Journal of Structural Biology 191 (2015) 341–351

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

The proteomics of wool fibre morphogenesis

Jeffrey E. Plowman [1,*], Duane P. Harland [1], Sivasangary Ganeshan, Joy L. Woods, Bede van Shaijik, Santanu Deb-Choudhury, Ancy Thomas, Stefan Clerens, David R. Scobie

Food & Bio-Based Products, AgResearch, Lincoln Research Centre, Christchurch, New Zealand

ARTICLE INFO

Article history:
Received 28 June 2015
Accepted 10 July 2015
Available online 21 July 2015

Keywords:
Wool
Keratin
KAP
Hair follicle

ABSTRACT

Gel and gel-free proteomic techniques have been used for the first time to directly study the proteins present in whole wool follicles and dissected portions of follicles that correlated with morphological changes in the developing fibre as determined by transmission electron microscopy. Individual wool follicles were dissected into four portions designated as the bulb, elongation, keratogenous and keratinisation portions. Gel-free proteomic analysis of dissected portions from 30 follicles showed that the first keratins to appear were K31, K35 and K85, in the bulb portion. The first epithelial KAP, trichohyalin, was detected in the bulb portion and the first cortical KAP, KAP11.1 was found in the elongation portion. Other major trichocyte keratins and cortical KAPs began to appear further up the follicle in the keratogenous and keratinisation zones. These results were consistent with what has been observed from gene expression studies and correlated well with the morphological changes observed in the follicle. Other proteins detected by this approach included the keratin anchor protein desmoplakin, as well as vimentin and epithelial keratins, histones, ribosomal proteins and collagens. Two-dimensional electrophoretic (2DE) analysis of dissected portions of 50 follicles revealed substantial changes in the position, number and intensity of the spots of the trichocyte keratins as they progressed through the follicle zones, suggesting that they are subject to modification as a result of the keratinisation process. Also present in the 2DE maps were a number of epithelial keratins, presumably from the inner and outer root sheaths, and the dermal components.

1. Introduction

The follicles in which mammalian fibres grow are miniature developmental systems that appear as epithelium-derived tubular down growths into the dermis of the skin (Orwin, 1979; Marshall et al., 1991). Follicles periodically alternate in morphology and activity, from active production of fibre (anagen) to quiescent (telogen) (Rogers, 2006; Schneider et al., 2009). In the follicles of most domestic sheep breeds, anagen typically lasts several years and the morphology of grown wool remains relatively constant over much of that time. For this reason sheep are an excellent model system for investigating the processes that underpin fibre assembly and, in particular, how the finer details of whole fibre phenotypes (e.g., fibre diameter or cuticle architecture) are defined via the organisation of follicle structural components across a wide range of spatial scales from molecular to multi-cellular (Hearle, 2003).

In this study we investigated proteomic changes that occur at different stages of fibre development and correlated this data with ultrastructural landmarks within the follicle. A robust framework for describing developmental stages is essential to facilitate study-to-study comparison. The fibre development process is conveniently linear in follicles. Specific stages in fibre development simply correspond to specific locations along the follicle. When fibres have a uniform morphology and when most follicles are in anagen, as in wool, it is possible to accumulate sufficient sample volumes to reliably investigate the proteome of different fibre development stages. Here we describe follicle morphology and fibre assembly using an ultrastructure-morphology-based scheme developed by Orwin (Orwin and Thomson, 1972), which was based on consistently observed features of the developmental process ranging in scale from nanometres to millimetres of both medullated and non-medullated wool follicles (Orwin, 1979; Marshall et al., 1991), and which divides the follicle into seven Zones named A through G (Table 1 and Fig. 1A).

Abbreviations: KAP, keratin-associated protein; IF, intermediate filament; ORS, outer root sheath; IRS, inner root sheath.

* Corresponding author at: AgResearch Ltd., Private Bag 4749, Christchurch 8140, New Zealand.

E-mail address: jeff.plowman@agresearch.co.nz (J.E. Plowman).

[1] These authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.jsb.2015.07.005
1047-8477/

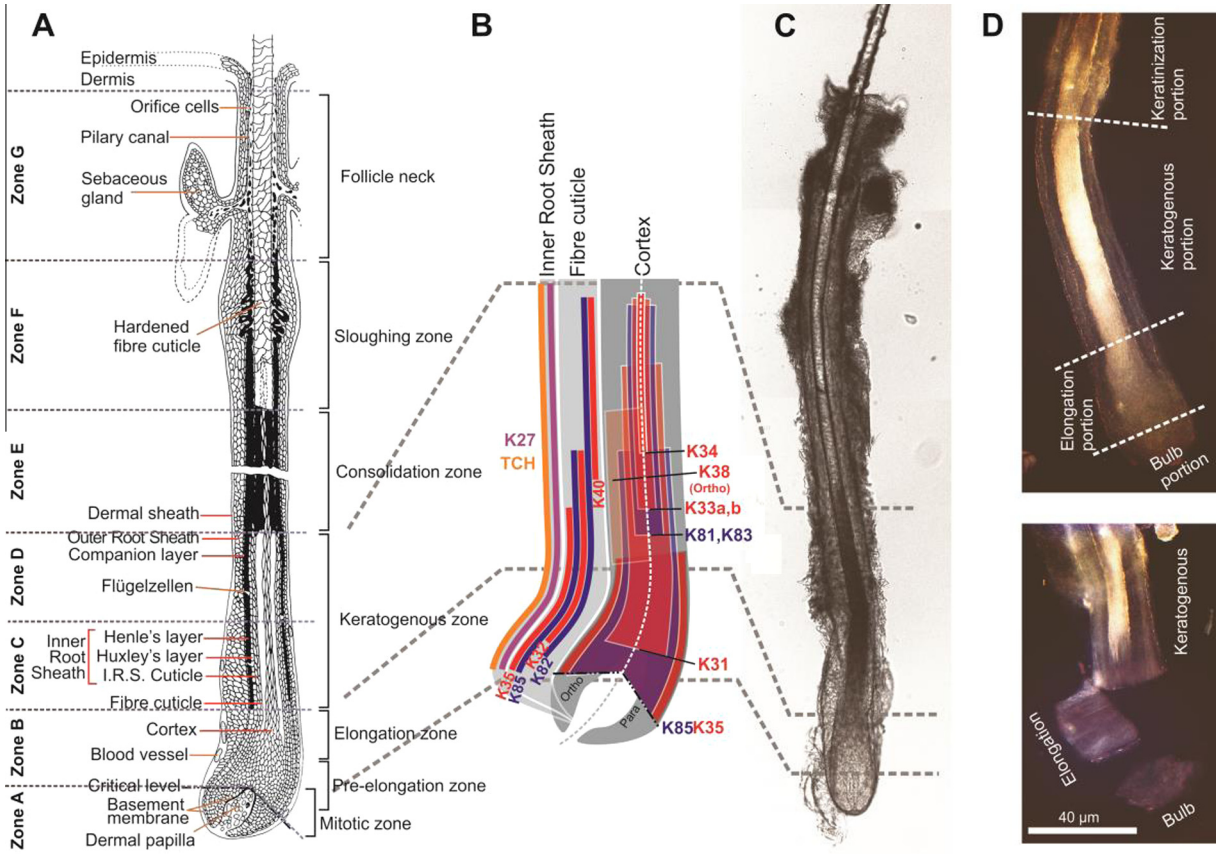

Fig. 1. Follicle morphology nomenclature gene, expression patterns and dissection. (A) Ultrastructural zones (left), histological zones and key features. (B) Pattern of keratin gene expression in wool follicle as determined from *in situ* hybridisation studies and showing overlap of Type I (red) and Type II (blue) keratins. (C) Example of a dissected whole wool follicle. (D) Example of dissection of follicle into portions. (A) Modified from an original illustration by Orwin and Woods. (B) Data from original micrographs used in Yu et al. (2011, 2009).

Table 1
Summary of ultrastructurally-defined zones of the wool follicle used in this study based on the study of Orwin (Orwin, 1979; Orwin and Thomson, 1972).

Zone	Markers	Main biological processes
Zone A	Mitosis and lack of clear cell line differentiation, distal boundary is a line angled proximally from the tip of the dermal papilla in non-medullated follicles or the widest part of papilla in medullated follicles	Basement membrane-bound stem cells (mother cells) around dermal papilla neck continuously bud off daughter cells which divide further (possible transient amplifying cells)
Zone B	Begins at first signs of cell line differentiation, in particular cell shape and alignment and first appearance of keratin in cortex	Cell line differentiation especially in cell junctions, shape and position, cytoplasmic expression of keratin and trichohyalin
Zone C	Begins where Henle's layer hardens	Most cell shape/position changes completed, major keratin and KAP synthesis in fibre, increase in particular of KAP species
Zone D	Begins where cuticle keratin layer is continuous along membrane apposed to IRS cuticle (fibre isolated from IRS)	Keratin and KAP synthesis continue in cortex and cuticle while non-keratin cell components broken down
Zone E	Begins where remaining IRS layers harden	Consolidation, keratinisation or hardening process associated with water loss, precipitation of keratin and internal changes to cortical IF structure
Zone F	Begins where osmium staining is no longer effective in cortex (osmiophilia lost)	Fibre is in mature form. Probable extraction of material from IRS layers occurs and IRS takes on aporous appearance before fragmenting to release the fibre
Zone G	Begins where fibre is fully free of IRS material	Sebaceous and sudoriferous (if present) glands coat fibre in material along with broken down remnants of IRS, the fibre emerges from the skin

Wool fibre development involves a plethora of protein and cellular differentiation processes. This begins with division from a monolayer of stem cells attached to a basement membrane surrounding the base of the dermal papilla in Zone A. Daughter cells from the stem cell layer continue to divide while they migrate around the dermal papilla into Zone B, where division stops and differentiation begins. Six cell lines, or seven in medullated fibres, develop in tightly coordinated cylindrical layers, with each having distinct gene expression patterns, morphological shaping and cell-to-cell junction development (Orwin, 1979; Langbein and Schweizer, 2005). Our focus was on fibre (trichocyte) keratins and keratin-associated proteins (KAPs) which make up the