Fig. 9.7D). It is likely that wedge cells, more so than supportive cells that undergo lipid-degeneration, contribute to mold the hooklets of the long barbules of pennaceous feathers. However, as wedge cells remain isolated, they do not form stable interbarb structures, and are eventually lost when the sheath breaks down and barbules distend to form the vane. Asymmetric close vanes presented aerodynamic properties and were later selected for flight.

The limited fusion of barb ridges into an imperceptible rachis in developing downfeathers is observed in various birds (Lucas and Stettenheim, 1972; Harris et al., 2002; Widelitz et al., 2003). This suggests that the evolving transition from downy to pennaceous feathers was very rapid, and explains why modern, bipinnate feathers are already present in the early fossil record (Brush, 2000; Martin and Czerkas, 2000; Prum and Brush, 2000; Wu et al., 2004).

## 9.5 The Evolution of Pennaceous Feathers is Related to Follicular Modulation of Barb Ridge Patterning

With the replacement of downfeathers by juvenile feathers (molt) a new population of feather-keratin containing cells and barb vane ridge cells is produced from the follicle (Chuong et al., 2003; see Fig. 9.7).

In the follicle of regenerating feathers, barb ridges are formed from the collar located around the dermal papilla, the size of which is larger than that in the previous downfeather (Fig. 9.8A–B).

The intense cell proliferation in the collar (Sengel, 1975; Chodankar et al., 2003) produces longer barb ridges and longer rami that contain a higher number of cells than in downfeathers (Fig. 9.8B1). The growth and size of barb ridges and of rami produced from the collar can be modulated inside the follicle by the action of hormones, growth and signalling factors (Chuong and Widelitz, 1999; Chuong et al., 2003; Harris et al., 2002; Widelitz et al., 2003). This modulation can produce new feather types that replace molted down feathers. According to Spearman and Hardy (1985), seven basic types of feathers, from which the other types can be derived, are present in modern birds: downs, powder down feathers, semiplumes, contour feathers (including remiges and rectrices), hypopennas, filoplumes, and bristles. The modulation in the pattern, size and shape of barb ridge formation can explain these seven basic types.

In down feathers, barb ridges are similar and do not merge before the collar (Fig. 9.8A–A2). No hooklets are formed and downs remain fluffy. Powder

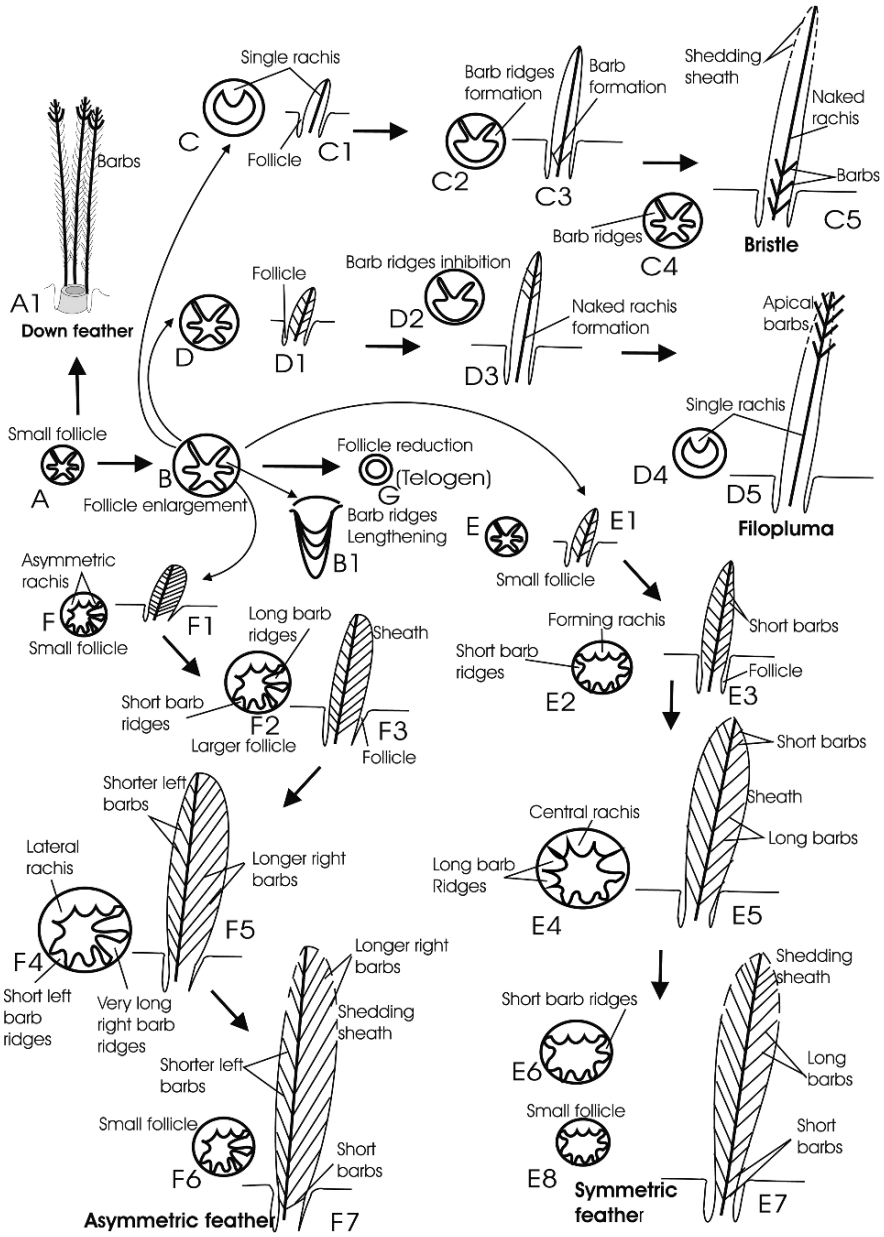


**Fig. 9.8** Schematic drawing illustrating some changes in the temporal patterns in the production of barb ridges from the initial pattern in downfeather (**A–A1)** to those more variable and complex in pennaceous feathers (**A–B–E–F**), in bristle (**B–C5**), and in filoplume (**B–D5**). The formation of barb ridges with different length and size (**B1**), and the variable number of barb ridges produced in different periods during the growth phase of feathers is represented (see text for details). A, small follicle of down with separate barb ridges; **B**, larger follicle of juvenile/adult feathers with rachidial

downfeathers develop in large follicles where long and folded barb ridges are formed without forming a rachis (Lucas and Stettenheim, 1972). Barbule cells are continuously lost from barbules and produce a powdery like material among the plumage, used for protection, colour production (by iridescence/interference) etc.. The other five feather types derive from a process of fusing barb ridges into a rachis (Fig. 9.8C–F).

The size of barb ridges becomes larger in germs of juvenile feathers produced underneath downfeathers before replacing them in the first molt (Fig. 9.8B–B1). At the end of growth the germinal epidermis does not form barb ridges, leaving a circular collar and a small dermal papilla (telogen, Fig. 9.8 G). Different patterns of barb ridges are produced leading to the formation of bristles (Fig. 9.8C–C5), filoplumes (Fig. 9.8D–D5), symmetric contour feathers (Fig. 9.8E–E7), asymmetric contour feather (Fig. 9.8F–F7).

In the follicle of bristles, the collar initially produces one rachidial ridge or most barb ridges merge into a compact rachis (Fig. 9.8C–C1). When some barb ridges are later produced in the follicle, some branching barbs appear at the base of the bristle (Fig. 9.8C2–C5). As opposed to follicles of filoplumes, initially numerous barb ridges merge with the dorsally-located rachidial ridge forming a rachis with varying long barbs (Fig. 9.8D–D1). When no more barb ridges are produced or all merge with the rachis, only a naked rachis remains in the growing feather (Fig. 9.8D2–D5). Finally at the end of feather growth (or during telogen, Fig. 9.8G) the follicle stops producing new cells for the continuation of the rachis, and moulting will later take place.

In the follicle of initially growing germs of symmetric feathers, small barb ridges of the same size are produced near the rachidial ridge (or are initially merging with a larger rachidial ridge, Fig. 9.8E–E1). The results of this fusion is the production of short barbs branching from the apical part of the rachis (Fig. 9.8E2–E3). In the following stages longer barb ridges are progressively produced, in particular producing longer rami so that barbs become longer and longer producing the widest portion of the forming feather (Fig. 9.8E4–E5). This process continues for a certain period producing the wider portion of the vane of the feather. The production of smaller barb ridges at later stages produces shorter barbs toward the base of the feather (Fig. 9.8E6–E8). The formation of smaller and smaller barb ridges by the end of feather growth (anagen) determines the formation of short barbule plates with few barbule cells. The latter process determines the formation of short barbules where hooklets may disappear leaving the last produced barbs isolated. This process produces an open (incoherent) vane at the base of the feather. At the end of feather

←

**Fig. 9.8** (continued) ridge in anagen (growing) stage. **C–C5**, formation and shaping of bristle; **D–D5**, formation and shaping of filopluma; **E–E7**, formation and shaping of symmetric contour feather; **F–F7**, formation and shaping of asymmetric contour feather; **G**, flat circular epidermis of the collar in telogen (resting phase) localized at the base of any feather at the end of the feather cycle

growth (or in telogen), the feather follicle terminates the production of barb ridges and the follicle becomes narrow and contains a cylindrical epidermis (Fig. 9.8G).

In follicles of germs growing into asymmetric feathers, initially short barb ridges tends to merge mainly on the dorso-lateral side of the collar forming a rachis localized on one side of the follicle (left in our example in Fig. 9.8F–F1). The resulting apex of the forming feather shows short barbs on the left side of the nascent vane and longer barbs on the right side. Progressively longer barb ridges are produced on both sides of the enlarged follicle and consequently barbs elongate, extending the width of the growing vane (Fig. 9.8F2–F5). When later barb ridges become shorter, small barb ridges are produced in the follicle and the width of the vane decreases again (Fig. 9.8F6–F7). At the end of growth (or in telogen, Fig. 9.8G) barb ridges are no longer produced leaving a circular collar with a small papilla.

## 9.6 Diversification of Pennaceous Feathers

The previous examples of variation in follicular patterns of barb ridge formation suggest that, after the origin of a follicle in primitive birds and/or theropods, the modulation of the mechanism of patterning and size of barb ridges determined the origin to all the known phenotypes present in modern feathers (Spearmann and Hardy, 1985). These types are schematically illustrated in Figs. 9.8 and 9VI.

In semiplumes, more elongated barb ridges than downy feathers were formed, and they merged with a rachis forming an open but planar vane. The lack of hooklets in these feathers impeded the formation of a close (coherent) vane. Morphogenesis of contour feathers determined the formation of long barb ridges from the ventral area of the collar which merged with the rachis in the dorsal part of the collar (helical displacement, see Lucas and Stettenheim, 1972; Prum, 1999). The formation of hooklets in the long barb ridges allowed the formation of a close and aerodynamic efficient vane. The production of symmetric or asymmetric contour feathers possibly derived from the process indicated in Figs. 9.8C–D and 9.9VI.

In symmetric contour feathers, barb ridges of similar size were produced along the whole collar, right and left from the forming ventral region (Figs. 9.8E–E7, 9.9 VI). The length of the resulting rami and barbules branching from the rachis was similar. Opposite, in asymmetric contour feathers rami generated on one side of

**Fig. 9.9** (continued) symmetric or asymmetric barb ridge production give origin to countour feathers of symmetric or asymmetric type respectively. The hyporachis is generally present opposite the main rachis. Hooklets are formed over most of the vane of these feathers. Hypopennas are formed from a follicle with two equally developed but opposed rachidial ridges that grow into more or less long feathers. Filoplumes are formed from follicles with the initial formation of barb ridges that produce an apical ramification. The following formation of a single rachidial ridge determines the formation of a non-branched rachis beneath the apical tufts of barbules. In the follicle of bristles in the collar a single rachidial ridge is initially formed that produces a non-branched rachis. Barb ridges appear later, so that some branching occurs and few barbules are formed at the base of the single rachis

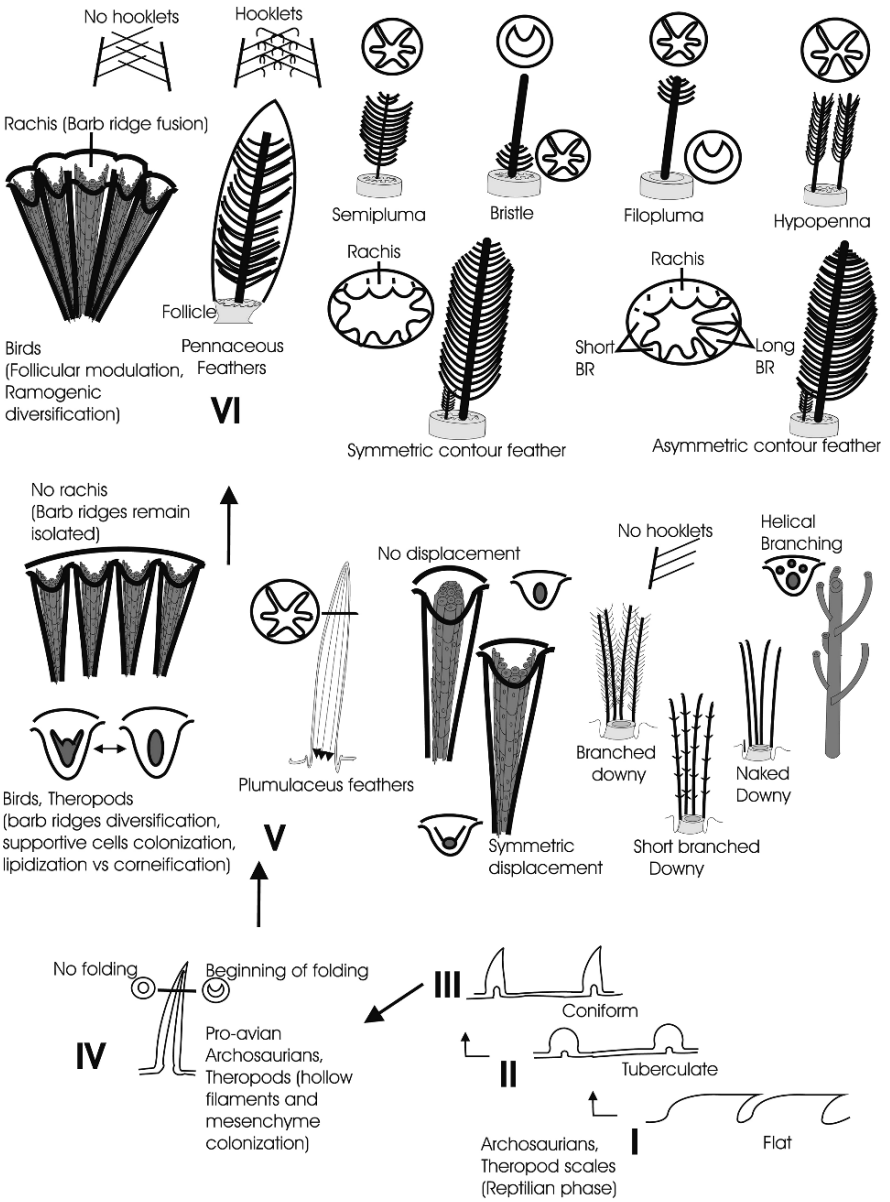


**Fig. 9.9** Summarizing scheme of proposed stages of feather evolution (see text for details). Stage **I**, flat scales; stage **II**, tuberculate scales; stage **III**, conic scales; stage **IV**, hollowed hairy-like filaments with no (*left section*) or beginning of folding (*right section*); stage **V**, plumulaceous feathers with isolated barb ridges (*left*); stage **VI**, origin (barb ridge fusion) of the rachis inside the follicle and diversification of pennaceous feathers. Schematic drawing featuring the diversification of seven types of pennaceous feathers derived from different patterns of barb ridge development) (see text for further explanation). In **VI**, the fusion of barb ridges (*left*) gives origin to different types of pennaceous feathers. Barbs with no hooklets produce semiplumes. Follicles with

the follicle were longer than in the opposite side (Figs. 9.8F–F7 and 9.9VI). The length of barb ridges with more barb and barbule cells can produce longer rami on one side of the rachis than on the other sides. As a consequence, longer rami and barbules were formed in the branching sides from the rachis, forming an asymmetric vane. The latter type presents the best aerodynamic properties, and was later selected during evolution of birds capable of flight.

A hyporachis might have formed with similar modalities of the main rachis on the opposite side of the follicle. The development of the hypopennae with hyporachis and rachis of equal dimension (Fig. 9.9VI) resembles that of contour feathers but the collar produces two rachidial ridges located in opposite regions of the follicle.

At the beginning of filoplume morphogenesis, numerous short barb ridges produced symmetric barbs (Figs. 9.8D–D5 and 9.9VI). Later, barb ridges no longer formed as separate entities, or merged into an unbranched rachis so that barb branching remained only at the tip. In comparison to filoplumes, an opposite process of timing was probably present during the modulation of barb ridge production in bristles (Figs. 9.8C–C5 and 9.9VI). Initially, only one rachidial ridge was formed (or it derived from the fusion of barb ridges into a non-branching rachis): this process produced a naked rachis. After various barb ridges were formed (or merged into a non-branching rachis) they produced short ramifications at the base of the bristle.

It is unknown how the rachidial ridge was selected or cytological details of the modifications of the rachis derived from the process of fusion with the other barb ridges. The continuous production of barb ridges from the ventral side of the follicle determined their fusion in the rachidial area for the vertical growth as a rachis. The molecular mechanisms of modulation and changing of barb ridge pattern growth inside the follicle are largely unknown, and represent an important area for future research in feather biology (Widelitz et al., 2003).

## 9.7 The Evolution of Feathers was a Consequence of the Diversification of Barb Ridges

From the previous discussion the following hypothesis is here proposed for feather evolution (Fig. 9.9). The hypothesis is based on the variation of the morphogenetic pattern of barb ridge formation that might have occurred in the skin of ancient theropods and birds of the Mesozoic Era.

Like previous hypothesis (Maderson, 1972; Prum, 1999; Brush, 2000; Maderson and Alibardi, 2000; Chuong et al., 2003; Wu et al., 2004) a reptilian phase is first considered, from large to conical scales (stages I, II, and III in Fig. 9.9). These stages were required to produce conical skin derivatives, a premise for the formation of tubular skin appendages (Alibardi, 2003, 2005c, 2006a, b). Stages I–III were present in archosaurian reptiles living during the Mesozoic Era, especially theropods: feathers were absent but conic scales were common skin derivatives in these reptiles, as is documented in the fossil record (summarized in Martin and Czerkas, 2000; Prum and Brush, 2000; Wu et al., 2004). An essential component of the epidermis

of archosaurian reptiles was the presence of beta-keratins, small proteins capable of forming long and resistant filaments (Sawyer and Knapp, 2003; Alibardi and Sawyer, 2006). Beta-keratins allowed cell elongation and the shortest version of all beta-keratins (feather-keratins) evolved in avian (theropods?) skin. Probably the latter keratin is better suited to forming long filaments with axial orientation for the elongation of barb and barbule cells, and to resisting to the digestive phase that eliminates supportive cells among barb and barbules.

The second requirement toward the emergence of feathers was the mesenchymal colonization of elongating, narrow cones on the surface of the skin in pro-avian reptiles (stage IV in Fig. 9.9). The latter stage definitely addressed the evolution of hairy-like appendages, made of beta-keratin, toward the formation of feathers. In fact, inside these hollowed appendages, the growing epidermis could become folded. The fold of the inner epidermis became the "joke" that created the incredible evolutionary potential leading to the evolution of barb ridges first, and from them to downfeathers. The present hypothesis on feather evolution is completely based on the origin and diversification of the morphogenetic process of barb ridge formation. Stage IV represents the formation of hollowed, hairy-like skin appendages, also found in theropods (e.g. *Sinosauropteryx*), and nourished by a vascular mesenchyme. These appendages, like hairs in mammals, were mainly used for thermoregulatory, sensory, and display activity. They might have remained as simple tubular outgrowths without epidermal folds (Fig. 9.9 stage IV). No differentiation of barb vane ridge cells was possibly present in these early folds. In this case, no sculpturing of separate barb and barbules and loss of the sheath was possible to allow the emergence of a feather branching. Conic or hairy-like appendages were protofeathers and not feathers, as they lacked the peculiar characteristics of feathers, i.e. the symmetric branching.

The following phase (stage V in Fig. 9.9) concerns the morphogenesis of barb ridges and their elaboration by displacement of embryonic layers into a V-shaped or a centred barb (Figs. 9.6 and 9.9V). Stage V concerns the process of barb ridge morphogenesis that allowed the "carving out" of barb and barbule cells through the action of barb vane ridge cells and of cylindrical cells in marginal plates. The detailed ultrastructural study of these supportive cells have revealed the intimate relationships with barb/barbule cells (Alibardi, 2005a, b, 2006a, b, c). According to the process of morphogenesis of barb ridges (Figs. 9.6 and 9.9V), naked or branched plumulaceous feathers were produced in both theropods and birds of the Mesozoic Era, as is indicated in the fossil record.

Plumulaceous feathers however produced a limited shape variation, related to branching patterns around the main axis, the ramus. Variations in the process of barb ridges morphogenesis formed variably branched barbs inserted in a basal calamus. Phenotype variations of plumulaceous feathers (Fig. 9.9V) were probably associated with thermoregulation or other functions such as mechanical reception, display, or sexual recognition. Long (Fig. 9.6F3) or short (Fig. 9.6E3) branching downs were better suited for thermical insulation than naked downs (Fig. 9.6D3), and nothing related to flight was possible at this stage. The use of feathers for flight was beyond this stage of feather morphogenesis and evolution (see later stage VI). Whether

naked or branched downs were more primitive is not known. Barbule and barb cells were formed at the same time and their appearence as barbs or barbules depended on the specific morphogenesis within barb ridges, not on the hypothesised, progressive complication of the branching pattern (Prum, 1999; Brush, 2000; Chuong et al., 2002; Wu et al., 2004).

Finally, the last step of feather evolution (stage VI in Fig. 9.9) was the formation of a follicle capable of regenerating a new feather and where barb ridges merged into a vertically growing rachis. Therefore, the formation of a follicle permitted the origin of the variety of feathers. The rachis became the axial element, essential for forming feathers with a "definite shape" (contour, display, hairy-like, etc.), and capable of more functions than plumulaceous feathers. Among one of these functions, the possibility to form planar, aerodynamic efficient feathers was specifically selected in a line of archosaurians from which birds originated.

According to the different types of barb ridge morphogenesis (Fig. 9.6), length of the barb ridge and presence of hooklets (Fig. 9.7), and pattern of barb ridge formation within follicles (Fig. 9.7), different feather phenotypes were produced (Figs. 9.8 and 9.9 stage VI). Some of these formed the variety of semiplumes, contour feathers, filoplumes, bristles, display feathers etc. present in modern birds. Barb ridges with no cell displacement produced naked barbs and formed open vanes: these are present, for instance, in hypopennas of some flightless birds. Whether naked pennaceous feathers were more primitive than branched pennaceous feathers (Prum, 1999; Brush, 2000; Chuong et al., 2003; Wu et al., 2004) is not known. Branching or non-branching feathers seems to be equally ancient according to the present hypothesis on barb ridge evolution.

Barb ridges with symmetric cell displacement produced long barbules overlapping those of the next barb (Fig. 9.9 stage VI). The latter process represented the beginning of formation of compact vane but hooklets might have been initially absent in these feathers. Within the long chains of barbule cells of large follicles, the formation of hooklets derived from the partial overlapping between barbule cells with the interposition of barb vane ridge or of wedge cells (Alibardi, 2005b). This process presents some aspects that resemble those present during setae formation in scales of some lizards. Hooklets were responsible for the formation of compact close vane, resistant to deformation and air currents, and later exploited for flight.

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