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RESEARCH ARTICLE

Immunolocalization of alpha-keratins and feather beta-proteins in feather cells and comparison with the general process of cornification in the skin of mammals

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SUMMARY

The maturation of the corneous material of feathers is a special case of cornification in vertebrate epidermis and is believed to occur mainly by the accumulation of small proteins of about 100 amino acids and a central beta-pleated sheet region known as feather keratins. The present immunocytochemical study carried out using double-labeling immunogold shows that a small amount of alpha-keratins of intermediate filament type form the early keratin clumps in barb and barbule cells. These initial nuclei of formation of corneous material are rapidly coated by the deposition of large amounts of small feather keratin-associated beta-proteins (feather keratins). The latter proteins turn the keratin bundles of barb and barbule cells into a compact and apparently amorphous mass of corneous material where no sign of intermediate filaments are seen. Feather beta-proteins however form an irregular filamentous network of 2–3 nm thick electron-pale filaments and produce the characteristic feather X-ray pattern due to their prevalent amount over any other protein present in feather cells. The modality of cornification in feathers is discussed in relation to the process of formation of corneous material in the skin of vertebrates in general that occurs by the association of intermediate filament proteins and keratin-associated proteins.

1. Introduction

The epidermis of vertebrates accumulates intermediate filament keratins, which became specialized in land vertebrates through evolution by the enrichment by hydrophobic amino acids such as glycine and valine for the formation of a resistant corneous layer (Fuchs et al., 1987; Steinert and Freedberg, 1991; Bragulla and Homberger, 2009; Vandebergh and Bossuyt, 2011). Different types of skin derivatives or appendages have been formed in land vertebrates, among which feathers represent the most complex case (Chuong and Widelitz, 1999; Maderson and Alibardi, 2000; Wu et al., 2004; Alibardi and Sawyer, 2006; Dhouailly, 2009; Maderson et al., 2009). The minute cell branching of feathers derives from the formation of barb ridges during development or regeneration (Fig. 1A–D; Matulionis, 1970; Alibardi, 2006a,b; Alibardi et al., 2009). Cells of barbules and barbs, calamus and rachis differentiate from the follicle, accumulate keratins, and eventually form the different types of feathers (Fig. 1E–I).

As intermediate filament proteins, keratins produce an alpha-X-ray pattern and most of the central region of their molecule forms alpha-helix, and these proteins are indicated as alpha-keratins (Rudall, 1947; Fraser et al., 1972; Fuchs et al., 1987; Steinert and Freedberg, 1991; Bragulla and Homberger, 2009; see Fig. 2). Other smaller types of epidermal proteins, very different from those of intermediate filaments were also synthesized especially in land vertebrates and these proteins reinforce the initial keratin meshwork (Fraser et al., 1972; Matoltsy, 1987; Powell and Rogers, 1994). The latter proteins are indicated as inter-keratin matrix proteins or keratin associated proteins (KAPs). The association of intermediate filament keratins and KAPs in the epidermis of all vertebrates produces a hard and hydrophobic corneous material in relation to their relative ratio in each different skin appendage (Alibardi, 2006a).

In reptiles and birds, beta- or phi-keratins produce a beta-X-ray pattern and contain a central beta-pleated region (Baden and Maderson, 1970; Fraser et al., 1972; Gregg and Rogers, 1986; Brush, 1983, 1993; Fraser and Parry, 1996, 2008; Sawyer et al., 2000; Dalla Valle et al., 2008, 2010). These beta-keratins are themselves capable of forming resistant filaments (Brush, 1983, 1993), and the association of alpha- and beta-keratins gives rise to the hard corneous layers of scales and claws. A specialized and short version of these small proteins includes the prevalent type of proteins present in feather cells indicated as “feather keratins” (Fraser et al., 1972; Brush, 1983, 1993; Gregg and Rogers, 1986; Sawyer et al., 2000, 2005; Fraser and Parry, 2008). The structural role of alpha-keratins

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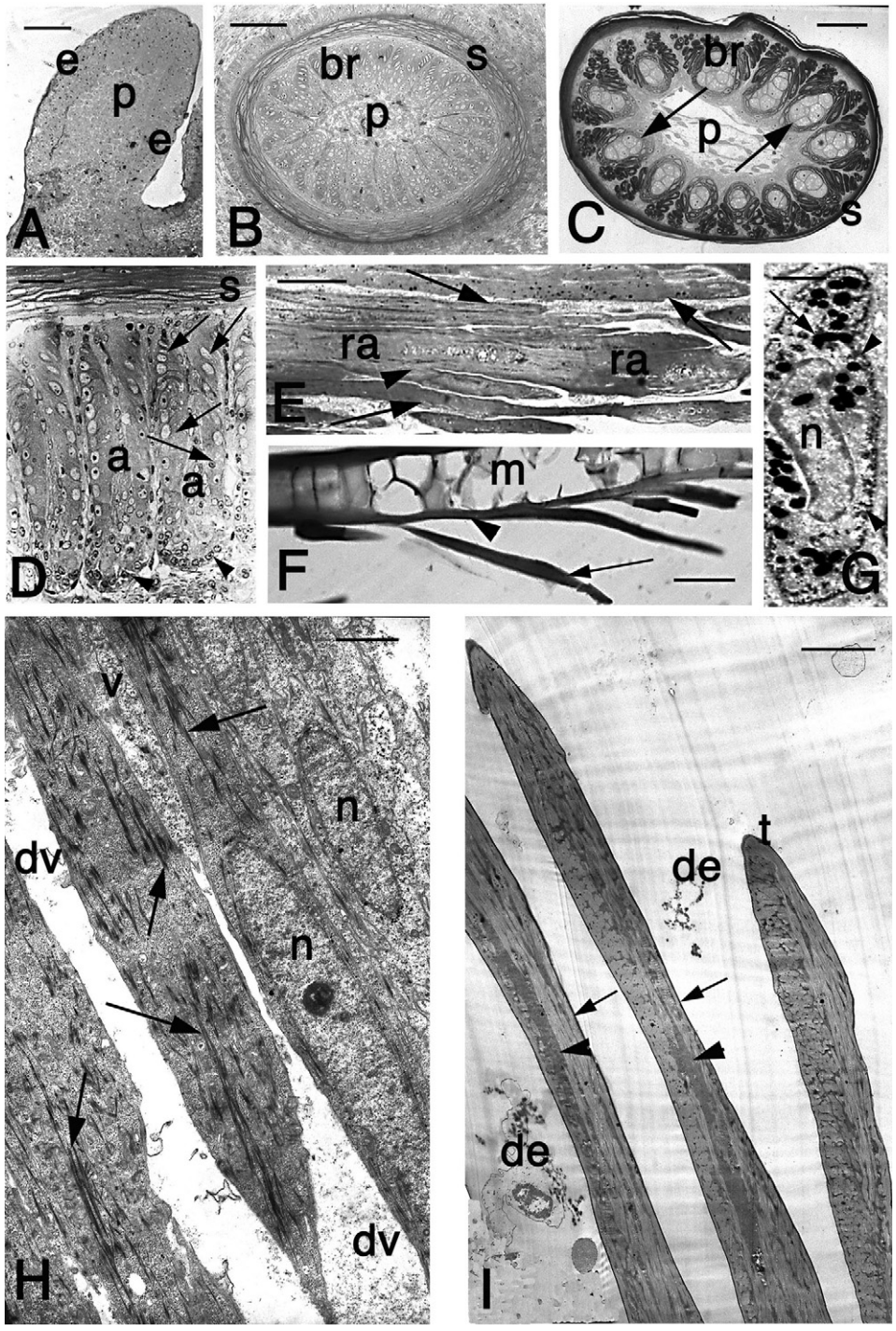

Fig. 1. Light (A–F) and electron microscopic (G–I) view of feather formation. A, feather germ at 12 days. Bar, 30 μm. B, cross-sectioned feather filament inside the follicle showing barb ridges surrounding the pulp at stage 38. Bar, 40 μm. C, cross-sectioned feather filament about 200 μm outside the follicle at stage 39 showing vacuolated cells of the barb-medulla (arrows) in maturing barb ridges (the dark cells are barbule cells stuffed with corneous material). D, detail of elongated barb ridges within the follicle of juvenile feather with numerous barbule cells along the two barbule plates in each barbule ridge (arrows). Bar, 20 μm. E, longitudinal section of barb ridge outside the follicle at stage 37 showing the axial ramus (ra) and the barbule branching (arrowheads). The arrow points to barb cortical cells. Bar, 15 μm. F, mature medullated ramus (the arrowhead indicates the external wall made of barb cortical cells) with branching barbules (arrow) at stage 39. Bar, 25 μm. G, differentiating barbule cell storing central (arrows) and peripheral (arrowheads) corneous material. Bar, 2.5 μm. H, longitudinally sectioned barbule cells accumulating bundles of corneous material made of alpha and feather keratin (arrows). The empty spaces between barbule cells derive from the degeneration of supportive cells. Bar, 2.5 μm. I, mature barbule cells separated by empty spaces containing some degenerated material at stage 39. Barbules show a thickened plasma membrane (arrows) and some dense areas (arrowheads) within the paler corneous material. Bar, 1 μm. *Legends*: a, axial plate; br, barb ridges; de, cell debris among mature barbule cells, dv, degenerating barb vane ridge cells leaving empty spaces; e, epidermis; m, medullated ramus; n, nucleus; p, pulp; ra, ramus; s, sheath; t, tip of barbules; v, barb vane ridge cell (supportive inter-barbule).

present in the corneous material of sauropsid scales, claws, rhamphoteca (beak), is not known but it is assumed these proteins acts as initial cytoskeletal elements for cell shaping (Fraser et al., 1972; Brush, 1993).

Also feathers, as specialized skin appendages containing prevalently feather beta-keratins, like in all skin appendages in vertebrates should contain intermediate filament keratins, at least at the beginning of their formation. While the small feather keratins