Journal of Anatomy

J. Anat. (2009) **214**, pp516–559 doi: 10.1111/j.1469-7580.2009.01066.x

REVIEW

Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia

Hermann H. Bragulla[1] and Dominique G. Homberger[2]

[1]*Department of Comparative Biomedical Sciences, School of Veterinary Medicine and*
[2]*Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana, USA*

Abstract

Historically, the term 'keratin' stood for all of the proteins extracted from skin modifications, such as horns, claws and hooves. Subsequently, it was realized that this keratin is actually a mixture of keratins, keratin filament-associated proteins and other proteins, such as enzymes. Keratins were then defined as certain filament-forming proteins with specific physicochemical properties and extracted from the cornified layer of the epidermis, whereas those filament-forming proteins that were extracted from the living layers of the epidermis were grouped as 'prekeratins' or 'cytokeratins'. Currently, the term 'keratin' covers all intermediate filament-forming proteins with specific physicochemical properties and produced in any vertebrate epithelia. Similarly, the nomenclature of epithelia as cornified, keratinized or non-keratinized is based historically on the notion that only the epidermis of skin modifications such as horns, claws and hooves is cornified, that the non-modified epidermis is a keratinized stratified epithelium, and that all other stratified and non-stratified epithelia are non-keratinized epithelia. At this point in time, the concepts of keratins and of keratinized or cornified epithelia need clarification and revision concerning the structure and function of keratin and keratin filaments in various epithelia of different species, as well as of keratin genes and their modifications, in view of recent research, such as the sequencing of keratin proteins and their genes, cell culture, transfection of epithelial cells, immunohistochemistry and immunoblotting. Recently, new functions of keratins and keratin filaments in cell signaling and intracellular vesicle transport have been discovered. It is currently understood that all stratified epithelia are keratinized and that some of these keratinized stratified epithelia cornify by forming a *Stratum corneum*. The processes of keratinization and cornification in skin modifications are different especially with respect to the keratins that are produced. Future research in keratins will provide a better understanding of the processes of keratinization and cornification of stratified epithelia, including those of skin modifications, of the adaptability of epithelia in general, of skin diseases, and of the changes in structure and function of epithelia in the course of evolution. This review focuses on keratins and keratin filaments in mammalian tissue but keratins in the tissues of some other vertebrates are also considered.

Key words epithelium; keratin; keratinization; keratin filaments; cornification.

Introduction

When we started to survey, collect and organize the current knowledge on keratins (unless mentioned otherwise, hereafter the term 'keratins' refers to keratin proteins) and keratin filaments for the invited review of keratins in soft-keratinized epidermis and epithelia, we soon realized that such a study would lead to a greater understanding only if the keratins were discussed as integral elements of cells, tissues and organs. As an analogy, a review of collagen would also make sense only within the context of connective tissue structures (Wang, 2006). In order to understand the functions of intermediate filaments as components of the cytoskeleton for the cells to respond to extracellular forces (Reichelt, 2007), to provide a network for organized processes of transportation and to participate in transmembrane signaling processes, an integrated approach to keratins, filament formation and assembly of a cytoskeleton in connection with keratin filament-associated proteins (KFAPs), focal cell membrane modifications, and intercellular cementing substances is necessary.

Corneous, or horny, tissues have a long history of interest due to their economic, practical and emotional value. For example, the sheaths of horns have been fashioned into drinking vessels; mammalian fur has been used for clothing; the skin of reptiles has been manufactured into leather for

Correspondence
Hermann H. Bragulla, Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Skip Bertman Drive, Louisiana State University, Baton Rouge, LA 70803,USA. E: bragulla@lsu.edu

Accepted for publication *30 January 2009*

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

clothing and pouches; mammalian hair has been used to make felt or to spin yarn for weaving and knitting; feathers have been used for various bedding materials and clothing; baleen has been used as whalebone in the fashion industry; 'tortoise shell' has been used for making combs and decorative objects; and hooves of farm animals have been used as slowly decaying fertilizers (Gupta & Ramnani, 2006; Thys & Brandelli, 2006). Keratin-rich tissues are studied for their economic importance in the wool industry, for cosmetics and dermatology (Er Rafik et al. 2004). Furthermore, the health of the hooves of farm and draft animals is of crucial economic importance to large animal producers and forms the basis of a longstanding interest in veterinary medicine concerning the structure and function of keratinized and cornified tissues. The complexion of the skin in humans contributes significantly to a person's appearance; it is a major indicator of a person's health status and, thus, a source of interest for medicine and dermatology. Finally, fundamental differences in the structure of the skin among various vertebrates have been used by traditional comparative anatomists as characters to conceptualize the evolution of the various vertebrate lineages. Recently, environmental problems arising from keratins as a byproduct of mass-produced poultry have been addressed (Werlang & Brandelli, 2005). As a result of the various interests in and uses of keratinized and cornified tissues, different research approaches and bodies of knowledge have developed over time, and these need to be integrated and synthesized to gain a coherent understanding of these tissues.

Corneous tissues hold a special place among the tissues of vertebrates as they cover the surface of animals and, thereby, represent the interface between an organism and its environment (Wu et al. 2004). Hence, both the underlying connective tissues of the organism and the environment directly influence these corneous tissues and the effects of these influences can often be observed *in vivo* (Homberger & Brush, 1986). Despite the great variety in appearance, it was recognized by early comparative anatomists that structures as diverse as hairs, feathers, hooves and baleen consist of a similar substance, which was called 'horn' or 'keratin' (Siedamgrotzky, 1871; Tullberg, 1883). Subsequently, it was recognized that corneous tissue can be relatively soft and pliable or relatively hard and stiff (Boas, 1881) and that these different properties can be correlated to different types of keratin molecules within the cells [e.g. α- and β-keratins, acidic vs. basic, soft vs. hard, various molecular weights (MWs)] (Fraser et al. 1972). As more data have become available, it has also become clear that the composition of keratins within each category of corneous tissue is more diverse than previously assumed (Moll et al. 1982; Schweizer et al. 2006), with various gradations between the categories (Hesse et al. 2004). Corneous tissues not only differ in their biochemical nature but also in their microarchitecture that results from the arrangement, shape and developmental state of their cells. The origin of this microarchitecture lies in the three-dimensional shape of the interface between the epidermis and the underlying connective tissue of the dermis (Budras et al. 1989; Homberger, 2001; Bragulla & Hirschberg, 2003; Homberger et al. 2009).

In the following review, we cast a wide net and cover a variety of topics, thus providing the necessary context for the discussion of the structure, function and evolution of keratins, keratin granules and keratin filaments. Our review is rooted in the context of function and morphology, with the cells and tissues viewed primarily as materials with distinctive physical properties. These physical properties are subject to selective regimes as a result of their interactions with their environment, so that the cells and tissues can adapt to new conditions and uses, usually through gradual changes (Chuong & Homberger, 2003).

Morphological classification of tissues

Tissues in general: types of tissues, cells and intercellular substances

Vertebrate tissues are traditionally divided into two major categories: (1) epithelial tissues of ectodermal or endodermal origin and with little intercellular substances (i.e. intercellular cementing substance); and (2) mesenchymal (connective) tissues of mesodermal origin with a substantial amount of extracellular substances (e.g. glycosaminoglycans, collagen fibers, etc.) (Banks, 1993). However, the distinction between these two categories is not as sharp as some textbooks report and some tissues, such as keratinized tissues, do not fit neatly within this scheme. All tissues consist of cells as well as of intercellular or extracellular substances, which are produced by the cells. One aspect in which these tissues differ is in the proportion of cells to the amount of intercellular or extracellular substances. For example, the epithelium lining the nasal cavity consists of tightly packed cells with little intercellular substances, whereas a tendon usually has widely spaced cells with a large amount of extracellular substances, such as collagen fibers. The intercellular or extracellular substances determine the properties of the particular tissue, especially in connective tissues, such as cartilage and tendons. Epithelial tissues, depending on the structure that they form, comprise a large number of tightly packed cells with a varying, but at most moderate, amount of intercellular cementing substances, which establish a semipermeable barrier between the cells (Elias & Friend, 1975).

Epithelial tissues: location, structure and function of epithelial tissues

Epithelia line surfaces, form glands and act as receptor cells in sensory organs (Fig. 1). Epithelial tissues line internal and external surfaces, such as the external surface of the

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

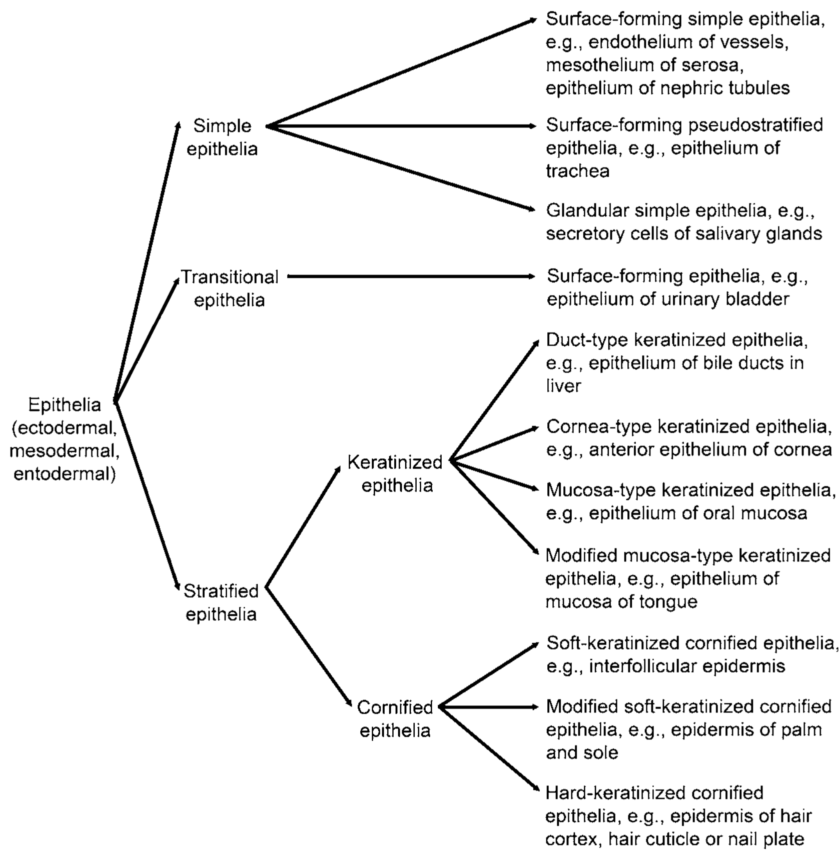

Fig. 1 Pre- and postnatal development of epithelial tissues. The first epithelia in a vertebrate embryo are simple epithelia such as the trophoblast epithelium or the epithelium of the vitelline sac. Epithelial tissues are derivatives of all three germ layers, i.e. the ectoderm, mesoderm and endoderm. All stratified epithelia start as simple epithelia and stratify as well as differentiate during the embryonic, fetal development and even postnatal development. The structures of epithelia reflect their various functions, e.g. semipermeable or protective barrier.

skin or the internal lining of the intestine. In addition, epithelia line the ducts and secretory units of glands, such as those of the liver or pancreas. Epithelia separate compartments by forming barriers (e.g. blood–urine barrier), regulate the exchange of molecules between compartments (e.g. between intestine and blood) and protect tissues and structures covered by stratified epithelia (e.g. epidermis).

Epithelial tissues consist of squamous, cuboidal, columnar or polyhedral cells that are attached to one another via short cell processes. These cells produce only a small amount of intercellular substances (Banks, 1993). The structure of epithelial tissues varies with its function. Cells of secretory epithelia are polarized in order to direct secretory vesicles to the apical surface and absorb metabolites on the basolateral surfaces (Frappier, 2006).

Morphological classification of epithelial tissues

Morphological characteristics, such as cell shape and stratification, are the basis for the classification of epithelial tissues (Frappier, 2006). In general, epithelia are distinguished as being simple, transitional or stratified (Fig. 1).

Simple epithelia

Two types of simple epithelia are distinguished, i.e. single-layered and multi-layered epithelia. In single-layered epithelia, all cells are attached to the basement membrane and extend to the surface of the epithelium (e.g. endothelium, mesothelium, epithelium in the renal tubules and alveoli). In multi-layered epithelia (i.e. pseudostratified epithelia), all cells are in contact with the basement membrane but do not necessarily extend to the surface of the epithelium (e.g. respiratory epithelium).

Transitional epithelium

In transitional epithelia, at least some cells attach to the basement membrane and also extend to the surface of the epithelium [e.g. epithelium of the renal pelvis, ureter and urinary bladder (also called 'urothelium' by Banks, 1993)].

Stratified epithelia

In stratified epithelia, only the basal cells are attached to the basement membrane and only the most superficial of the suprabasal cell layers form the surface of these epithelia. Only the cells in the basal stratum are mitotically active and replenish the loss of cells on the surface of the superficial stratum. In the intermediate stratum of a stratified epithelium, the cells undergo various processes of differentiation, such as keratinization. In general, two types of stratified epithelia are distinguished, namely the stratified keratinized but non-cornified epithelia and the stratified keratinized-cornified epithelia.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Stratified, non-cornified epithelia
In the superficial stratum of a stratified, non-cornified epithelium, the cells (i.e. keratinocytes) are living and keratinized but not cornified (e.g. epithelium of the oral cavity, esophagus, vagina, urethra).

Stratified-cornified epithelia
The epithelial cells in the superficial stratum (i.e. the *Stratum corneum*), the corneocytes, are cornified and dead. Cornification requires the previous keratinization of cells, including the addition of a proteinaceous layer (i.e. the cornified envelope) on the cytoplasmic surface of the cell membrane. In general, two types of stratified-cornified epithelia are distinguished, namely the soft-cornified epithelia (e.g. the epidermis), and the hard-cornified epithelia (e.g. the plate of the the human fingernail).

Stratified soft-cornified epithelia
The suprabasal cell layers include a *Stratum granulosum* characterized by the presence of basophilic keratohyalin granules, which store the KFAPs that are synthesized by the keratinizing cells. In the process of soft cornification, the KFAPs form a filament–matrix complex and a proteinaceous cellular envelope on the inside of the cell membrane. The superficial cornified cells of stratified non-modified soft-cornified epithelia (e.g. epidermis) desquamate continuously and readily, whereas those of modified stratified soft-cornified epithelia (e.g. cuticle of the fingernail, periople of the hoof) do so only after accumulating for a certain time.

Stratified hard-cornified epithelia
In stratified hard-cornified epithelia, the suprabasal cell layers do not include a *Stratum granulosum* (e.g. cortex of hair, plate of the human fingernail, cornified sheath of a cat claw, cornified sheath of a bird beak, wall of the horse hoof, mechanical filiform papillae of the tongue, palatal rugae, baleen plates of mysticete whales). The superficial cells of the *Stratum corneum* of hard-cornified epithelia do not desquamate but are worn off.

Constituents of the cytoskeleton in epithelial cells

There are three types of filaments, each with specific properties, which interact with one another in the formation of the cytoskeleton of epithelial cells (Frappier, 2006). They are classified on the basis of diameter and physicochemical properties as microfilaments, microtubules and intermediate filaments.

Microfilaments

Microfilaments are the smallest filaments of the cytoskeleton with a diameter of 7 nm. They are assembled from globular actin molecules, which join to form filamentous actin by using energy from adenosine triphosphate or guanosine triphosphate. Two filamentous actin molecules then combine into a helical microfilament. Microfilaments are polarized so that while one end is elongated by the addition of more filamentous actin molecules, the opposite end is disassembled. Therefore, actin microfilaments can act as conveyor belts for micro-motor molecules, such as myosin. Actin filaments are anchored via actin-binding proteins (e.g. plectin) to the cell membrane by focal adhesions, which are part of tight junctions.

Microtubules

Microtubules are the largest filamentous structures of the cytoskeleton with a diameter of about 20 nm. They are assembled from α- and β-tubulin molecules, which form heterodimers. These heterodimers are assembled using energy from guanosine triphosphate to form protofilaments, which in turn are combined to form a hollow microtubule. Microtubules are polarized and, thus, can act as conveyor belts for micro-motor molecules (e.g. dynein, kinesin) similar to microfilaments. Microtubule formation is initiated by so-called microtubule-organizing centers, such as the basal bodies in kinocilia or the centrosomes involved in the formation of the spindle apparatus of cells. The network of microtubules is stabilized within a cell by the surrounding microfilaments and intermediate filaments. The microtubule-organizing centers also interact with keratin filaments (see below) by binding to them through mediator proteins and thereby maintaining the polarity of certain epithelial cells.

Intermediate filaments in general

Intermediate filaments differ fundamentally from microfilaments and microtubules. Unlike microfilaments and microtubules, intermediate filaments can aggregate into bundles of varying diameter, ranging from 7 to 12 nm. Intermediate filaments are not polarized. Therefore, intermediate filaments are not involved in intracellular transport but serve as a scaffold for the cytoskeleton. Recently, however, keratin filaments in mammals have been suggested to be involved in the transport of melanosomes from their site of endocytosis in the cell periphery to the cell center (Planko et al. 2007). Keratins that form intermediate filaments are expressed exclusively in epithelial cells *sensu lato* (Moll et al. 1982; Steinert, 2001), regardless of the germ layer origin of these cells. In non-epithelial cells, there are various types of intracellular intermediate filaments, including desmin (in myogenic cells), vimentin (in fibroblasts and fibrocytes), lamin (in cell nuclei) and neurofilaments (in neuronal cells) (Moll et al. 1982). In addition, cells in connective tissues (e.g. fibroblasts, chondroblasts) produce extracellular intermediate filaments (i.e. the collagen filaments).

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Previously, it was assumed that the process of filament aggregation could start anywhere in a cell. Recent observations suggest, however, that the formation of intermediate filaments requires some kind of organizer, such as the focal adhesions of microfilaments to the cell membrane (Gu & Coulombe, 2007). The assembly and turnover of intermediate filaments require the assistance of enzymes, such as kinases and phosphatases, as well as chaperone molecules, such as heat shock proteins (Herrmann et al. 2007).

Intermediate filaments are a crucial component of the so-called cell signalosome, the multifunctional protein complex that is essential for cell development and possibly for the regulation of protein degradation, which translates environmental cues into modifications of gene expression (Herrmann et al. 2007). In the cytoskeleton, intermediate filaments are attached to the nuclear membrane next to nuclear pores and to cell membrane modifications, such as desmosomes and hemidesmosomes (Steinert, 2001). Electron microscopic images suggest that intermediate filaments loop through the attachment plaques of desmosomes (Pekny & Lane, 2007).

Intermediate filaments in epithelia: keratin filaments

Intermediate filaments in epithelial cells (i.e. keratin filaments) are made of keratins and these keratins are found only in vertebrates (Steinert et al. 1982; Fuchs, 1983). Keratins account for about 80% of the total protein content in differentiated cells of stratified epithelia (Pekny & Lane, 2007). In both human and murine stratified epidermis, keratins account for 25–35% of the extracted proteins (Bowden et al. 1984).

The expression of proteins forming intermediate filaments can change when epithelial cells develop into mesenchymal cells and vice versa (Moll et al. 1984). For example, during neural tube formation, keratin-producing ectodermal cells change into vimentin-producing mesenchymal cells, whereas during the formation of renal tubules vimentin-producing mesenchymal cells change into keratin-producing epithelial cells (Moll et al. 1984).

Definition and nomenclature of keratins

'Keratin' is often misunderstood to be a single substance, even though it is composed of a complex mixture of proteins, such as keratins, KFAPs and enzymes extracted from epithelia (Tomlinson et al. 2004). Keratins are found only in epithelial cells and are characterized by unique physicochemical properties (Steinert et al. 1982; Sun et al. 1983). They are resistant to digestion by the proteases pepsin or trypsin and are insoluble in dilute acids, alkalines, water and organic solvents (Block, 1951; Steinert et al. 1982). Keratins are insoluble in aqueous salt solutions but these proteins are soluble in solutions containing denaturing agents, such as urea (Steinert et al. 1982). Keratins in aqueous solution are able to reassemble intermediate filaments (Steinert et al. 1982; Sun et al. 1983). Keratins are specifically classified according to their molecular structure, physicochemical characteristics (see section 'Physicochemical characteristics of keratins'), the epithelial cells producing them and the epithelial type containing the keratin-producing cells (Steinert et al. 1982). It is worth noting that keratins expressed by human and bovine tissues are very similar in electric charge, size and immunoreactivities (Cooper & Sun, 1986). In addition, keratins of hard-cornified tissues and dental enamel show remarkably constant molecular ratios of histidine, lysine and arginine (Block, 1951).

Keratins can be extracted from various tissues by using reducing agents, such as thioglycollate, dithiothreitol or mercaptoethanol, which cleave disulfide bonds (Brown, 1950; Sun & Green, 1978; Steinert et al. 1982). The first keratin protein nomenclature was published by Moll et al. (1982) and it has been repeatedly updated in recent years (Hesse et al. 2001, 2004; Schweizer et al. 2006) to accommodate the results of ongoing research in humans and other vertebrates. The comprehensive nomenclature of keratins follows the guidelines issued by the Human and Mouse Genome Nomenclature Committees (Schweizer et al. 2006) and is an adaptation of various older keratin nomenclatures. Szeverenyi et al. (2008) published a comprehensive catalogue of the human keratins, their amino acid sequence, the nucleotide sequence of the keratin genes in humans as well as the same data of the orthologue keratins and keratin genes in various vertebrate species.

Physicochemical characteristics of keratins

The physicochemical characteristics [i.e. MW in kDa and isoelectric point (pI)] of keratins in various tissues and different species determine the physical properties of keratins in epithelial cells and tissues. To determine their physicochemical properties, keratins need to be placed in solution first by extracting them from epithelial cells using solvents (at a particular pH and a specific concentration) of urea and reducing agents (e.g. mercaptoethanol or dithiothreitol) to break the disulfide bonds that link these keratins to each other and to KFAPs (Moll et al. 1982; Sun et al. 1983). These solubilized keratins are then separated according to MW and pI using one- and two-dimensional gel electrophoresis (O'Farrell, 1975; O'Farrell et al. 1977). Differences in the MW and pI of orthologous keratin protein in various species are due to slight differences in the keratin genes, post-transcriptional processing of the messenger RNA, post-translational processing of the protein or variations in the number of phosphorylated or glycosylated amino acid residues (Eckert, 1988).

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Molecular weight

The MW of keratins in mammals ranges from 40 to 70 kDa (Sun et al. 1983). Based on two-dimensional gel electrophoresis and immunoblotting, Cooper & Sun (1986) separated and compared human and bovine keratins and found that although some keratins in corresponding tissues had the same MW, others did not. For example, the suprabasal keratin K10 (see below) has the same MW (56.5 kDa) in both human and bovine epidermis, whereas K13 has a MW of 51 kDa in humans and 43 kDa in bovines (Cooper & Sun, 1986). Our interpretation of this apparent paradox is that keratins in corresponding cells and tissues of different mammalian species may have similar functions within the cells as well as antigenetic epitopes that bind the same antibody but that they actually differ in molecular structure and amino acid composition.

Isoelectric point

Keratins can be distinguished according to their pI (Moll et al. 1982). The distinction between type I (acidic or subfamily A) keratins and type II (basic or subfamily B) keratins is based on the pH at which the proteins are neutral (i.e. have a net charge of 0). This pH is regarded as the pI and is abbreviated as pI or pKi. In humans, type I keratins have a pI of 4.9–5.4, whereas type II keratins have a pI of 6.5–8.5 (Bowden et al. 1984). In bovines, type I keratins have a pI < 5.6 and type II keratins have a pI > 6.0 (Cooper & Sun, 1986). Keratins that are specific to hair, nail or wool have a pI of 4.7–5.4 (Marshall, 1983). The pI of keratins can be changed due to post-translational modifications of their amino acids (Bowden et al. 1984). Despite the differences in the pI, 30% of the amino acid types and sequences are the same in the acidic and basic types of keratins and in other intermediate filaments (Hanukoglu & Fuchs, 1983).

Types of keratins

Different types of keratins are distinguished according to various characteristics, such as physicochemical properties, or according to the cells and tissues that produce certain keratins. Keratins in simple, non-stratified epithelia are of different types than those in stratified epithelia. Epithelial cells in simple as well as in stratified epithelia always synthesize particular keratins on a regular basis. These keratins are referred to as the primary keratins of epithelial cells, such as K8/K18 in simple epithelia (Pekny & Lane, 2007) or K5/K14 in stratified epithelia (Moll et al. 1982). These epithelial cells can also produce other keratins in addition to or instead of the primary keratins and these keratins are referred to as secondary keratins, such as K7/K19 in simple epithelia or K15 and K6/K16 in stratified epithelia. Keratins produced in the suprabasal cells of the soft-keratinizing and cornifying epidermis of the skin differ from the keratins produced in the suprabasal epithelial cells of the hard-keratinizing and cornifying epidermis of the hair cortex, hair cuticle or plate of the human fingernail.

The following sections present a compilation of currently available basic information about various keratins. The amount of available information varies among the keratins. The MW (in kDa) and the pI mentioned refer to the keratins in humans unless mentioned otherwise. The old labels of the various keratins are given in parentheses besides the new labels issued by the Human and Mouse Genome Nomenclature Committees in 2006.

Basic keratins (i.e. keratins of type II). Basic keratins, i.e. K1–K8, K71–K80 and K81–K86 in humans (Coulombe et al. 1989; Coulombe & Omary, 2002; Schweizer et al. 2006) are summarized as keratins of type II with a MW ranging from 52 to 67 kDa (Fuchs et al. 1981; Moll et al. 1982). Some of these keratins are specific for the process of soft keratinization, whereas others are unique for hard keratinization.

Basic keratins in soft keratinization (K1–K8 and K71–K80). Keratin K1: The MW of K1 is variable among mammals (Moll et al. 1982). For example, in human epidermis K1 has a MW of 65 kDa (Moll et al. 1982), whereas the orthologue in rat epidermis is 66 kDa (Kopan & Fuchs, 1989). K1 and its partner K10 are produced in the suprabasal cells of the epidermis and are therefore regarded as important for postmitotic differentiation in stratified keratinizing and cornifying epithelia (Kartasova et al. 1993; Pekny & Lane, 2007). The expression of K1 precedes that of K10 and K1 is therefore first associated with preformed K5/K14 filaments. After the expression of K10, both K1 and K10 are integrated into the keratin filament network (Kartasova et al. 1993).

Keratin K2 (K2e): The MW of K2 is 61 kDa in rats, 65.5 kDa in humans and 70 kDa in mice (Moll et al. 1982; Kopan & Fuchs, 1989; Mahler et al. 2004). The amino acid sequence of K2 is similar to that of the epidermal K1 and K5 and to that of the corneal K3 (Collin et al. 1992). K2 is a regular component of the epidermal cell cytoskeleton and is expressed most commonly in the suprabasal cells of the third or fourth layer of the *Stratum spinosum* (Smith et al. 1999). In mechanically stressed epithelia (e.g. ear, foot pad and tail of mouse), the synthesis of K2 is increased and induced by the underlying dermis (Mahler et al. 2004). K2 can also be expressed in the superficial cells of the epithelium of the oral mucosa, which indicates an advanced orthokeratinization of those epithelial cells (Bloor et al. 2003).

In rat embryos, K2 starts to be produced in epidermal cells with the onset of epidermal stratification (Kopan & Fuchs, 1989). Similarly, the expression of K2 starts in the intermediate cells of the developing epidermis of human fetuses at about 87 days of estimated gestational age (Smith et al. 1999). In fetuses of 70 days estimated

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

gestational age, the epithelial cells of the presumptive nail bed already produce K2. In fetuses older than about 94 days estimated gestational age, K2 is produced in epidermal cells of the proximal nail fold but no longer in the nail matrix or nail bed epidermis (Smith et al. 1999). In the hair of humans, K2 is expressed together with the KFAP trichohyalin in the soft-keratinizing and cornifying epidermal cells of the inner root sheath (Smith et al. 1999). The basic K2 can form heterodimers with the acidic K9 or K10 (Moll et al. 1987).

Keratin K3: K3 has a MW of 63 kDa and an isoelectric pH of 7.5 (Moll et al. 1982). The gene encoding K3 (*KRT3*) is active in humans, chimpanzees, dogs, cows and rabbits but not in mouse, guinea pig and pig (Hesse et al. 2004; Lu et al. 2006). In the mouse at least, *KRT3* has become inactive and K3 is substituted by K4 (Lu et al. 2006). The epithelial cells of the stratified anterior epithelium of the human cornea synthesize K3 and its partner K12 (Eichner & Kahn, 1990; Pekny & Lane, 2007).

Keratin K4: K4 has a MW of 59 kDa and an isoelectric pH of 7.3 (Moll et al. 1982). K4 and its partner K13 are produced in the differentiating suprabasal cells of the oral epithelium (Pekny & Lane, 2007). Small amounts of K4 are also produced in the cells of the transitional epithelium of the renal pelvis, ureter and urinary bladder (Achtstätter et al. 1985).

Keratin K5: K5 has a MW of 56 kDa and an isoelectric pH of 7.4 (Moll et al. 1982). It is produced together with its partner K14 as the primary keratins of the basal cells in the stratified epidermis (Pekny & Lane, 2007). Small amounts of K5 have also been detected in the cells of the transitional epithelium of the renal pelvis, ureter and bladder (Achtstätter et al. 1985). K5 is produced in the mitotically active basal cells of stratified epithelia (Byrne et al. 1994). The production of this keratin is correlated with the mitotic activity of epithelial cells but not with the differentiation of epithelial cells or the stratification of the epithelium because suprabasal cells of stratified epithelia can still produce K5 provided that they have not started yet the tissue-specific differentiation processes (Byrne et al. 1994).

Keratin K6: K6 has a MW of 56 kDa and an isoelectric pH of 7.8 (Moll et al. 1982). K6 and its partners K16/K17 (i.e. functional redundancy; Coulombe et al. 2004) are expressed in a variety of internal stratified epithelia, such as those of the tongue, palate and female genitalia (Navarro et al. 1995). Additionally, K6 is produced in the suprabasal cells of the palmar and plantar epidermis, of the nail and of the cells in the outer root sheath of the hair follicle (Navarro et al. 1995). Isoforms of K6, such as K6hf (new name: K75), serve different functions in various cells, such as the cells of the companion layer of the inner root sheath of hair follicles (Winter et al. 1998). K6 is also produced in the hyperproliferative epithelial cells of cancer and in wound healing (Navarro et al. 1995). The expression of this keratin is upregulated in wound repair and in skin diseases (Coulombe et al. 2004). K6 and its isoforms are special because they are encoded in several genes (Navarro et al. 1995), which form a subfamily of genes that encode slightly different K6 polypeptides (see section 'Keratins as products of a multi-gene family of intermediate filament genes').

Keratin K7: K7 has a MW of 54 kDa and an isoelectric pH of 6.0 (Moll et al. 1982). In human, mouse and the marsupial *Pototrous tridactylus*, the amino acid sequence of the part that determines the rod domain (see later) of the keratin is very similar (i.e. highly conserved) (Smith et al. 2002); 88%, 82% and 84%, respectively, of the amino acid sequence of the rod domain of K7 are similar in these three species but the amino acid sequence of the tail domain of K7 is highly variable in these three species (Smith et al. 2002). This explains why anti-keratin antibodies directed against an epitope in the tail domain of a keratin are highly species-specific, whereas antibodies directed against an epitope of the rod or head domain of a keratin cross-react with the same keratin or with other keratins even in different species. K7 is a secondary keratin of simple epithelia (Pekny & Lane, 2007). It is also expressed in the cells of the renal tubule and the collecting duct of the kidney, as well as in the cells of the transitional epithelium of the mucosa of the renal pelvis, ureter and bladder (Achtstätter et al. 1985). K7 is also expressed in the epithelial cells of the nail bed epidermis (Smith et al. 2002).

Keratin K8: K8 has a MW of 52.5 kDa and an isoelectric pH of 6.1 (Moll et al. 1982). In rats, its orthologue has a MW of 55 kDa and its pI is 6.4 (Franke et al. 1983). K8 forms heterodimers with the acidic K18 or K19 (Dale et al. 1985). K8 and its acidic partner K18 are considered primary keratins because they are the first to be produced in the simple epithelia of embryos (Pekny & Lane, 2007). In human fetal skin, the epithelial cells of the ectoderm and periderm express K8 (Moll et al. 1984; Kopan & Fuchs, 1989). Based on the cross-reactivity with the antibody against human K8, epithelial tissues of the clawed frog (*Xenopus laevis*) and goldfish (*Carassius auratus*) are supposed to contain a K8 orthologue (Franz & Franke, 1986; Giordano et al. 1989).

The following four basic keratins K71–K74 (K6irs1-4) and K75 (K6hf; hf = hair follicle) are specific for the soft-keratinizing and -cornifying cells forming the inner root sheath of hair follicles in humans. Orthologue keratins have been extracted and analysed in the inner root sheath of hair follicles of the mouse and sheep (Porter et al. 2004).

Keratin K71 (K6irs1): K71 has a MW of 57.3 kDa and an isoelectric pH of 6.5; it is expressed in all compartments of the inner root sheath, i.e. in the cuticle as well as in the Henle and Huxley layer (Langbein et al. 2002, 2003; Schweizer et al. 2007). This basic keratin was also detected in the human palmar and plantar epidermis (Porter et al.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

2001). K71 can form heterodimers with each of the three acidic K25, K27 and K28, which are also specific for the inner root sheath of the human hair follicle (Schweizer et al. 2007). The orthologue protein mK6irs1 (MW 57 kDa) was described in the inner root sheath of murine anagen hair follicles (Aoki et al. 2001). Deficiency of this orthologue protein causes alopecia (Peters et al. 2003).

Keratin K72 (K6irs2): K72 has a MW of 55.8 kDa and an isoelectric pH of 6.9; it is expressed late in the keratinizing cells of the cuticle of the inner root sheath (Langbein et al. 2003). The orthologue (mIRSa2) was described in the inner root sheath of the mouse hair (Porter et al. 2004).

Keratin K73 (K6irs3): K73 has a MW of 58.9 kDa and an isoelectric pH of 7.3; it is produced in the suprabasal cells of the root sheath cuticle (Langbein et al. 2003). An orthologue was found in the inner root sheath of murine hair follicles (Porter et al. 2004).

Keratin K74 (K6irs4): K74 has a MW of 57.8 kDa and an isoelectric pH of 7.8; it is expressed exclusively in the keratinizing epithelial cells of the Huxley layer of the inner root sheath of hair follicles (Langbein et al. 2003).

Keratin K75 (K6hf): K75 has a MW of 59.5 kDa and an isoelectric pH of 7.9; it is characteristic of the cells forming the companion layer of the inner root sheath of hair follicles (Gu & Coulombe, 2007). The keratin filaments containing K75 are oriented perpendicularly to the longitudinal axis of the hair like the 'hoops of a barrel' (Winter et al. 1998). This basic (type II) keratin consists of 551 amino acids and 80% of the sequence of these amino acids is the same as in the keratin K5 (Winter et al. 1998). In contrast, the amino acid sequence of K75 is only 74% similar to that of K6a and K6b (Winter et al. 1998).

The following basic keratins are minor constituents of the cytoskeleton in suprabasal cells of various stratified soft-keratinizing epithelia.

Keratin K76 (K2p): K76 has a MW of 65.8 kDa and an isoelectric pH of 8.2; it is produced in the suprabasal cells in the upper layers of the stratified epithelium of the hard palate (Rogers et al. 2004) and gingiva (Collin et al. 1992). The amino acid sequence of K76 is only 71% similar to that of K2, which is produced in suprabasal cells of the epidermis (Collin et al. 1992). The canine orthologue to the human K76 contains 21 additional amino acids (Miller et al. 2001).

Keratin K77 (K1b): K77 has a MW of 61.8 kDa and an isoelectric pH of 5.8; its protein chain contains 578 amino acids (Rogers et al. 2005). The mRNA encoding K77 is weakly expressed in the suprabasal cells of the human epidermis (Rogers et al. 2005). K77 is also expressed in the epithelial cells of sweat gland ducts (Langbein et al. 2005).

Keratin K78 (K5b): K78 has a MW of 56.8 kDa and an isoelectric pH of 5.8; its protein chain contains 520 amino acids). The mRNA encoding K78 is produced in the cells of the epithelial covering of the human tongue (Rogers et al. 2005).

Keratin K79 (K6l): K79 has a MW of 57.8 kDa and an isoelectric pH of 7.2; its protein chain contains 535 amino acids). The mRNA encoding K79 has been isolated from human epidermis and skeletal muscle (Rogers et al. 2005).

Keratin K80 (Kb20): K80 has a MW of 50.5 kDa and an isoelectric pH of 5.0; its protein chain contains 452 amino acids. It is produced in the suprabasal cells of the epithelium of the human tongue (Rogers et al. 2005).

Basic keratins in hard keratinization. The following six basic hard keratins K81–86 (hHb1–hHb6) are produced in the hard-keratinizing cells of the cortical cells of human hairs (Langbein & Schweizer, 2005) but K81, K85 and K86 are also produced in the cells of the nail matrix in humans (Perrin et al. 2004). The MW of these hair keratins ranges from 54 to 57 kDa (Langbein et al. 2001). K81, K83 and K86 are structurally similar but the amino acid sequences of K82, K84 and K85 are rather distinct (Langbein et al. 2001). The transition from anagen to telogen hair follicle is marked by the downregulation of these keratins (Bowden et al. 1998).

Keratin K81 (hHb1): K81 has a MW of 54.8 kDa and an isoelectric pH of 5.2; it is expressed in the advanced differentiation of the cortical cells of human hairs (Langbein et al. 2001). The transcripts of this keratin gene are produced simultaneously with those of K83 (Rogers et al. 1997). The amino acid sequence of K81 is very similar to that of the hair keratins K83 and K86. K81 is also synthesized in the keratinizing cells of the hair medulla (Langbein et al. 2001).

Keratin K82 (hHb2): K82 has a MW of 56.6 kDa and an isoelectric pH of 6.7; it is coexpressed with the acidic hair-specific K32 early in the development of the suprabasal cells of the hair matrix forming the hair cuticle (Langbein et al. 2001). The amino acid sequence of K82 is very similar to that of K84 and K85 in the human hair (Langbein et al. 2001).

Keratin K83 (hHb3–I and hHb3–II): K83 has a MW of 54.1 kDa and an isoelectric pH of 5.1; it is expressed simultaneously with K81 and K86 in the advanced processes of keratinization in the suprabasal cells forming the cortex of human hairs (Langbein et al. 2001).

Keratin K84 (hHb4): K84 has a MW of about 65 kDa and an isoelectric pH of 7.6; it is not expressed in the hair follicle but has been detected in extracts of the dorsal epithelium of the human tongue (Langbein et al. 2001). K84 is produced in the aboral epithelium of the filiforme papillae in the caudal one-third of the tongue. The amino acid sequence of K84 is very similar to that of the hair-specific K82 and K86 (Langbein et al. 2001).

Keratin K85 (hHb5): K85 has a MW of 55.7 kDa and an isoelectric pH of 6.5 (Langbein et al. 2001). K85 shows up as a series of isoelectric variants in two-dimensional gel electrophoresis. It is produced in the keratinizing cuticular cells of the human hair (Langbein et al. 2001). In the hair

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

matrix, K85 forms filaments with its acidic keratin partner K35. In the lower portion of the hair cortex, K85 forms keratin filaments with the acidic keratin K31 (Langbein et al. 2007).

Keratin K86 (hHb6): K86 has a MW of 53.5 kDa and an isoelectric pH of 5.3; it is produced in the suprabasal cells of the cortex in human hairs (Langbein et al. 2001). The transcripts encoding K86 are produced after the onset of the synthesis of the transcripts encoding K81 or K83 in the cortical cells of the hair (Rogers et al. 1997).

Acidic keratins (i.e. keratins of type I). The soft keratins K9–K23 and K25–K28 (K25irs1–4), which are specific for the inner root sheath (irs) of hairs, and the hair-specific keratins K31–K38 (hHa1–hHa8) (Coulombe et al. 1989; Langbein et al. 1999; Coulombe & Omary, 2002) are small acidic keratins. They are classified as keratins of type I with a MW of 40–56.5 kDa in humans.

Acidic keratins in soft keratinization. Keratin K9: In humans, K9 has a MW of 64 kDa and an isoelectric pH of 5.0 (Moll et al. 1987; Langbein et al. 1993; Pekny & Lane, 2007). In bovines, the orthologue K9 has a MW of 60–64 kDa and an isoelectric pH of 5.4. It is found in the muzzle and heel epidermis of bovines (Knapp et al. 1986; Langbein et al. 1993). The difference in the MW of the orthologue keratins is probably the result of various degrees of post-translational phosphorylation (Knapp et al. 1986). Starting at the gestational age of 15 weeks, K9 is produced in the suprabasal cells of the palmar and plantar epidermis, in which the expression of K9 is induced by mechanical stress (Yamaguchi et al. 1999). K9 is also found in suprabasal cells surrounding the intraepidermal portion of the ducts of sweat glands (Moll et al. 1987). The cross-reactivity of anti-human K9 antibody with bovine and equine tissues suggests that K9 of human, cow and horse have a similar epitope despite the considerable differences in their amino acid sequence and type (Knapp et al. 1986; Bragulla & Hirschberg, 2002). The amino acid sequence of K9 shows a remarkable similarity to that of K10 but also reveals striking differences in the subdomain 1A of the rod domain (Langbein et al. 1993). The K9 gene (*KRT9*) is about 2353 bp long. The head domain of K9 contains 153 amino acids, its α-helical rod is made out of 306 amino acids and its tail is formed by 163 amino acids (Langbein et al. 1993). K9 forms heterodimers with K1 or K2 (Knapp et al. 1986). The expression of K9 in keratinocytes of the palmar and plantar epidermis can be maintained in cell culture (Yamaguchi et al. 1999). K9 expression in keratinocytes can be induced by fibroblasts of the palmar and plantar dermis (Yamaguchi et al. 1999).

Keratin K10: K10 has a MW of 56.5 kDa and an isoelectric pH of 5.3 (Moll et al. 1982). It is the main type I keratin expressed in the postmitotic keratinizing cells in the suprabasal layers of the epidermis and other cornifying stratified epithelia (Coulombe & Omary, 2002). The presence of K10 in a keratinocyte apparently prevents further cell divisions (Koch & Roop, 2004). The expression of K10 is downregulated in injured epidermis (Koch & Roop, 2004). In differentiating suprabasal keratinocytes of the epidermis, K10 is expressed prior to K9 (Moll et al. 1987). K10 forms heterodimers with the basic K1 or K2.

Keratin K11: K11 turned out to be a polymorphic variant of K10 due to changes in the tail domain of K10 (Moll et al. 1982; Korge et al. 1992a,b). Therefore, this label is no longer used to identify keratins.

Keratin K12: K12 has a MW of 55 kDa and an isoelectric pH of 4.9; it is expressed only in the keratinizing suprabasal cells of the external stratified epithelium of the human cornea (Moll et al. 1982). Its partner for the formation of heterodimers is the basic K3. In the mouse and pig, the K3 gene is inactive and therefore K4 forms heterodimers with the acidic keratin K12 (Hesse et al. 2004).

Keratin K13: K13 has a MW of 54 kDa and an isoelectric pH of 5.1 (Moll et al. 1982). It is expressed in the cells of the transitional epithelium of the mucosa of the renal pelvis, ureter and urinary bladder (Achtstätter et al. 1985). K13 is also expressed in the suprabasal cells of the oral mucosa, esophagus and forestomach. K13 is the characteristic acidic keratin produced in the suprabasal cells of non-cornified stratified epithelia (Waseem et al. 1998). In the esophageal epithelium of the rabbit, K13 is cross-linked via disulfide bonds to the basic K4 to form heterodimers (Pang et al. 1993).

Keratin K14: In humans, K14 has a MW of 50 kDa (Dale et al. 1985). Its isoelectric pH is 5.3 (Moll et al. 1982). K14 appears to be a fundamental keratin of all keratinocytes in stratified epithelia (Porter et al. 2000). The expression of K14 (and of its partner K5) increases in embryonic epithelial cells, as these cells become basal cells of stratified epithelia (Coulombe et al. 1989). K14 is synthesized in the basal cells of the interfollicular epidermis and upper part of the outer root sheath of hair follicles. The basal cells of the distal part of the outer root sheath of hair follicles and the basal cells bordering epidermal wounds produce only small amounts of K14 but increase the synthesis of K14 as they differentiate (Coulombe et al. 1989). A decrease or even lack of immunoreactivity of K14 in the suprabasal cells of stratified epithelia, however, could be due to proteolysis of K14 or to a masking of the antibody-binding epitope by newly formed keratins (Coulombe et al. 1989). According to Coulombe et al. (1989), the expression of K14 is correlated with the mitotic activity and the degree of pluripotency of the basal cells in stratified epithelia.

Keratin K15: In humans, K15 has a MW of 50 kDa and an isoelectric pH of 4.9 (Moll et al. 1982). Its mouse orthologue has a MW of 48 kDa (Lloyd et al. 1995). K15 is produced in basal keratinoblasts of stratified epithelia of the fetal epidermis and nail in humans (Waseem et al. 1999). K15 is also synthesized in a subset of keratinoblasts in the

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

outer root sheath of the human hair follicle (Lyle et al. 1998). The synthesis of K15 may be upregulated in the absence of K14 (Waseem et al. 1999). K15 seems to be a secondary keratin in the basal keratinoblasts of the epidermis (Pekny & Lane, 2007). The expression of K15 appears to be associated with a more mature type of basal cells that is more stable and has a lower rate of cell division (Porter et al. 2000). K15 is present in larger amounts in the basal cells of thin skin than, for example, in those of the thick plantar skin with its rapid turnover (Porter et al. 2000). In addition, K15 is a characteristic keratin in epidermal stem cells (Waseem et al. 1999) and in the limbal stem cells of the human cornea (Lyngholm et al. 2008). Stem cells expressing K15 reside between basal cells of the hair medulla, sebaceous glands and interfollicular epidermis (Whitbread & Powell, 1998). The expression of K15 is downregulated when the basal keratinoblasts begin to differentiate (Waseem et al. 1999). K15 partners with the basic K5 to form heterodimers (Lloyd et al. 1995; Whitbread & Powell, 1998). The keratin filaments comprised of K5 and K15 have a smaller diameter than those comprised of K5 and K14 (Lloyd et al. 1995). As a further indication of differences between K15 and K14, the antibody raised against the carboxy terminal of K15 does not cross-react with that of K14 (Lloyd et al. 1995). However, the amino acid sequences determining the rod domain of K15 and K14 are 76% identical (Whitbread & Powell, 1998).

Keratin K16: K16 has a MW of 46 kDa and an isoelectric pH of 5.1 (Moll et al. 1982). The amino acid sequence of K16 is 82% identical in the human and mouse (Wawersik & Coulombe, 2000). K16 expression defines a subset of epithelial cells during the morphogenesis of the skin and of cells in the companion layer of the outer root sheath of hairs (Bernot et al. 2002; Larouche et al. 2008). The expression of K16 does not coincide with the expression of its usual binding partner for heterodimerization, K6, during the embryonic development of the murine skin (Bernot et al. 2002). K16 marks cells that are presumably in an intermediate state of cell differentiation between basal and suprabasal cells, and it may provide sufficient structural stability by allowing the flexibility required for cell movement or mitosis (Bernot et al. 2002). K16 (and its basic partner K6) is a marker for the activated state of a keratinocyte, which is able to migrate, produce extracellular matrix and hyperproliferate (Freedberg et al. 2001). K16 is also produced in the suprabasal epidermal cells of the proximal nail fold and in the epidermal nail bed in humans (Bernot et al. 2002). K16 can polymerize to form keratin filaments with the basic keratins K5, K6b and K8 (Coulombe et al. 1998). K16 is involved in the hair cycle, and the expression of K16 peaks between the catagen and telogen phases of the hair cycle.

Keratin K17: K17 has a MW of 48 kDa in both humans and mice and an isoelectric pH of 5.1 (McGowan & Coulombe, 1998). The amino acid sequences of both orthologous keratins are very similar: 88% in the head domain, 96% in the rod domain and 97% in the tail domain (McGowan & Coulombe, 1998). This great similarity in all three domains is unusual among orthologue keratins and points to the possibility that K17 is subject to a strong stabilizing selection, at least partly because it is involved in wound-healing and in developmental processes (e.g. the formation of ectodermal placodes for hair, glands, thymus and teeth, as well as for the formation of modified skin of the palms and soles) (McGowan & Coulombe, 1998). K17 has been observed in small amounts in the cells of the simple epithelium of the seminal vesicular gland and epididymis (Achtstätter et al. 1985), in basal cells of transitional and pseudostratified epithelia, in myoepithelial cells of secretory units of exocrine serous glands, and in injured human interfollicular epidermis (Komine et al. 1996; Freedberg et al. 2001; Kim et al. 2006). In the interfollicular epidermis, K17 is expressed in the suprabasal cells only when the epidermis is injured and, in this case, it is induced by interferon-γ that is secreted by invading T-lymphocytes (Komine et al. 1996). K17 promotes hair follicle growth by attenuating the pro-apoptotic signal tumor necrosis factor-α (Gu & Coulombe, 2007). K17 is expressed at the same time as K6, with which it forms heterodimers, although it can also combine with K5 and K8 (McGowan & Coulombe, 1998).

Some epithelial cells (e.g. myoepithelial cells of serous glands) that express K17 are contractile or motile (McGowan & Coulombe, 1998). This fact raises the possibility that K17 allows basal epithelial cells to be actively involved in the development of a complex three-dimensional interface between the epidermis and dermis (i.e. the dermal papillary body).

Keratin K18: In humans, K18 has a MW of 44 kDa and an isoelectric pH of 5.5 (Hutton et al. 1998). According to Dale et al. (1985), K18 has a MW of 45 kDa and, according to Debus et al. (1982), it has an isoelectric pH of 5.7. These differences in the MW and pI can be attributed to differences in the methods applied for the chemical analysis of K18. In the rat, K18 has a MW of 49 kDa and an isoelectric pH of 5.38 (Franke et al. 1983). The epithelial cells of the ectoderm and periderm of the human fetal skin express K18 (Kopan & Fuchs, 1989). K18 is also expressed in most of the simple epithelia of the human male urogenital tract (Achtstätter et al. 1985). K18 is a constituent of the cytoskeleton in the cells of simple epithelia, such as the hepatocytes, the cells lining the bile duct and renal tubules, and the cells of the intestinal, bronchial and alveolar epithelia (Debus et al. 1982). K18 seems to restore some, but not all, deficiencies caused by the absence of K14 in a knockout mouse (Hutton et al. 1998), which suggests that K18 can partly substitute for K14. Several antibodies raised against K18 in human tissues react with the corresponding tissues of some, but not all, other mammals, thereby indicating that species-specific differences exist in K18 (Debus et al. 1982).

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Keratin K19: K19 has a MW of 40 kDa (Eckert, 1988; Fradette et al. 1998) and an isoelectric pH of 5.2 (Moll et al. 1982). The amino acid sequences of human K19 and of the corresponding bovine orthologue are 89% identical (Eckert, 1988). K19 is the smallest keratin because it is lacking the typical tail domain (Bader et al. 1986). K19 is the only 'simple' keratin produced in the cells of stratified epithelia (Bosch et al. 1988). K19 is rapidly inducible in a variety of epithelia, such as human epidermal keratinocytes and conjunctival epithelial cells (Bader et al. 1986). K19 can form intermediate filaments with K8 (Bader et al. 1986; Eckert, 1988; Fradette et al. 1998) but it can also partner with K5 (Fradette et al. 1998) and K7 (Eckert, 1988). K19 is also expressed in the basal layer and periderm of the skin in a human fetus (Kopan & Fuchs, 1989). It is also expressed in the pseudostratified epithelium of the bovine urinary bladder (Bader et al. 1986). K19 is characteristic of and, thus, a marker of epidermal stem cells but is also expressed in Merkel cells (Michel et al. 1996; Watt, 1998). At this point, it is unclear whether all stem cells express K19 (Michel et al. 1996). The number of epidermal cells expressing K19 diminishes with age. For example, 84% of the basal cells in the epidermis of 2–7-day-old boys express K19, whereas only 9% of the basal cells in 2–5-year-old boys do so, and 0.1% of the basal cells synthesize K19 in 17–42-year-old men. This age-related decrease of basal cells producing K19 suggests that the number of stem cells in the interfollicular epidermis diminishes with age (Michel et al. 1996). K19 heterodimerizes with K5. This heterodimer is less stable and, *in vitro*, shorter and narrower than that of K14 and K5 (Fradette et al. 1998). Hence, the keratin composition of the keratin filaments influences their structure and most probably their mechanical properties. The expression of *KRT19* is regulated by retinoic acid, which downregulates the expression of *KRT19* at the same time as the level of *KRT19* mRNA in the cytoplasm increases (Crowe, 1993). This apparent paradox could mean that a member of the family of cytoplasmic retinoic acid binding proteins binds to the *KRT19* mRNA to make it less vulnerable to proteolytic decay (Crowe, 1993).

Keratin K20: K20 has a MW of 48.5 kDa. Its amino acid sequence is quite different from all other type I keratins (Moll et al. 1993). K20 has been found in epithelial cells of the murine lung and rat placenta (Hesse et al. 2004). K20 is produced in the epithelial cells of the gastric mucosa (Jovanovic et al. 2002). K20 is also expressed in the epithelium of the intestinal mucosa (Moll et al. 1993) and of the uterus and urinary bladder (Zhou et al. 2006). The cells of the intestinal epithelium produce K20 in addition to K18 (Zhou et al. 2003). K20 is also expressed in Merkel cells (Moll et al. 1995). In the developing oral mucosa, K20 is exclusively specific to taste bud anlagen (Witt & Kasper, 1999) and bipolar cells (Zhang & Oakley, 1996).

Keratin K21: K21 has a MW of 48 kDa (Quaroni et al. 1991). It is a constituent of cells of simple epithelia in the mucosa of the rat intestine. K21 is produced only in the differentiated cells of the intestine and not in the proliferating cells in the intestinal crypts (Quaroni et al. 1991). K21 is very similar to other type I keratins of simple epithelia, such as the human K16, K18 and K19 (Chandler et al. 1991). K21 of epithelial cells in the rat intestine could be the orthologue to human K20 (Chandler et al. 1991; Quaroni et al. 1991; Moll et al. 1993; Zhou et al. 2003) and it is therefore not listed in the actual catalog of human keratins (Schweizer et al. 2006).

Keratin K22: This label has not been assigned to any keratin.

Keratin K23: K23 has a MW of 48.1 kDa. It is part of the cytoskeleton in pancreatic cells (Hesse et al. 2001). The expression of K23 in the pancreatic cells can be induced by sodium butyrate and can be blocked by actinomycin D (Zhang et al. 2001). This fact may be useful for the treatment of pancreatic cancer.

Keratin K24: K24 has a MW of 55.1 kDa; it is associated with simple glandular or stratified non-cornified human epithelia (Rogers et al. 2004). The expression of K24 is strong in the epithelium of the tongue, placenta, eye and colon but only relatively weak in the epithelia of the thymus, lung and small intestine (Rogers et al. 2004). The expression of *KRT24* was observed in keratinocytes, as well as in the epithelial cells of the colon, spleen, thymus, testis and placenta (Sprecher et al. 2002).

The following four acidic keratins K25–28 (K25irs1–K25irs4) are produced in the soft-keratinizing suprabasal cells of the inner root sheath in the hair follicle (Langbein et al. 2006). K25, K27 and K28 also occur in the epithelial cells of the medulla of human beard hairs (Langbein et al. 2006). Orthologues of these keratins have been detected in the inner root sheath of the hair in the mouse [mIRS1, mIRS3.1 and mIRS3.2 (Porter et al. 2004)] and in the sheep [oIRSa1, oIRSa3.2, oIRS3.2 and oIRSa2 (Bawden et al. 2001)].

Keratin K25 (K25irs1): K25 has a MW of 49.3 kDa and an isoelectric pH of 4.7; it is produced in the epithelial cells of all three compartments of the inner root sheath of the hair follicle (i.e. root sheath cuticle, Henle layer and Huxley layer) (Langbein et al. 2006). Orthologue proteins have been found in the hairy skin of the mouse, rat and chimpanzee (Szeverenyi et al. 2008).

Keratin K26 (K25irs2): K26 has a MW of 51.8 kDa and an isoelectric pH of 4.6; its expression is restricted to the cells forming the root sheath cuticle (Langbein et al. 2006). Orthologue proteins have been found in the hairy skin of the mouse, rat and chimpanzee (Szeverenyi et al. 2008).

Keratin K27 (K25irs3): K27 has a MW of 49.8 kDa and an isoelectric pH of 4.8 (Szeverenyi et al. 2008). Like K25, it is produced in the epithelial cells of all three compartments of the inner root sheath of the hair follicle [i.e. root sheath cuticle, Henle layer and Huxley layer (Langbein et al. 2006)].

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Keratin K28 (K25irs4): K28 has a MW of 50.5 kDa and an isoelectric pH of 5.1 (Szeverenyi et al. 2008). Its expression pattern resembles that of K25 and K27 in the three compartments of the inner root sheath of the hair follicle (Langbein et al. 2006).

Acidic keratins in hard keratinization. The acidic keratins in the follicular epidermis are characterized by a large number of cysteine and proline residues in their head and tail domains (Langbein et al. 2007). In humans, there are 10 hair-specific keratins (i.e. K31–K40), which are synthesized in the keratinizing cells of the various compartments of the human hair follicle (Langbein et al. 2007).

Keratin K31 (hHa1): K31 has a MW of 47.2 kDa and an isoelectric pH of 4.5 (Szeverenyi et al. 2008). It is expressed in the hair cortex of human hair follicles (Langbein et al. 2001; Cribier et al. 2004). Its partner for the formation of heterodimers is the basic keratin K85 (Langbein et al. 2007). Its orthologue is found in the hair cortex of the mouse, as revealed by the cross-reactivity of antibodies (Bertolino et al. 1990; Meier et al. 1999). K31 is also expressed in the epithelial cells of the nail matrix (De Berker et al. 2000). A lymphoid enhancer binding factor (LEF)-binding site resides in the promoter region of *KRT31* (Cribier et al. 2004).

Keratin K32 (hHa2): K32 has a MW of 50.3 kDa and an isoelectric pH of 4.5 in humans; it is expressed at the bottom and mid-height of the hair cuticle (Langbein et al. 2007). Its partners are the basic keratins K82 or K85 (Langbein et al. 2001, 2007). Orthologue keratins have been described in the mouse, rat, dog and chimpanzee (Szeverenyi et al. 2008).

Keratins K33a and K33b (hHa3-I and -II): K33a and K33b have similar physicochemical properties and are therefore difficult to separate via gel electrophoresis. Based on the amino acid sequence, K33a and K33b were originally thought to be subtypes encoded by a single keratin gene. K33a has a MW of 45.9 kDa and an isoelectric pH of 4.8, whereas K33b has a MW of 46.2 and an isoelectric pH of 4.5 (Szeverenyi et al. 2008). In the mouse, its orthologue (i.e. mHa3) is expressed in the hair cortex and in the outer root sheath but not in the inner root sheath of hairs (Meier et al. 1999). The keratin mHa3 is also produced in the suprabasal cells of the murine nail matrix (Meier et al. 1999). Orthologues of K33b have been described for the mouse, rat, dog and chimpanzee (Szeverenyi et al. 2008).

Keratin K34 (hHa4): In humans, K34 has a MW of 49.4 kDa and an isoelectric pH of 4.7; it is expressed in the upper cortex of hair (Langbein et al. 2007). Its partner is the basic keratin K86 (Langbein et al. 2007). In the mouse, the orthologue of K34 (i.e. mHa4) is produced in the suprabasal cells forming the hair cortex and outer root sheath of the hair (Meier et al. 1999).

Keratin K35 (hHa5): K35 has a MW of 50.3 kDa and an isoelectric pH of 4.5; it is expressed in the bottom of the hair cuticle of human hairs (Cribier et al. 2004; Langbein et al. 2007). Its partner is the basic keratin K85 (Langbein et al. 2007).

Keratin K36 (hHa6): K36 has a MW of 52.2 kDa and an isoelectric pH of 4.6; it is expressed in the advanced differentiation of the cortical cells of the hair (Langbein et al. 1999).

Keratin K37 (hHa7): K37 has a MW of 49.7 kDa and an isoelectric pH of 4.6; it is expressed in the hair medulla (Jave-Suarez et al. 2004). The basic hair keratins K81, K85 and K86 are putative partners for the formation of heterodimers with K37 (Jave-Suarez et al. 2004).

Keratin K38 (hHa8): K38 has a MW of 50.5 kDa and an isoelectric pH of 4.5; it is expressed in the cortex of hairs (Langbein et al. 1999; Langbein et al. 2007). Orthologues to K38 have been described in the hairy skin of the dog and chimpanzee (Szeverenyi et al. 2008).

Keratin K39 (Ka35): K39 has a MW of 55.6 kDa and an isoelectric pH of 4.99; it is expressed in the upper hair cuticle, upper hair cortex and medulla of terminal hairs of humans (Langbein et al. 2007). Its partner in forming keratin filaments is the basic keratin K82 (Langbein et al. 2007).

Keratin K40 (Ka36): K40 has a MW of 48.2 kDa and an isoelectric pH of 4.17 (Langbein et al. 2007). K40 is expressed only in the upper hair cuticle (Langbein et al. 2007). Its partner is the basic keratin K82 (Langbein et al. 2001, 2007).

Structure of keratins and keratin filaments

Keratins in different vertebrates have similar amino acid sequences as inferred from the observation that epithelial tissues of various species of teleost fishes, amphibians, reptiles, birds, and marsupial and placental mammals cross-react with anti-human keratin antibodies (Gigi et al. 1982; Fuchs & Marchuk, 1983; Groff et al. 1997; Alibardi et al. 2000). Unlike in other vertebrates, non-epithelial cells in teleost fishes also cross-react with anti-human keratin antibodies (Markl & Franke, 1988; Groff et al. 1997; Schaffeld et al. 2002a,b). This suggests that keratins are not restricted to epithelial tissues in teleost fishes. In addition, extracellular keratins (i.e. 'thread keratins') have been described in hagfishes, lampreys, teleosts fishes and amphibians (Schaffeld & Schultess, 2006). All these keratins and keratin filaments have a similar basic structure.

Primary structure of keratins

Each keratin is characterized by a chain of amino acids as the primary structure of the keratin protein, which may vary in the number and sequence of amino acids, as well as in polarity, charge and size (Brown, 1950; Makar et al. 2007). However, the amino acid sequence of a particular keratin is remarkably similar in different species (Fuchs,

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland
المنارة للاستشارات

1983). For example, bovine, rat and human skin keratins are all rich in the amino acids glycine, serine, leucine and glutamic acid (Fuchs, 1983). The amino acid sequences of keratins in soft- or hard-cornified epithelia of mammals are also similar (Fuchs, 1983). This similarity is the basis for the concept of orthologous keratins (i.e. similarity in immunological epitope, MW, pI, amino acid sequence and location within tissues).

The amino acid sequence of a keratin influences the properties and functions of the keratin filament (Roop et al. 1984). Furthermore, the position of a particular amino acid within the chain of amino acids can influence the entire three-dimensional architecture of the keratin molecule (Wu et al. 2000). For example, lysine in position 23 and glutamate in position 106 are required for the proper assembly of K5/K14 keratin filaments *in vitro* and *in vivo* (Wu et al. 2000).

The amino acid sequence of a specific keratin determines the molecular structure and properties of the secondary, tertiary and quaternary structures of keratins, as well as the nature of the bonds (e.g. covalent or ionic) to other components of the cytoskeleton (Steinert et al. 1984; Coulombe & Omary, 2002). In particular, the sulfur-containing amino acids methionine, histidine and cysteine are instrumental in establishing disulfide bonds within an amino acid chain, between two keratins or between keratins and KFAPs (e.g. filaggrin, involucrin, see section 'Keratins and keratin filament-associated proteins') in keratinized cornifying cells of stratified epithelia.

The amino acid chain of β-keratins [see section 'Other keratins (e.g. β-keratin and thread keratins)'], which are characteristic of hard-keratinized and hard-cornified modified epidermis in reptiles and birds, is shorter than that of α-keratins (which are characteristic of the soft-keratinized and soft-cornified epidermis of reptiles and birds, as well as of all keratins in mammals). For example, in the β-keratin of the emu feather, only 32 amino acids form the central rod domain, 23 amino acids form the head domain and 47 amino acids form the tail domain (Fraser & Parry, 2008). Feather keratins also characteristically contain large amounts of the amino acids serine, proline, valine, leucine, glutamate and aspartate (Woodin, 1956).

Post-translational changes of the primary structure of keratins

The amino acid sequence (i.e. the primary structure of a keratin encoded by a gene) is slightly longer than the amino acid sequence of the mature keratin, which indicates a post-translational modification of the keratin prior to the formation of the keratin filaments (Hesse et al. 2001).

Comparative analyses of keratins extracted from the living cells of the *Stratum spinosum et granulosum* and from the dead cells of the *Stratum corneum* in humans and mice demonstrate a precursor/product relationship, also indicating post-translational modifications as well as other modifications during cell differentiation (i.e. keratinization and cornification) (Bowden et al. 1984). The MW of a keratin extracted from the cells of the *Stratum corneum* is about 2–5 kDa smaller than that of its precursor in the living epidermal cells, this possibly being the result of a loss of amino acids from the tail domain (Bowden et al. 1984). The MW of keratins can also increase as a result of their post-translational modification, sometimes altering the pI (Schweizer & Winter, 1982). Post-translational modifications influence the physicochemical properties of keratins and their assembly to form heterodimers and tetramers (Yamada et al. 2002). Such alterations primarily affect the head and tail domains of keratins, whereas the rod domain may be modified by caspase-mediated cleavage (Coulombe & Omary, 2002).

Post-translational modifications of keratins, such as the formation of disulfide bonds, phosphorylation, glycosylation, deimination or inter- and intrachain peptide bonds, can influence the conformation of the molecule and the formation of keratin filaments.

Cross-linking of keratins via inter- and intrachain peptide bonds occurs during epidermal differentiation (Kubilus & Baden, 1983) by connecting an ε-lysine to a γ-glutamyl amino acid residue mediated by transglutaminases (Buxman & Wuepper, 1975; Baden et al. 1976; Ahvazi et al. 2003). Keratins may be cross-linked by transglutaminases to one another or to constituents of the cornified envelope, such as the *Stratum corneum* basic protein or involucrin to form insoluble aggregates (Kubilus & Baden, 1983). For example, transglutaminases cross-link a lysine residue of the V1 subdomain in the head domain (see section 'Primary structure of keratins') of the type II keratins K1, K2e, K5 and K6 to involucrin and loricrin in the cornified envelope in stratified squamous epithelia (Candi et al. 1998; Eckert et al. 2005). Three different transglutaminases have been identified so far in the epidermis of mammals (Eckert et al. 2005), all of which depend on calcium as a co-factor (Ogawa & Goldsmith, 1976). Transglutaminases are also present in the avian epidermis (Alibardi & Toni, 2004).

Intra- and intermolecular disulfide bonds are formed in keratins by connecting two sulfhydryl residues of two amino acids (such as two cysteines) enzymatically via the enzyme sulfhydryl oxidase (Hashimoto et al. 2000). This enzyme (MW 65 kDa) is expressed in keratinocytes of the *Stratum granulosum* of the rat and mouse epidermis (Hashimoto et al. 2000; Matsuba et al. 2002). Disulfide bonds are also formed in the rod domain of K4 and K13, which are produced by suprabasal cells of the esophageal epithelium (Pang et al. 1993). In addition to intrachain disulfide bonds, interchain disulfide bonds are formed in heterodimers of keratins (Pang et al. 1993). All keratins in the cornified cells of the human epidermis are cross-linked

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

by interchain disulfide bonds, whereas the keratins in the keratinizing epidermal cell are not (Sun & Green, 1978).

Phosphorylation and dephosphorylation are perhaps the most important post-translational modifications of keratins because they affect the equilibrium of soluble keratins deposited in granular aggregates (Strnad et al. 2002). The degree of phosphorylation is variable (Sun & Green, 1978) and may enable or prevent the interaction of keratins with other molecules, such as signaling molecules, receptor molecules, etc. (Kirfel et al. 2003). Potential consequences of keratin phosphorylation include changes in the solubility of keratins, in the organization of keratin filaments and in the interactions with other proteins (Liao et al. 1995; Strnad et al. 2002). Phosphorylation modifies the conformation of keratin filaments as a prerequisite for remodeling the cytoskeleton (Owens & Lane, 2003; Pekny & Lane, 2007). Specifically, the phosphorylation of amino acids in the head domain changes the overall net charge of this region and, thus, prevents any interactions with the rod domains of adjacent keratins, thereby preventing the assembly of keratin filaments (Wöll et al. 2007). The head domains of type II keratins are more easily phosphorylated enzymatically than those of type I keratins (Liao et al. 1995). For example, the amino acids serine or threonine of the head domain of type II keratins K4, K5 and K6 are phosphorylated enzymatically in epithelial cells of the esophagus and epidermis (Ku & Omary, 2006). The enzyme p38 mitogen-activated phosphokinase catalyses the phosphorylation of serine in the head domain of the type II keratin K8 (Wöll et al. 2007). Similarly, the serine or threonine residues of both the type II keratin K8 and type I keratin K18 are phosphorylated in hepatocytes (Ku et al. 2002). Phosphorylation of K8 and K18 in the liver prevents the attachment of ubiquitin molecules and therefore protects them from proteolysis (Ku & Omary, 2000). Phosphorylation also contributes to the depolymerization (i.e. disassembly) of keratin filaments in hepatocytes. As a result of this depolymerization, the pool of soluble tetramers increases (Ku et al. 1996; Steinert, 2001). Enzymes, such as mitogen-activated protein kinase and protein kinase C-related kinase, are involved in the phosphorylation of keratins, which are then able to bind to heat-shock proteins and to signaling molecules in order to intercept signals (Ku et al. 1996). In addition, keratin phosphorylation sequesters phosphates, thereby influencing signaling cascades (Ku et al. 1996, 2002). In contrast to phosphorylation, dephosphorylation promotes keratin filament reassembly (Steinert, 2001).

Glycosylation is another post-translational modification of proteins. The glycosylation of keratins has been documented in human hepatocytes (Chou et al. 1992) and in the epidermis of pigs (King, 1986). For example, a sugar moiety, such as *N*-acetylglucosamine, is enzymatically linked to the amino acid residue of serine or threonine of K8 and K18 in hepatocytes (King & Hounsell, 1989; Chou et al. 1992). Glycosylation has also been observed in K13 in the esophageal epithelium (King & Hounsell, 1989). Glycosylated amino acid residues are not available for other chemical processes, such as phosphorylation, which results in an alteration of the binding or signaling functions of keratin (Chou et al. 1992).

Deimination, also known as citrullination, is another form of post-translational modification of keratins and keratin filaments (Kubilus et al. 1980). During this process, the enzyme peptidylarginine deiminase converts arginine to citrulline. Because citrulline is neutral, the loss of a positive charge may lead to conformational changes of the keratin and keratin filaments. For example, deimination of K1 takes place during terminal differentiation of human epidermal cells (Senshu et al. 1996). K10 and the KFAP filaggrin are also deiminated (Senshu et al. 1996).

Proteolytic cleavage of proteins may also occur post-translationally. Keratins and keratin filaments are quite resistant to cleavage by common proteolytic enzymes (e.g. trypsin and pepsin) due to their stabilization by numerous cross-linking disulfide bonds (Gupta & Ramnani, 2006). Proteolytic cleavage of keratins and keratin filaments is achieved by specific enzymes, such as caspase or cathepsin, which are released from lysosomes (Presland & Jurevic, 2002). Proteolysis can occur in the proteasome as part of the disassembly of keratin filaments and proteins in simple epithelia (e.g. hepatocytes) or as part of the processes of keratinization and cornification in stratified epithelia (e.g. the epidermis).

Keratin proteolysis (i.e. keratinolysis) is also achieved by specific proteases (i.e. keratinases) that are produced by keratinolytic fungi or bacteria known to affect fingernails, hairs, beaks and feathers (Gupta & Ramnani, 2006; Thys & Brandelli, 2006). Such keratinases are related to serine proteases and have important biotechnological applications, such as for the degradation of feathers as a waste byproduct in the poultry industry (Gupta & Ramnani, 2006).

Secondary structure of keratins

All proteins that form intermediate filaments have a tripartite secondary structure consisting of an N-terminal head domain, a central α-helical rod domain and C-terminal tail domain, and all proteins are able to self-assemble into filaments (Steinert et al. 1984; Coulombe et al. 2004). The secondary structure of keratins is also divided into three parts (Fig. 2a), i.e. the head domain (towards the N-terminal of the molecule), the rod domain in the center and the tail domain (Steinert et al. 1984; Presland & Dale, 2000; Parry et al. 2007). Each of these three domains is divided into subdomains (Steinert et al. 1985; Hatzfeld & Burba, 1994). Domains and subdomains are determined by the amino acid sequence of the keratin and serve various functions in the assembly of keratin filaments and in the binding of keratins and keratin filaments to cell adhesion

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

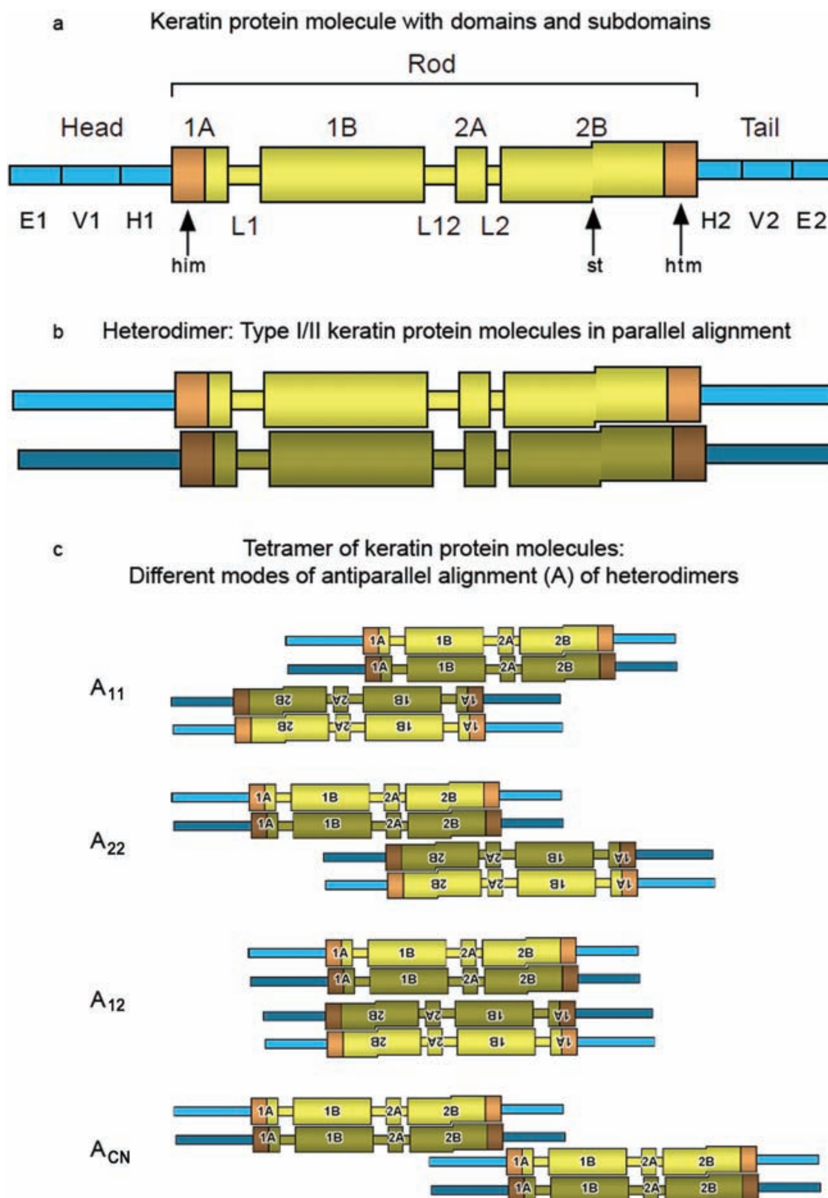

Fig. 2 Schematic depictions of the secondary structure of a keratin molecule (a), the heterodimer (b) and the different modes of alignment of heterodimers to form tetramers (c). (a) The X-ray diffraction analyses of purified keratins as well as the analysis of the sequence of the amino acids in the chain of a keratin revealed the secondary structure of keratins molecule. This molecule forms three domains, the head domain at the N-terminal end of the molecule, the central rod domain and the tail domain at the C-terminal end of the molecule. The head domain of a keratin molecule consists of three subdomains, i.e. the end subdomain (E1), the variable subdomain (V1) in the middle and the homologous subdomain (H1). The central rod domain consists of four α-helical subdomains, which are connected by β-turn linker regions (L). The subdomains 1A and 1B are connected via linker L1 and the subdomains 2A and 2B are connected via linker L2. The subdomains 1B and 2A of the rod domain are connected via the linker L12 (linker 'one-two'). The N-terminal part of the subdomain 1A is called helix initiating motive (h i m) and the C-terminal part of the subdomain 2B functions as a helix terminating motif (h t m). In addition, the α-helix of this subdomain 2B in the rod domain of a keratin molecule is interrupted by a stutter (st). The subdomains (H2, V2, E2) of the tail domain of a keratin molecule are similar to those in the head domain. (b) One acidic keratin molecule, i.e. a type I keratin, and one basic keratin molecule, i.e. a type II keratin, align in parallel to form a heterodimer. (c) Two of the same heterodimers of keratins associate in antiparallel and staggered alignment modes (A_{11}, A_{22}, A_{12}) or in the end-to-end alignment mode A_{CN} to form a tetramer (see section 'Quaternary structure of keratins' for details).

complexes or to signaling molecules (Steinert et al. 1984; Hatzfeld & Burba, 1994). The distribution of ionic charges may vary in the different domains and subdomains, with the head and tail being positively charged (Steinert et al. 1984).

Head domain of keratin molecules

The head domain of a keratin molecule consists of a highly variable number of amino acids (e.g. 50–100) with an overall positive charge (Herrmann & Aebi, 2004) that interacts with other molecules, such as the membrane-bound molecules of desmosomes (Kazerounian et al. 2002). This molecular interaction depends on the chemical properties of the constituent amino acids. For example, positively charged arginine residues in the head domain interact with acidic residues of the rod domain to form coiled-coil heterodimers (Herrmann & Aebi, 2004). The highly conserved lysine residue in the head domain of the basic K1, K2, K5 and K6 binds to proteins of the cornified envelope, such as loricrin, involucrin and envoplakin (Kirfel et al. 2003). The serine residue of a head domain can become negatively charged by phosphorylation, resulting in the disassembly of the keratin filament. Therefore, this residue is instrumental in the disassembly and reassembly of keratin filaments (Herrmann & Aebi, 2004). The head domain of hard-cornified epithelial cells, including those of the hair cortex, are rich in cysteine, which forms disulfide bonds with other keratins or with KFAPs (Jones et al. 1997; Steinert, 2001).

The head domain has a globular, non-helical structure (Er Rafik et al. 2004) with β-turns (Steinert et al. 1985). In type II keratins, it is subdivided into the subdomains E1

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

(end), V1 (*v*ariable) and H1 (sequence of *h*omology) (Steinert et al. 1985; Steinert & Roop, 1988; Steinert, 2001). In type I keratins, the head domain contains only the V1 and E1 subdomains, which may explain why the MW of type I keratins is 6–9 kDa lower than that of the corresponding type II keratins (Steinert et al. 1985).

The amino acid sequence in the H1 subdomain is relatively constant in basic type II keratins but variable in acidic type I keratins (Hatzfeld & Burba, 1994). For example, the H1 subdomain of K1 contains many threonines and prolines (Steinert et al. 1985) and may play an important role in the correct parallel alignment of keratin polypeptide chains (i.e. one chain of type I keratin and one of type II keratin) during the formation of the coiled-coil heterodimer (Hatzfeld & Burba, 1994). The H1 subdomain may also stabilize the tetramers by binding to a specific part of the 2A/L2 subdomain of the rod domain in the coiled-coil of a heterodimer. It may also be involved in the assembly (i.e. nucleation and elongation) of keratin filaments (Hatzfeld & Burba, 1994).

The V1 subdomain of the head domain is more variable than the H1 subdomain and contains many glycine residues (Steinert et al. 1985). In the basal cells of stratified epithelia, this V1 subdomain is connected via a lysine residue to desmosomal proteins, whereas in suprabasal cells it is attached to proteins of the cornified envelope of keratinocytes and corneocytes (Candi et al. 1998). Cross-linking of the V1 subdomain lysine residue to a glutamine residue of the cornified envelope, such as involucrin, is promoted by the enzyme epidermal transglutaminase (Steinert et al. 1998). The V1 subdomain is also essential for keratin filament elongation (Hatzfeld & Burba, 1994).

Rod domain of keratin molecules

The rod domain of α-keratins comprises roughly 310 amino acids and consists of four right-handed α-helical subdomains 1A, 1B, 2A and 2B, which are separated from one another by non-helical β-turns called 'linker' regions (L1, L12 and L2) (Hanukoglu & Fuchs, 1983; Steinert, 2001; Coulombe & Omary, 2002; Herrmann & Aebi, 2004). About 38–45% of the structure of a keratin molecule is in the α-helical conformation (Steinert, 1975). In type I (acidic) keratins, all subdomains of the rod domain are negatively charged. In type II (basic) keratins, the only acidic subdomains are 1B and 2A, whereas the 2B subdomain is neutral and the 1A subdomain is positively charged (Steinert et al. 1984).

The α-helical subdomains 1A, 1B, 2A and 2B of the rod domain are composed of repeats of seven amino acids in positions labeled a–g (i.e. a heptad pattern). These heptads are prerequisites for the formation of the α-helix and coiled-coil heterodimer (Eckert, 1988). A heptad forms the backbone of one turn of an α-helix (Smith et al. 2004). Specific hydrophobic apolar amino acids in positions a and d, and charged amino acid residues in positions e and g, stabilize the α-helix (Steinmetz et al. 1998). The first 13 amino acid residues at the N-terminal of the rod domain form a so-called trigger site (i.e. helix-initiating motif), which initiates the formation of the α-helix of the rod domain (Kammerer et al. 1998; Coulombe & Omary, 2002). This sequence provides stability to the α-helical conformation and to the coiled-coil formation of the heterodimer through interchain ion pairings and is also able to start the formation of the coiled-coil heterodimer (Coulombe & Omary, 2002; Herrmann & Aebi, 2004). In addition, special amino acid sequences have been identified in the subdomains 1B and 2B, which favor and stabilize the formation of an α-helix as the secondary structure of a keratin molecule (Wu et al. 2004). Similarly, at the C-terminal of the rod domain is a helix-terminating amino acid motif, which connects the α-helical rod domain to the non-helical tail domain of the keratin protein. Overall, this rod domain is negatively charged (Herrmann & Aebi, 2004). The α-spiral configuration of keratins is maintained by hydrogen bonds and by intrapeptide and disulfide bonds (Makar et al. 2007). The physical properties of the rod domain and its subdomains define the mechanical properties of intermediate filaments (Herrmann et al. 2007). For example, the subdomain 2B of basic soft and hard α-keratins has a conserved amino acid sequence close to the C-terminal of the rod domain, which influences the lateral packing of the heterodimers in the formation of tetramers (Herrmann et al. 2000). In addition, subdomains of the rod domain attach keratin filaments to cell membrane modifications, such as desmosomes. For example, the subdomain 1B of K18 binds to the C-terminal of the protein desmoplakin in the dense plaque of a desmosome (Stappenbeck et al. 1993).

The non-helical β-turns, called 'linker' regions (i.e. L1, L12 and L2; see Fig. 2), of the rod domain differ in their amino acid sequence and length (Parry et al. 2002). For example, the length of the linker L1 in the rod domain of keratins varies from eight to 16 amino acids and this linker forms a flexible hinge (Parry et al. 2002). The linker L12 is usually the longest non-helical region of the rod domain, whereas the linker L2 contains only seven residues in human keratins (Szeverenyi et al. 2008).

Depending on the amino acid sequence, the secondary structure of the central rod domain of a keratin has one of two basic forms, the α-helix or β-sheet. These two forms of the secondary structure of keratins are the basis for the classification of keratins as α-keratins or β-keratins. The β-keratins are unique to hard-cornifying tissues of sauropsids (i.e. claws and scales of reptiles and beaks, claws and feathers of birds), whereas all vertebrates produce α-keratins (Fraser et al. 1972).

Mutations of keratin genes (*KRT*), which alter the amino acid sequence that determines the secondary structure of the rod domain, may disrupt the formation of the coiled-coil and, hence, of heterodimers and tetramers, and, as a

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

consequence, may alter the mechanical properties of the keratin filament. At present, there is no explanation for how mutations of nucleotides in the keratin gene affect the secondary, tertiary or quarternary structure of keratins and keratin filaments (Smith et al. 2004).

Tail domain of keratin molecules

The tail domain (i.e. the C-terminal of the amino acid chain) is globular and non-helical (Er Rafik et al. 2004). In type II keratins, it is subdivided into the three subdomains H2, V2 and E2 (Steinert et al. 1985). In type I keratins, the tail domain consists of only the V2 and E2 subdomains (Steinert et al. 1985). The V2 subdomains of K1 and K10 contain numerous glycines and have a structure of repetitive loops. These loops are required for the tissue-specific role of keratin filaments in the process of cornification (Parry & Steinert, 1999). Although the tail domain is often involved in the formation of a keratin filament (Parry et al. 2002), it may not always be needed in this role, as the tail-less K19 is able to partner with a basic keratin to form keratin filaments (Bader et al. 1986). The interaction between the tail domain of one keratin and the rod domain of a companion keratin determines the diameter of a keratin filament (Herrmann et al. 2000, 2004). At least for K14, the tail domain also promotes filament bundling by interacting with a rod domain of a neighboring keratin, thus enhancing the mechanical properties of keratin filaments at least *in vitro* (Bousquet et al. 2001; Coulombe & Omary, 2002). The tail domain of K5 and K14 is required for the stabilization of keratin filaments *in vitro* (Wilson et al. 1992), whereas the V2 subdomain of K1 facilitates the parallel alignment of keratin filaments in filament bundles, as well as the translocation of loricrin, a KFAP, to the cell membrane (Sprecher et al. 2001). In acidic hair-specific keratins, the tail domain is more variable than the head and rod domains (Bertolino et al. 1990). The tail domain of keratins in hard-keratinizing-cornifying cells is rich in cysteine, which forms disulfide bonds with other keratins or with KFAPs (Jones et al. 1997). In type I hard α-keratins, the amino acid chain of the tail domain forms a left-handed, tri-folded helix, whereas in type II hard α-keratins, it forms a four-stranded antiparallel β-sheet with an apolar surface (Parry et al. 2002).

Changes in the secondary structure of keratins

The secondary molecular structure (e.g. α-helix, β-sheet or β-turn) of keratins and filaments can be changed under the influence of physicochemical forces, such as mechanical forces or chemical processes.

Mechanical forces, such as tension, compression and probably also shearing, can alter the secondary molecular structure of keratins. For example, the secondary molecular structure of keratin filaments in sheep hair is changed from an α- to a β-conformation when a hair bundle is stretched (Bendit, 1957). As revealed by X-ray diffraction pattern analysis, low humidity accelerates this transformation, possibly through changes of the internal hydraulic pressure within the cells. X-ray diffraction patterns prior to and after release from stretching revealed no significant differences, suggesting that the original secondary molecular structure of the keratins is recovered (Bendit, 1957). If a horse hair is stretched for more than 60% of its length, the α-helix of the keratin molecule is transformed into a β-sheet, possibly due to an unraveling of the coiled-coil of the heterodimer (Kreplak et al. 2001).

Certain direct modifications (e.g. phosphorylation, intra- and interchain peptide bonds) of keratins or indirect changes in the chemical milieu (e.g. pH, ionic strength, osmolarity, etc.) of the cell compartments affect the molecular structure and functions of keratins (Wilson et al. 1992).

Functions of the domains and subdomains of keratins

The domains and subdomains of one keratin molecule (Fig. 2a) will interact with those of adjacent keratin molecules in the formation of subunits, such as heterodimers, tetramers and finally of keratin filaments. Although the α-helical rod domain is sufficient for the formation of heterodimers (Fig. 2b) or tetramers (Fig. 2c), the assembly of keratin filaments requires the non-helical head and tail domains (Wilson et al. 1992).

Heterodimers are formed by a pair of keratins that are oriented parallel so that the head of one adjoins the head of the other. The rod domain assists in the formation of the *c.* 45 nm long coiled heterodimer. The amino acid sequences in the rod domain are largely homologous or, in other words, the rods are only minimally variable. This means that their structure is highly deterministic of its function and that variants may not be able to function with partner keratins (Steinert, 2001).

The head and tail domains establish the correct parallel and staggered orientation of the rod domains in a heterodimer and possibly also in a tetramer. In addition, the head domain of type II keratins is covalently bound to proteins in the cornified envelope of keratinizing epithelial cells, whereas the tail domain anchors the intermediate filaments to the desmosome via so-called linker proteins (Candi et al. 1998). The tail also interacts with KFAPs, such as filaggrin or trichohyalin, forming a filament–matrix complex that is similar to reinforced concrete to impart stability to the cytoskeleton (Hatzfeld & Burba, 1994).

Tertiary structure of keratins: heterodimer

The tertiary structure of keratins is a heterodimer (Fig. 2b) that is formed by the rod domains of one acidic and one basic keratin in parallel orientation (Er Rafik et al. 2004). This heterodimer is the first building block of a keratin

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

filament (Eichner et al. 1986). Keratin filaments are obligatory heteropolymers containing equimolar amounts of type I and type II keratins (Moll et al. 1982; Hatzfeld & Franke, 1985). The heteropolymeric nature of the keratins is established at the level of the double-stranded coiled-coil (i.e. a heterodimer) (Steinert, 1990), as predicted by Crick (1953) and Pauling & Corey (1953). Therefore, synthesis of keratins in the cells is tightly controlled in order to obtain stoichiometry of the acidic and basic keratin pairs and to produce the keratin filaments that are specific to particular stages of the differentiating epithelial cells (Navarro et al. 1995). All epithelial cells at all stages of differentiation express mRNA sequences of both acidic and basic types of keratins in a coordinated fashion (Fuchs & Marchuk, 1983; Fuchs et al. 1983).

Formation of a heterodimer by the parallel alignment of a single pair of type I and type II keratin is the first step in the assembly of a keratin intermediate filament (Coulombe & Fuchs, 1990; Steinert, 2001). In a heterodimer, only the α-helical rod domains of the keratins align with each other (Steinert et al. 1984; Hatzfeld & Burba, 1994; Kammerer et al. 1998) and the alignment and structure are stabilized by the hydrophobic interactions of certain amino acid residues (Coulombe & Fuchs, 1990; Meng et al. 1998). Jones et al. (1997) observed that the keratin molecules in heterodimers of soft-keratinizing-cornifying cells are aligned in parallel but slightly out of phase. This shift of about seven to eight amino acids in the alignment allows an overlap of the head and tail of the keratins where two heterodimers come together. In contrast, the keratins in heterodimers of hard-keratinizing-cornifying cells are aligned in register and do not overlap the tail and head domains where two heterodimers come together. *In vitro*, Coulombe & Fuchs (1990) found that heterodimers (and tetramers) are the most stable building blocks of keratin filaments. The heterodimers of keratins are still soluble in the cytoplasm but this solubility depends on the type of keratin and on the physicochemical characteristics of the cytoplasm (Herrmann & Aebi, 2004). For example, the K8/K18 heterodimers are soluble *in vitro* in a medium with a pH of 9, whereas, in the same conditions, the heterodimers K5/K14 extensively form filaments (Herrmann & Aebi, 2004).

Quaternary structure of keratins forming keratin filaments

Keratins are characterized by the capacity for forming keratin filaments with a complex quaternary structure (Steinert, 1991; Makar et al. 2007), including the formation of a tetramer, i.e. a protofilament with a diameter of 2 nm (Aebi et al. 1983; Eichner & Kahn, 1990), an octamer, i.e. a protofibril (two protofilaments) with a diameter of 4.5 nm (Herrman & Aebi, 2004), and 'unit length filaments' (ULFs) with a diameter of 20 nm (Herrmann et al. 2002) comprising four bundled protofibrils (Parry et al. 2001).

Tetramer

The formation of a 10 nm wide keratin intermediate filament starts with the formation of an approximately 60 nm long tetramer by the half-staggered antiparallel alignment of two heterodimers (Herrmann et al. 2002), thereby forming a protofilament about 70 nm long and 2 nm in diameter (Steinert, 2001). The driving forces supporting this alignment and, hence, the formation of tetramers, are electrostatic bonds between the charged amino acid residues on the surface of the heterodimers (Meng et al. 1998). However, the exact mode of alignment to create the tetramers is not known. Four suggested modes (Fig. 2c) are: A_{11} (alignment of the α-helical subdomains 1 of adjacent rod domains), A_{22} (alignment of the α-helical subdomains 2 of adjacent rod domains), A_{12} (alignment of the α-helical subdomain 1 with the subdomain 2 of an adjacent rod domain) and A_{CN} (connection between the tail end, i.e. the C-terminal, of one rod domain and the head-end, i.e. N-terminal, of another rod domain) (Parry et al. 2001; Steinert, 2001; Herrmann et al. 2007).

The formation of tetramers takes place in the cell periphery, and the solubility of these building blocks, as well as their further assembly to filaments, is influenced by keratin phosphorylation (Windoffer & Leube, 1999), pH, as well as salinity (i.e. the amount of ions available for interacting with the residues of the amino acids in the keratins) (Herrmann et al. 2002). For example, tetramers containing K16 are less stable than those containing K14, K17 and K19 (Coulombe et al. 1998). The tetramers are the stored building blocks that can be recruited to form keratin filaments when the need for them arises (Coulombe et al. 1998).

Unit length filament

In mammals, keratins form soluble short full-width filaments called 'unit length full-width particles' (Parry et al. 2007) or 'ULFs' (Herrmann et al. 2002) [i.e. intermediate filament-like particles (Steinert, 1991)], through a lateral association of tetramers. These ULFs are produced, for example, during the formation of epidermal keratin filaments by the alignment of K1 and K10 (Herrmann et al. 2002). The time needed to assemble ULFs *in vitro* depends on the keratins involved and on the physicochemical characteristics of the medium such as pH and osmolarity (Herrmann et al. 2002). The heterodimers K8/K18 assemble ULFs in 2 s, whereas the heterodimers K1/K10 need about 20 min to form ULFs (Herrmann et al. 2002). Each UFL is about 70 nm long with a diameter of about 20 nm and this diameter shrinks during the formation of keratin filaments (Parry et al. 2001). The MWs of ULFs are variable (Parry et al. 2001). According to X-ray diffraction studies, eight tetramers are thought to align side-by-side to form a tube-like structure called 'unit length full-width particles' (Parry et al. 2007), which join end-to-end to form keratin filaments in non-cornified cells (Steinert, 2001). In cornified cells of hair and wool with the oxidized form of keratin

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

filaments, the tetramers are thought to be arranged in a ring of seven protofilaments (i.e. tetramers) with the eighth protofilament in the center of the ring (Parry et al. 2007). Thus, the question arises how this structural change in the architecture of keratin filaments is achieved in the process of cornification.

Keratin filament

Mammalian keratin filaments are made by lateral and longitudinal aggregations of subunits, such as protofilaments (i.e. tetramers) and protofibrils (i.e. octamers) (Aebi et al. 1983). The capacity for self-bundling of these protofilaments and protofibrils depends on the local pH and osmolarity of the cytoplasm (Yamada et al. 2002; Magin et al. 2007). In cross-section, a mammalian keratin filament has 32 keratin molecules, i.e. 16 heterodimers forming eight tetramers (Jones et al. 1997). The exact architecture of the subunits in a keratin filament is still under debate (Watts et al. 2002). Keratin filaments can be stretched because the primary and secondary building blocks (e.g. the heterodimers and tetramers) can slide relative to each other (Magin et al. 2007). The lack of polarity in keratin filaments is the result of the particular architecture of the keratins and how they are assembled into heterodimers, tetramers and keratin filaments. This in turn influences the mechanical properties of keratin filaments and their interaction with other molecules in the cytoplasm.

Keratin filaments (and keratins) are dynamic structures as was shown *in vitro* by using keratins with the green fluorescent protein attached to their tail domain (Windoffer & Leube, 1999). The dynamic assembly and disassembly of keratin filaments depend on the interaction of keratins and keratin filaments via special molecules, such as plectin, with microfilaments and microtubules (Windoffer & Leube, 1999). The assembly of keratin filaments is spatially and temporarily organized in distinct compartments of the cytoplasm (Windoffer et al. 2004). In the cell periphery, an organizing center for the formation of keratin filaments has been postulated but remains unidentified (Windoffer et al. 2004). Keratin granules, which consist of heterodimers and tetramers, are located close to the cell membrane (Windoffer & Leube, 1999). Therefore, specific plasma membrane domains or focal adhesion sites of microfilaments at the plasma membrane may initiate the formation of keratin filaments at the cell periphery (Windoffer et al. 2006). Other more centralized organizing centers, where the formation, elongation and degradation of keratin filaments take place, have also been postulated based on the observation that the network of keratin filaments changes not only in the cell periphery but also in the center of the cell (Windoffer et al. 2004).

Keratin filaments are densely packed in bundles of varying diameter, also called tonofilament bundles (Norlen et al. 2003), and are the building blocks of the cytoskeleton in epithelial cells. The arrangement of keratin filament bundles depends on external forces exerted on the epithelial cell. Each filament bundle is surrounded by polyribosomes and by multivesicular or multifolded membrane complexes, which form a branched tubular network (Norlen, 2003). In the *Stratum corneum*, keratin filament bundles are thought to be packed in a cubic rod pattern with lipid membranes acting as a scaffold (Norlen & Al-Amoudi, 2004). Keratin filaments, filament-associated proteins and the lipid membrane form a composite structure that almost completely fills the cornified cell (Norlen & Al-Amoudi, 2004).

In reptiles and birds, hard-cornified β-keratins form filamentous structures made of polypeptide chains folded into β-sheets. Two β-sheets are stacked on top of each other, forming a dimer, and are twisted to form a helical structure (Fraser & Parry, 2008). Hence, the filamentous structure of reptilian and avian hard keratins is quite different from that of mammals (Fraser & Parry, 1996).

Keratins and cell functions

Keratins perform instrumental functions within epithelial cells to ensure not only their physical integrity but also their metabolic processes (Vaidya & Kanojia, 2007). To elucidate the functions of keratins, the morphology of normal epithelial organs, tissues and cells has been compared with that of abnormal conditions (Porter & Lane, 2003). Some keratins are redundant, whereas others such as K5 have a specific function and cannot be replaced by other keratins (Porter & Lane, 2003).

Mechanical functions of keratins

Keratins fundamentally influence the architecture (e.g. cell polarity and cell shape) and mitotic activity of epithelial cells (Magin et al. 2007). The best-known function of keratins and keratin filaments is to provide a scaffold (through self-bundling and by forming thicker strands) for epithelial cells and tissues to sustain mechanical stress, maintain their structural integrity, ensure mechanical resilience, protect against variations in hydrostatic pressure and establish cell polarity (Coulombe & Omary, 2002; Gu & Coulombe, 2007). Keratin filaments can be rapidly disassembled and reassembled, thereby providing flexibility to the cytoskeleton. Keratins as building blocks of keratin filaments are concentrated in the cell periphery near the focal adhesions of the cell membrane, and the polymerization of these keratins forming filaments is regulated by signaling molecules (e.g. heat shock proteins, 14-3-3 regulatory proteins, and various kinases and phosphatases) (Magin et al. 2007).

Keratins are functionally redundant, hence they can assemble in various combinations to form keratin filaments (e.g. in the epidermis K1/K10, K1/K9, K2/K9 or K2/K10), and some keratins, at least, can be replaced by others

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

without a loss of functionality of the keratin filaments (Coulombe & Omary, 2002). The regulation of keratin function is achieved by the post-translational modification of the keratins (Coulombe & Omary, 2002). Keratins and keratin filaments interact with cell-adhesive complexes, i.e. desmosomes, hemidesmosomes and focal adhesions (Maniotis et al. 1997; Coulombe & Omary, 2002). Keratin filaments are also connected to microtubules, which indicates that the constituents of the cytoskeleton interact with one another (Kim & Coulombe, 2007; Oriolo et al. 2007). This interaction of keratin filaments with constituents of the microtubule organizing center is involved in the apicobasal orientation of microtubules, which maintains the cell polarity of enterocytes, i.e. epithelial cells of the intestinal mucosa (Oriolo et al. 2007).

To adapt to changing conditions within cells, keratins interact with various other proteins, such as heat shock proteins (i.e. chaperones), desmoplakins of desmosomes, tubulin complex proteins, etc. (Izawa et al. 2000; Garmyn et al. 2001; Oriolo et al. 2007).

Non-mechanical functions of keratins

Keratins and keratin filaments are involved in other than mechanical functions, such as cell signaling, cell transport, cell compartmentalization and cell differentiation (Oshima, 2007; Vaidya & Kanojia, 2007). For example, K10 inhibits cell proliferation in suprabasal cells but induces cell differentiation (Paramio et al. 1999). Keratin filaments also influence the cell responses to intrinsic and extrinsic signals, such as pro-apoptotic signals and the proper distribution of membrane proteins in polarized epithelial cells (Coulombe et al. 2004; Kim & Coulombe, 2007). For example, in hepatocytes, K8 and K18 bind to signaling molecules, thereby disrupting the apoptotic signaling cascade that would initiate apoptosis (Gu & Coulombe, 2007). The rod domain of K18 can bind to the C-terminal of a cell membrane-bound signal receptor, thereby blocking the activation of the second messenger, i.e. an enzyme involved in the apoptosis in hepatocytes (Inada et al. 2001). Similarly, K8 and K18 bind to the tumor necrosis factor receptor 2 and, thereby, modify the apoptotic signaling cascade (Paramio & Jorcano, 2002). Keratins bind various signaling molecules, such as protein kinases, phosphatases and proteins of the 14-3-3 family (Paramio & Jorcano, 2002). In epidermal wounds, K1 and K10, which are normally expressed in suprabasal cells, are down-regulated, whereas the expression of K6, K16 and K17 is initiated (Coulombe et al. 2004). Keratins in modified epithelial sensory cells (e.g. Merkel cells, hair cells) could be involved in the signal transduction from the apical part to the basolateral part, where transmembrane channels or transmitter-containing vesicles are opened to release signaling molecules transducing the signal to associated neurons (Lumpkin & Caterina, 2007). K8 is a marker for cells developing taste buds (Mbiene & Roberts, 2003), and denervation of the gustatory fibers eliminates the keratins that are specific for taste buds (Oakley, 1993). In certain supporting epithelial cells in the organ of Corti, keratin filaments may be involved in micromechanical functions of transmitting vibrations to the sensory hair cells (Mogensen et al. 1998).

Keratin filaments also influence cell metabolic processes by regulating protein synthesis and cell growth (Gu & Coulombe, 2007). K17 regulates the protein synthesis and cell growth in injured stratified epithelia by binding to a signaling molecule (Kim et al. 2006).

Keratins may also be involved in the transport of membrane-bound vesicles in the cytoplasm of epithelial cells. For example, the distribution of membrane-bound melanosomes in epidermal basal cells is disrupted in cells that produce a depleted K5 due to a 'loss of function' mutation in the gene affecting the amino acid sequence of the head domain of K5 (Planko et al. 2007).

Keratins and cell metabolism

Keratins that are not polymerized in heterodimers are broken down (Kulesh et al. 1989). Keratins that need to be recycled in hepatocytes are marked for proteolysis in the proteasome by being tagged with ubiquitin (Inoue et al. 2001). In hepatocytes, the dephosphorylated K18 is broken down enzymatically by the proteases caspase-3 and -7 but the hyperphosphorylated forms of K18 cannot be cleaved by these caspases (Ku & Omary, 2001). The disassembly of K8/K18 in hepatocytes may fail due to an overload of the proteasome and these keratins are then stored in mallory bodies in hepatocytes (Zatloukal et al. 2007).

Keratins characteristic of certain types of epithelia

Historically, epithelia have been classified according to their morphology. Although this type of classification remains useful, it has been refined by incorporating additional criteria, such as chemical, biochemical and biophysical properties, as well as the type of keratin that is produced by the cells. Some morphologically defined epithelia are already known to differ in their mode of keratinization and cornification, the expression of keratins, and the organs that they form (Achtstätter et al. 1985). For any given epithelium, there are local differences in the qualitative expression of keratin and the processes of keratinization and cornification. Keratins in the epidermis vary between different regions and structures, such as hairs, epidermal glands, palm and sole skin, claws, hooves, fingernails, etc. Even within a particular organ (e.g. hair, hooves and claws), there are differences in keratin expression, which are related to different mechanical properties. Historically, the mechanical attributes of an organ (e.g.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

hooves, claws, etc.) were interpreted as a simple combination of soft keratinization and hard cornification. Recently, a greater appreciation of the complexity of such organs has emerged (De Berker et al. 2000; Perrin, 2007). For example, it is now understood that different parts of the bovine hoof and human fingernail produce various keratins that are characteristic of soft-cornified and hard-cornified epithelia (De Berker et al. 2000). Similarly, the degree of cornification also differs between the filiform papillae and the epithelium between them of the tongue of cats. The types of keratins within the mucosa of the palate of herbivores also vary, along with the degree of cornification, between the crests of the palatal rugae and the sulci between them. Hence, keratins are not characteristic of entire tissues but more probably of particular functional properties of cells and tissue regions. For example, Langbein et al. (2001) found that certain keratins that are expressed in the root of hairs are also expressed in the tongue of rats.

During embryonic development of simple to stratified epithelia, different keratins are expressed (Banks-Schlegel, 1982). Cells of the single-layered precursor of the human epidermis produce the same types of keratin that are characteristic of simple epithelia, namely K8, K18 and K19 (Dale et al. 1985). With the onset of stratification, different keratins are expressed in the basal and suprabasal layers, e.g. K5 is produced instead of K8. With the onset of keratinization, K1 and K10 are added to the cytoskeleton in the suprabasal cell layers. Concurrently, the expression of certain keratin genes changes, with large keratins being produced with the onset of keratinization and cornification, and smaller ones no longer being synthesized (Banks-Schlegel, 1982). These developmental changes are similar to the differentiation processes that take place in the interfollicular epidermis and hair follicles of adult mammals.

Keratin filaments can be created *in vitro* by combining proteins from both simple and stratified epithelia (Hatzfeld & Franke, 1985), which may indicate that keratin filaments *in vivo* preferentially combine with particular proteins for reasons other than only molecular structure.

Keratins in simple epithelia

Keratins of simple epithelia make up less than 5% of the total protein content in an epithelial cell (Nelson & Sun, 1983). Simple epithelia usually express the basic keratin K8 and its acidic partner K18, along with various other keratins (e.g. K7, K19, K20 and K23) during cell differentiation following cell division (Achtstätter et al. 1985; Bosch et al. 1988; Kirfel et al. 2003; Owens & Lane, 2003; Lu et al. 2005; Pekny & Lane, 2007). In venules, lymphatics and capillaries, endothelial cells express K7 and K18 but not K8 and K19, which are characteristic of many simple epithelia, or K14, which is characteristic of basal cells of all stratified epithelia (Miettinen & Fetsch, 2000). During the development of simple epithelia, the translation of mRNAs encoding the basic keratins K7 and K8 precedes that of the acidic partners K18 and K19, and consequently there are no keratin filaments in the cell periphery at first but instead many granulated aggregations of K7 and K8 (Lu et al. 2005). Different keratins are produced in various parts of the simple epithelia lining sweat glands (Schön et al. 1999). Cells in the secretory unit synthesize K7, K8 and K19, whereas cells forming the duct produce K5, K14 and K19 (Schön et al. 1999). Unlike the keratins of cornified stratified epithelia, those of simple epithelia are not cross-linked by disulfide bonds (Owens & Lane, 2003). In apoptotic cells of simple epithelia, keratin filaments and proteins are digested enzymatically. For example, K18 in apoptotic hepatocytes is cleaved by the enzyme caspase generating a neoepitope of K18, and this neoepitope can be recognized immunohistochemically to detect apoptotic hepatocytes for diagnostic purposes (Leers et al. 1999).

Keratins in stratified epithelia

Keratins in stratified epithelia (Fig. 3) make up 30–40% of the total protein content in an epithelial cell (Nelson & Sun, 1983), with different sets of keratins being synthesized at specific stages of cell differentiation. Keratins in stratified epithelia are classified according to their position within a stratum of keratin-producing cells. Basal keratins (i.e. 'B-type keratins') are produced in the basal stratum, whereas the differentiation-specific keratins (i.e. 'D-type keratins') are produced in suprabasal strata (Schermer et al. 1989). Suprabasal cells can also produce 'hyperproliferative' keratins (i.e. 'H-type' keratins), such as K6 and K16 (Schermer et al. 1989). Stratified epithelial cells produce keratins (e.g. K1, K5, K10, K14) in such a manner that each of the acidic keratins (e.g. K1, K5) is always matched by a particular basic keratin (e.g. K10, K14) to form a heterodimer (Steinert, 2001). Keratins characteristic of simple epithelia, such as K8 and K18, can be co-expressed with keratins characteristic of stratified epithelia, such as K4, K13, K14 and K15 (Bosch et al. 1988).

Keratins typical of basal cells of stratified epithelia include the basic keratin K5 and the acidic keratin K14, which combine to form heterodimers (Nelson & Sun, 1983; Dale et al. 1985). Stratified epithelia express additional keratins starting in the suprabasal cell layers (Bowden et al. 1984). Different types of stratified epithelia are characterized by different keratin pairs in the suprabasal cells (Eichner & Kahn, 1990). There are at least four differentiation types for the suprabasal cells of stratified epithelia (Sun, 2006), i.e. the skin type, cornea type, esophagus type and hyperproliferative type. For example, the suprabasal cells in the epidermis of the skin specifically produce K1 and K10, whereas those of the anterior corneal epithelium produce K3 and K12, and those of the esophageal epithelium

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

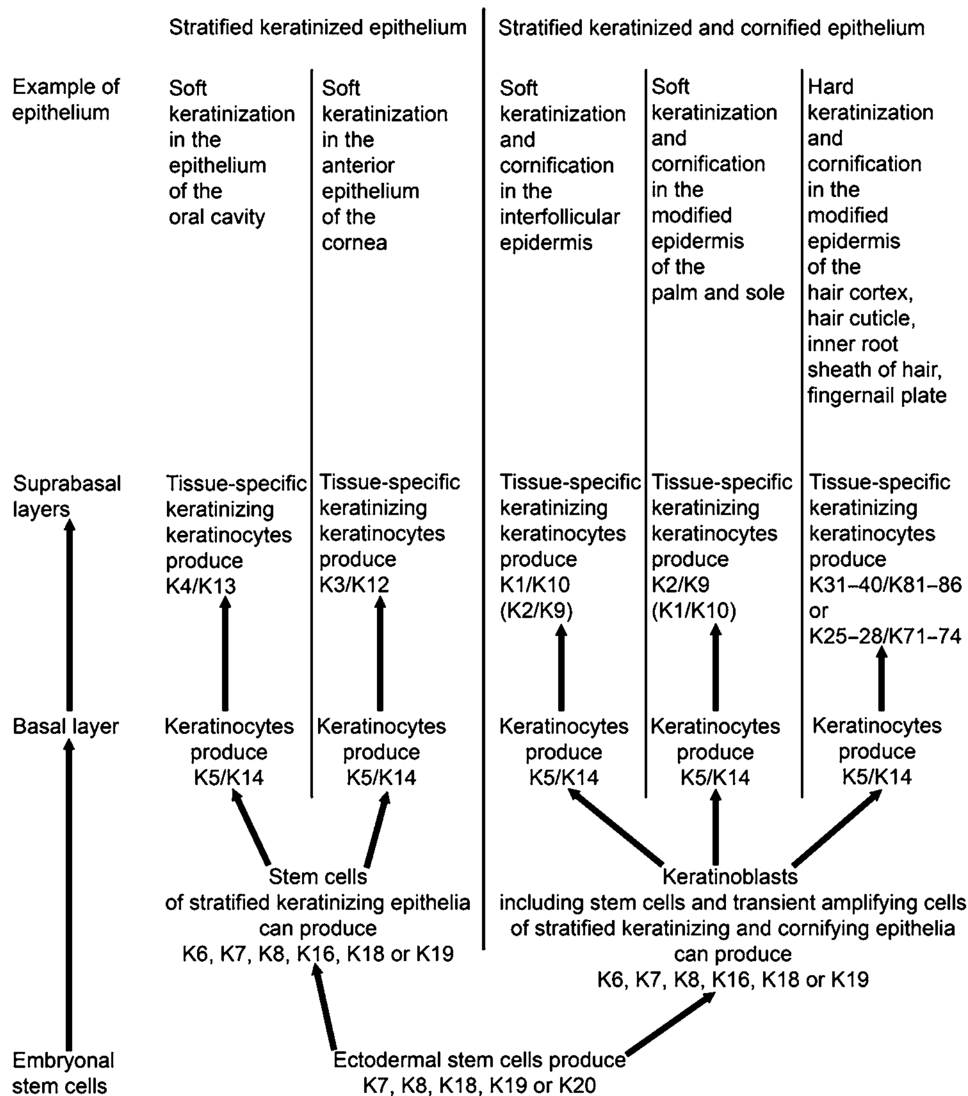

Fig. 3 Ectodermal stratified epithelia and the keratins produced in the basal and suprabasal cells. The basal cells of all stratified epithelia produce K5 and K14 but the suprabasal cells of stratified epithelia produce specific keratins, which are characteristic of the type of epithelium, e.g. the suprabasal cells of the anterior epithelium of the cornea produce K3/K12.

produce K4 and K13 (Eichner & Kahn, 1990). The hyperproliferative type of suprabasal cells is characterized by K6 and K16 (Sun, 2006).

The synthesis of the keratins that are characteristic of a specific type of differentiation of suprabasal cell may be reversible. For example, the expression of K1 and K10 in the suprabasal cells of the epidermis can be modulated reversibly (Nelson & Sun, 1983), so that the suprabasal cells of the keratinizing and cornifying epidermis can produce K2 and K9 in addition to K1 and K10 (Moll et al. 1987). The suprabasal cells of esophageal epithelium produce K4 and K13 but are also able to co-express K6 and K16 (Pang et al. 1993).

Keratins in non-cornified epithelia

K4 and K13 are produced in the suprabasal cells of the transitional epithelium of the renal pelvis, urinary bladder and entrance to the urethra (Achtstätter et al. 1985). The suprabasal cells of the stratified keratinizing epithelium (Banks, 1993) of the glans penis express the differentiation-specific K1 (Achtstätter et al. 1985). The suprabasal cells of the stratified epithelium of the vagina, exocervix (i.e. the distal one-third of the cervix), navicular fossa of the glans penis, glans penis itself, gingiva and thymic reticulum express K1 and K10 (Moll et al. 1987), suggesting that K1 and K10 are characteristic of keratinization (Nelson & Sun, 1983) but not cornification. In the developing teeth of humans, certain keratins (i.e. K7, K13, K14 and K19 but not K8, K10, K16 or K17) are expressed in the developing stratified enamel organ (Domingues et al. 2000). During ameloblast differentiation in the enamel organ, the change of the cell polarity is accompanied by a change of keratin expression, i.e. K14 is

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

downregulated, whereas K19 is upregulated (Domingues et al. 2000).

Keratins in soft-cornified epithelia

The most extensive stratified soft-cornified epithelium is the epidermis (Tobin, 2006). Keratins make up about 30% of the volume of its basal cells and about 80% of the volume of its cornified cells. In humans, the processes of keratinization and cornification last about 30 days for a keratinocyte to become a mature corneocyte (Tobin, 2006). These differentiation processes start in the suprabasal cells of the epidermis with the synthesis of K1 (Troy & Turksen, 2005), as K1 and K10 are typical for suprabasal cells of stratified and cornifying epithelia (Dale et al. 1985). Different heterodimers of keratins, e.g. K5/K14 and K1/K10, are characteristic of different stages of cell differentiation within the stratified epidermis (Lynch et al. 1986). Keratin filaments based on the heterodimers of K1 and K10 are built on the scaffold of K5 and K14 heterodimers, which were produced by the epidermal basal cells (Kartasova et al. 1993). In skin wounds, the epidermal suprabasal cells produce K6 and K16, whereas the production of K1 and K10 is downregulated (Wawersik & Coulombe, 2000). The keratins in the epidermal *Stratum corneum* are cross-linked by intermolecular disulfide bonds, whereas those in the living epidermal cells are not cross-linked (Sun & Green, 1978). In addition, the keratins in the *Stratum corneum* of the epidermis are linked to proteins of the cornified envelope via secondary peptide bonds connecting the ε-amino group of the amino acid lysine to the γ-carboxy group of the amino acid glutamate (Sun & Green, 1978).

The permeability barrier of cornified epithelia (i.e. the *Stratum corneum*), which is formed by two components (i.e. the corneocytes and the intercellular lipids), regulates the process of epidermal cornification (Debus et al. 1982). For example, the increased water loss following tape-stripping of the *Stratum corneum* in the mouse epidermis initiates the premature cornification of the uppermost cell layers of the epidermal *Stratum granulosum* (Debus et al. 1982).

Keratins in soft-cornified skin modifications

Soft-cornified skin modifications are specialized regions of the skin in which the thickened epidermis keratinizes and cornifies in a similar, yet also distinct, manner to that of the interfollicular epidermis. These skin modifications are subjected to increased mechanical loading [e.g. the palms of hands and the soles of feet in humans, the various pads (i.e. carpal/tarsal, metacarpal/metatarsal and digital pads) in mammals, the bovine muzzle] (Banks, 1993). The epidermal stem cells of soft-cornified skin modifications are located in the deep ridges of the epidermis and produce K15 (Webb et al. 2004). The basal cells of skin modifications synthesize K5 and K14 but the suprabasal cells synthesize the same keratins as do the suprabasal cells of the interfollicular epidermis (i.e. K1, K2 and K10) but, in addition, also K6, K9, K16 and K17 (Swensson et al. 1998). The suprabasal cells near the transition to the *Stratum corneum* produce K2 and K9 (Kirfel et al. 2003).

Other modifications of the soft-cornified epidermis are the outer and inner root sheaths in the hair follicle (Heid et al. 1986). The epithelial cells of the outer root sheath of the human hair follicle produce K5 and K14, which are also synthesized in the cells of the interfollicular epidermis, as well as the keratins K6, K16 and K17. In contrast, the epithelial cells of the inner root sheath of the human hair follicle produce special (hair-related) keratins, the type II keratins K71–K74 (Langbein et al. 2003, 2006). The four basic keratins K71–K74 of the inner root sheath of the hair follicle have very similar amino acid sequences in their rod domain and in their H1 and H2 subdomains (Langbein et al. 2003). K71 is expressed in all compartments of the inner root sheath, K72 and K73 are specific for the cuticular cells of the inner root sheath, and K74 is expressed only in the cells of the Huxley layer of the inner root sheath (Langbein et al. 2003; Perrin, 2007). The partners for the basic keratins K71–K74 of the inner root sheath in human hair to form heterodimers are the acidic keratins K25–K28, which are expressed in the various layers of the epithelial cells of the inner hair root sheath (Langbein et al. 2006). The keratins that are specific for the inner root sheets are very similar in their amino acid sequence but differ from other keratins in soft- and hard-cornified tissues (Langbein et al. 2003). The epithelial cells of the companion layer of the inner root sheath of body hairs and of the medulla of the terminal hairs specifically express K75 (Langbein et al. 2003, 2006). The early production of K75 in the cells of the companion layer of the inner root sheath may induce the specific cornification of the other compartments in the hair follicle (Roh et al. 2004). The cells of the companion layer produce K75 and K6a at different times and in different cells (Gu & Coulombe, 2007).

During the fetal development of the human skin, the keratinization of the follicular epidermis in the hair germs precedes that of the interfollicular epidermis (Dale et al. 1985). Furthermore, modified hairs (e.g. the vibrissae, the hairs of the mane and tail of equine fetuses) develop earlier than the hairs of the pelage (Zietzschmann & Krölling, 1955; Bragulla, unpublished observations). Other skin modifications (e.g. the carpal and tarsal pads, the human fingernail, the equine and bovine hooves) also start to keratinize and cornify earlier than the unmodified interfollicular epidermis (Smith et al. 1999; Bragulla unpublished observations).

Keratins in hard-cornified skin modifications

The processes of hard cornification in mammalian skin modifications, such as hairs and nails, are characterized by the intensive concentration of sulfur through the amino

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

acids cysteine and methionine in the keratogenous zone, as was revealed by radio-autographic studies (Bern et al. 1955). A high content of the amino acid cysteine in the head and tail domains is characteristic of the hard keratins in mammals, which are α-keratins (Rogers et al. 1995). Furthermore, hard keratins contain several residues of the amino acid cysteine in their rod domain (Wang et al. 2000). The amino acid sequence of the tail domain is the most specific part of a hard α-keratin (Rogers et al. 1995). In the human hair, bovine hair and in the wool of sheep, as well as in the hair of mice, four basic and four acidic hard α-keratins are produced (Heid et al. 1986; Rogers et al. 1995). The murine hair-specific keratins mHa1, mHa3 and mHa4, which are orthologues of the human hair-specific hard keratins K31 (i.e. hHa1), K33 (i.e. hHa3) and K34 (i.e. hHa4), are produced in the suprabasal cells forming the cortex of the hair, and mHa2 or K32 (i.e. hHa2) is expressed in the cells of the hair cuticle of the mouse or human, respectively (Rogers et al. 1995). These acidic hard keratins can partner with a variety of basic partners, just like the soft keratins do. For example, the acidic hard keratin K31 partners with the basic hard keratins K81 or K86 (Bowden et al. 1998), and the acidic hard keratin K37 may heterodimerize with the keratins K81, K85 or K86 (Jave-Suarez et al. 2004). The hair-specific hard keratins K31–K40 and K81–K86 are produced only once the hair follicle is fully developed (Kopan & Fuchs, 1989). The expression of the specific hair keratins ceases at the end of the hair cycle from the katagen to the telogen stage (Bowden et al. 1998).

Hair-specific hard keratins (e.g. K31, K34, K81, K85) are also produced in the suprabasal epidermal cells of the nail matrix (Perrin et al. 2004) but the expression of hair-specific and epidermal keratins differs regionally in the human fingernail (De Berker et al. 2000). In the nail matrix, the hair-specific hard keratin K31 is expressed, whereas in the proximal nail fold, the soft K1 and K10 are produced and, in the nail bed epidermis, the basic K6 and its acidic partners K16 and K17 are expressed (De Berker et al. 2000). In addition, the suprabasal cells of the nail bed epidermis produce K75 (Perrin, 2007). The cornifying cells distal to the nail bed epidermis produce K10 (Perrin, 2007), which is characteristic of the soft-cornifying epidermis.

Kitahara & Ogawa (1991) extracted K10, which is specific for keratinizing cells of the interfollicular epidermis, in addition to the hair-specific keratins from the cornified nail plate, which indicates that the human nail contains both skin-type and hair-type cornified cells. The nail plate in humans consists of 10–20% of the keratin heterodimers K5/K14 and K6/K16, which are characteristic of epidermal keratinoblasts, whereas the heterodimers K1/K10 and K2/K10, which are characteristic of epidermal keratinocytes, are absent (Lynch et al. 1986). Keratins that are specific for the hair cuticle (e.g. K25–K28 and K71–K74) are absent in the nail (Perrin et al. 2004).

The expression of genes encoding hair-specific keratins in the suprabasal cells of hair follicles and skin modifications is to some extent exclusive and suppresses the activation of genes that encode epidermal keratins (Bowden et al. 1998). However, in the medulla of hairs, the keratinocytes express both keratins typical of hard-cornifying epithelia (i.e. follicular or trichocytic keratins) as well as keratins typical of soft-cornifying epithelia (i.e. interfollicular epidermal keratins) (Heid et al. 1988a,b; Langbein et al. 2004). The keratinizing cells in the inner root sheath of the human hair follicle produce the epidermal K1 and K10 (Stark et al. 1990) as well as the special keratins K25–K28 and K71–K74 (Langbein et al. 2003; Langbein et al. 2006). It is unclear whether two keratin types (i.e. the soft epidermal and hard trichocytic keratins) can be expressed at the same time by a keratinocyte.

The assembly of keratin filaments from hair-specific hard α-keratins (= trichocytic keratins) in the epithelial cells (= trichocytes) of hair follicles follows the same pattern as the assembly of keratin filaments from soft α-keratins (Wang et al. 2000). The formation of disulfide bonds between hard α-keratins may influence the alignment mode (Fig. 2c) of the two heterodimers in the formation of the tetramers (Wang et al. 2000). In cell culture, hard α-keratins can form keratin filaments in combination with soft α-keratins (Yu et al. 1991).

The various acidic hair-specific hard α-keratins are very similar in their amino acid sequence not only to one another but also across mammals as revealed by the cross-reactivity of antibodies (Bertolino et al. 1990). For example, the acidic hair-specific hard mHa1 (i.e. murine Hair acidic 1) and mHa4 in mice have a 92% similarity with each other (Bertolino et al. 1990). These acidic hard keratins are even more similar to one another across species than to the acidic soft keratins in the cornifying epidermis of the same species (Bertolino et al. 1990).

The production of keratins changes over the course of development in hair and skin modifications (Heid et al. 1986; Bragulla, 1991; Bragulla & Hirschberg, 2002). For example, in a hair germ in its late 'bulbous-peg stage' stage of fetal development, the suprabasal cells of the developing hair cone, which covers the dermal papilla, change their expression of keratins by ceasing to express epidermal keratins and starting to produce keratins that are typical for hard-cornifying skin modifications (Moll et al. 1988). Similarly, in human fetuses, the epidermal cells that form the primordial nail plate express first keratins that are typical for soft cornification (e.g. K1, K5, K6, K10, K14, K15) and subsequently, in the advancing prenatal development, keratins that are specific for hard cornification (e.g. K31, K32) (Moll et al. 1988). K19, a marker for epidermal stem cells, is also expressed in the fetal human fingernail (De Berker et al. 2000). In the adult human nail plate, the epidermal cells express only keratins that are typical for basal cells (e.g. K5, K6, K14 and K16), and the

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

suprabasal cells produce the same hard keratins (e.g. K31, K32, K33, K34, and K81) that are specific for hard-cornifying suprabasal cells in hairs (Moll et al. 1988). At this point, the signal that induces the change in keratin expression from soft keratins to hard keratins is still unknown.

Proper keratinization and cornification processes are crucial for the health of cornified organs (Bragulla & Mülling, 1997). For example, in the dyskeratotic sole epidermis of the bovine hoof, the suprabasal keratinocytes continue to produce keratins that are characteristic of the basal cells and also produce K16 instead of the usual K1 and K10 (Hendry et al. 2001).

Calcification of keratins in hard-cornified skin modifications

Keratinization and cornification of epidermal cells in hard-cornified skin modifications may be accompanied by calcification (Toto et al. 1967). For example, keratin filaments of the hard-cornified horn of the Japanese serow (*Capricornis crispus*) contain crystalline inorganic components, including tricalcium phosphate and hydroxyapatite (Hashiguchi & Hashimoto, 1995). In the Saiga antelope (*Saiga tatarica*), the hard-keratinized *Stratum corneum* is mineralized with deposits of octacalcium phosphate (Hashiguchi et al. 2001). The nasal and frontal horn of the white rhinoceros (*Ceratotherium simum*) (Hieronymus et al. 2006) and the baleen plates of baleen whales (Pautard, 1970) are also calcified. The presence of calcium salt in hard-cornified materials is not pathological and has also been reported for hairs and fingernails (Hieronymus et al. 2006). The calcium concentration of human fingernails is negatively correlated with age and may be used as an indicator of the mineral metabolism of bone (Ohgitani et al. 2005). In contrast, the correlation of calcium levels in human toenails and bone tissue is low and not useful for measuring the degree of osteoporosis (Vecht-Hart et al. 1995).

Calcification of epithelial tissue may result from injuries, as in epithelia of hair follicles in rats (Pearce et al. 1972), although similar mineralization may also occur in intact skin (Pearce & Smillie, 1973). Calcification of keratin filaments has also been described in epithelial cancer. For example, keratin filaments in the horn pearls of a carcinoma of the *Glans penis* are calcified (Bonucci et al. 1979).

Diminished levels of calcification in the cornified horns and hooves of lactating cows could explain the presence of horn rings. The cornified sheath of cat claws is also calcified as indicated by the affinity of the stain Alizarin Red to the cornified cells (Bragulla, unpublished observations).

Keratins in stem cells

Stem cells of epithelia reside in niches, such as the deep epidermal ridges or the bulge of the hair follicle, where they are insulated from physical and mechanical influences (Watt, 1998). These stem cells produce specific keratins, such as K15 and K19 (Narisawa et al. 1994; Watt, 1998; Waseem et al. 1999; Webb et al. 2004). Originally, all basal cells in stratified epithelia (Fig. 4) were thought to be able to produce new cells to maintain the epidermis because mitotic figures were observed in the entire basal layer of the fetal human epidermis, in which all basal cells produce the keratin K15, which is characteristic of epidermal stem cells (Webb et al. 2004). However, the concepts of epidermal stem cells, transient amplifying cells and epidermal proliferative units were developed by Alonso & Fuchs (2003) when it was recognized that the telomeres of chromosomes limit the number of possible cell divisions to about 30 within the lifetime of a cell lineage and that this number of epidermal cell divisions would not suffice to compensate for the loss of cells through normal desquamation and shedding (Harley, 1991; Ueda, 2000).

Under the new concept, stem cells have a special capacity to renew themselves over the lifetime of an organism, and they divide into two different daughter cells, namely one stem cell and one transient amplifying cell (Watt, 1998; Alonso & Fuchs, 2003; Webb et al. 2004). Primary cell cultures of stem cells from hair follicle and the epidermis generate a variety of cell types, namely stem cells (10%), transient amplifying cells (40%) and keratinocytes (50%), with each cell type having different potentials to proliferate and generate new cell clones (Papini et al. 2003). In the interfollicular epidermis, 2–5% of the basal cells are stem cells and produce K15 (Webb et al. 2004). Some basal cells in the interfollicular epidermis cells are mitotically active keratinoblasts and produce the keratin K14. Some other basal cells are differentiating cells or keratinocytes that downregulate the production of K14 and upregulate the production of K10 (Webb et al. 2004). Drawing upon the observations by Alonso & Fuchs (2003), the new model of epidermal renewal proposes that the basal layer of stratified epithelia is comprised not only of stem cells and transient amplifying cells but also of cells that have already started their differentiation into keratinocytes. The transient amplifying cells replicate themselves four to eight times before they finally divide into keratinocytes. The descendants of one stem cell (i.e. the transient amplifying cells and keratinocytes) are collectively called an 'epidermal proliferative unit' (Strachan & Ghadially, 2008). Hence, the presence of transient amplifying cells would generate 480–1920 keratinocytes from a single stem cell over the lifetime of an organism (i.e. 16×30 to 64×30), which is about 16–64 times more than if stem cells produced only keratinocytes without transient amplifying cells. The epidermal proliferative unit is not simply a theoretical concept but also explains the occurrence of distinctly localized skin modifications in skin diseases.

In contrast, Clayton et al. (2007), using a different approach, proposed a model of epidermal cell amplification and

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

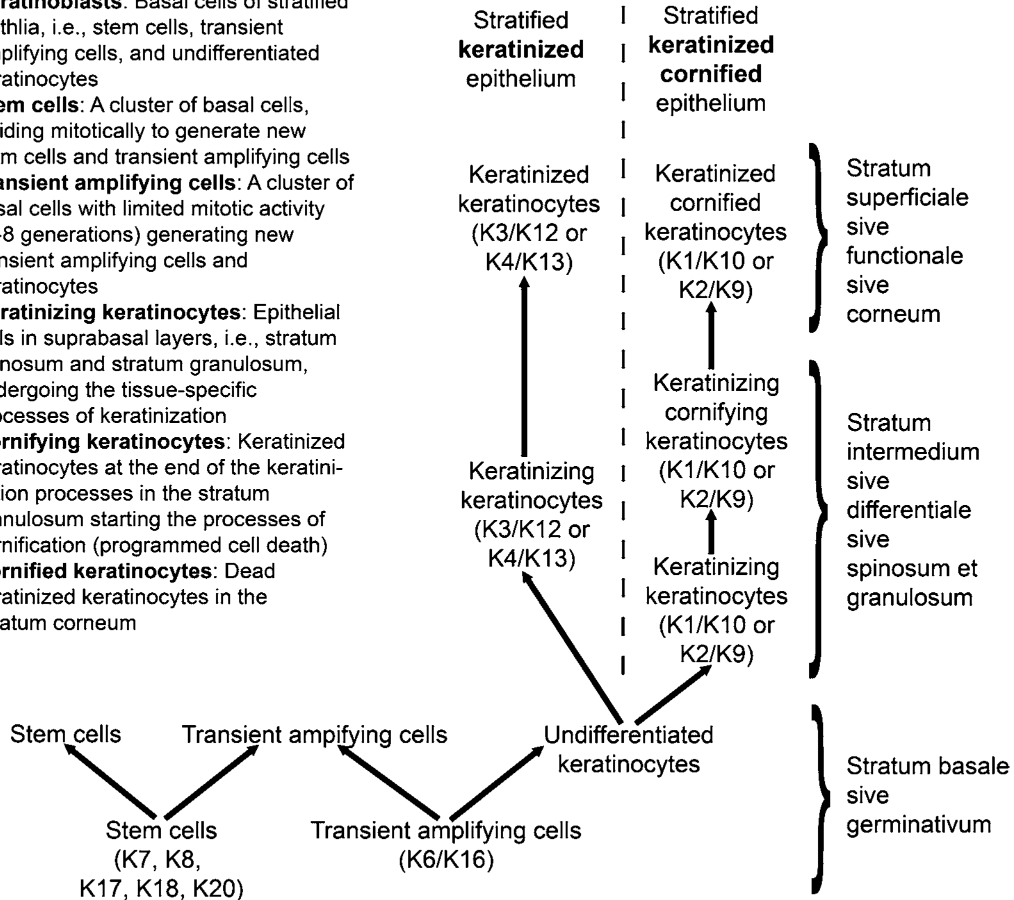

Fig. 4 The cells in the strata of stratified keratinized vs. stratified keratinized and cornified epithelia: similarities and differences. The *Stratum basale* of stratified epithelia houses the keratinoblasts, i.e. stem cells and transient amplifying cells as well as undifferentiated keratinocytes. As soon as the keratinocytes are pushed upwards by the following cell generation thereby losing contact with the basal lamina, they begin the specific differentiation process and produce specific keratins such as K1 and K10. The cells of the keratinizing and cornifying epidermis and of its derivatives, e.g. hair and nail, die in a programmed cell death and become the corneocytes in the superficial *Stratum corneum*. In contrast, the keratinocytes of keratinized, non-cornifying epithelia are viable cells forming the superficial stratum. These keratinized, non-cornifying epithelia, e.g. epithelia of the oral and vaginal mucosa, can cornify in reacting to changes in mechanical or hormonal signals.

maturation that includes only stem cells and keratinocytes. In this model, stem cells divide symmetrically into stem cells (8%) or keratinocytes (8%) and asymmetrically into a stem cell and a keratinocyte (84%). Thus, one cell produces maximally 1 billion keratinocytes over the lifetime of an organism [i.e. $2^{30} = (1024)^3 \sim 10^9$].

Epidermal stem cells, which are also called keratinoblasts, produce the keratins K5, K14, K15 and K19 (Lyle et al. 1998). Stores of these keratinoblasts are found in the bulge of hair follicles, in epidermal glands (i.e. sebaceous, sweat and mammary glands) and possibly in the interfollicular epidermis (Jones, 1996). In the hair bulge, the stem cells in the basal layer synthesize the keratins K5, K15, K17 and K19 but not K14 (Larouche et al. 2008). The keratinoblasts in the first suprabasal layer of the hair bulge express K5 and K17 but not K14, K15, K16 or K19. The keratinoblasts in the second suprabasal layer of the hair bulge express K5, K14, K15 and K17 but not K19 (Larouche et al. 2008). These variations in keratin expression may support the concept of different cell types (i.e. stem cells, transient amplifying cells or undifferentiated keratinocytes).

The keratinocytes are pushed towards the surface of the epidermis by the newly produced cells in the basal layer and go through various stages of maturation (i.e. keratinization only or keratinization and cornification). However, there are several issues that remain poorly understood. One issue is the lifespan of an individual cell lineage and the duration of the various maturation stages of the tissue-specific cells. Another issue is whether keratinocytes are still mitotically active or not, although the models by Alonso & Fuchs (2003) and Clayton et al. (2007) assume that they are not as indicated by the production of K1/K10 instead of K5/K14.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

Keratins in epithelial cells with special functions

Some epithelial cells do not fit within the traditional concept of epithelial tissues because they perform specialized tasks but nevertheless express keratins that are also expressed in epidermal cells. This fact provides a possible explanation for the connection between certain epidermal disorders and disorders of other organs, such as infertility. For example, spermatids express the keratins SaK57 (i.e. a homologue to the epidermal K5) and K9, which are expressed in the course of spermiogenesis (Kierszenbaum, 2002). Myoepithelial cells of the secretory units of exocrine serous glands, such as sweat, mammary, lacrimal and salivary glands, produce K17 (Freedberg et al. 2001). Merkel cells express K8, K18 and K19 (Moll et al. 1984; Narisawa et al. 1994; Larouche et al. 2008), and smooth muscle cells in the human placenta cross-react with an anti-K8 antibody, thus indicating that they contain K8 (Miettinen & Fetsch, 2000) or at least a molecule with an epitope that binds to this antibody. Similarly, in the transition from a non-sensory to a specialized sensory cell, the synthesis of keratins is downregulated (Cyr et al. 2000).

Other keratins (e.g. β-keratin and thread keratins)

In extant therapsids (the lineage leading to and including mammals), the keratins that stabilize the soft-cornified epidermis and the hard-cornified epidermal appendages have an α-helical secondary molecular structure. In extant sauropsids (i.e. reptiles and birds), only the keratins in soft-cornified epidermal structures have an α-helical secondary molecular structure. The keratins of hard-cornified epidermal structures, such as beaks, claws, scales and feathers, have a twisted β-sheet-like secondary structure (Fraser et al. 1972). More than 30 β-keratins have been extracted so far from epithelia of the chicken (Sawyer et al. 2003). The MWs of β-keratins in scale-forming cells range from 17 to 20 kDa and those of β-keratins in feather-forming cells range from 10 kDa (Woodin, 1956) to 14 kDa (Shames et al. 1989, 1991). The β-keratins of sauropsid hard-cornified tissues resemble the KFAPs of mammals (i.e. 'matrix proteins') in both MW and sulfur content (Fraser et al. 1972). Hence, the so-called β-keratins of birds and reptiles more closely resemble mammalian KFAPs and not α-keratins (Fraser et al. 1972). This concept raises the question: where are the actual filamentous keratins of sauropsid hard-cornified tissues?

Another difference between reptilian β-keratins and mammalian α-keratins is that the filamentous structure of the former has a diameter of 4 nm, whereas that of the latter is 7–10 nm (Fraser & Parry, 1996). The molecular structure of β-keratins is poorly known compared with that of mammalian α-keratins, although the secondary structure of β-keratins and of the keratin filaments in feathers has recently been described (Fraser & Parry, 2008). The secondary structure of a β-keratin molecule is characterized by a central domain of 32 amino acids forming four β-sheets (Fraser et al. 1972), each of which are four amino acids long and are separated from one another by β-turn regions of four amino acids (Fraser & Parry, 2008). The antiparallel β-sheet structure of the rod domain in β-keratins is maintained by hydrogen bonds (Fraser et al. 1972).

The current distinction between hard α- and β-keratins may be questionable considering the possibility of α-helical keratin filaments to be stretched into β-sheets (Toto et al. 1967; Kreplak et al. 2004). For example, based on limited observations, it has been shown that the secondary molecular structure of keratins can be modified by chemical or mechanical forces (Feughelman et al. 2002; Kreplak et al. 2002).

In addition to α- and β-keratins forming intracellular keratin filaments as part of the cytoskeleton, extracellular keratins (i.e. thread keratins TKα and TKβ) are produced and secreted by specialized epidermal cells in hagfishes, lampreys, teleost fishes and amphibians (Schaffeld & Schultess, 2006; Schaffeld et al. 2007). The nucleotide sequence of the genes encoding these thread keratins, as well as the amino acid sequence deduced from the mRNA, supports a close relationship of the extracellular thread keratins to the intracellular α-keratins. The thread keratins may have a function as a protective covering during the larval development of amphibians (Schaffeld & Schultess, 2006).

Keratins and the process of keratinization

Besides the syntheses of keratins and their assembly into keratin filaments and filament bundles, other processes of cell differentiation take place inside the epithelial cell during keratinization, such as the syntheses of KFAPs, the synthesis of intercellular cementing substances that are stored in membrane-coated vesicles, and signaling molecules, such as metalloproteinases, that control, regulate and synchronize the complex processes of keratinization. In addition, the process of keratinization is modulated and synchronized by environmental changes and cues (Eichner et al. 1984).

Signaling molecules influencing and regulating the keratinization

The matrix metalloproteinase 19 is expressed in the basal keratinocytes of the epidermis. This metalloproteinase affects numerous processes in the epithelial cells of the epidermis, such as adhesion, proliferation, migration and cell differentiation (Beck et al. 2007). A decrease in the synthesis of matrix metalloproteinase 19 results in a decrease of cell proliferation and in an enhancement of cell differentiation, which is accompanied by an altered synthesis of keratins, of KFAPs, such as involucrin and

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

loricrin, and of intercellular cementing substances such as sphingolipids, glucosylceramides and cholesterylsulfate.

The membrane-bound enzyme phosphatidylinositol 3-kinase is another regulator of differentiation of keratinocytes (Sayama et al. 2002). This enzyme mediates an adhesion signal in keratinocytes attached to the basement membrane suppressing the beginning of keratinization processes.

Keratins in different cell layers

Keratinizing keratinocytes of stratified non-cornifying or cornifying epithelia produce specific keratins that differ from those produced in the basal cells. The synthesis of K3 and K12 is characteristic of the keratinization in the stratified epithelium of the cornea, whereas the synthesis of K4 and K13 is characteristic of the keratinization of the stratified oral epithelium. The process of keratinization in the suprabasal cells of the stratified epidermis is defined by the production of K1 and K10. In parallel to the synthesis of those specific keratins, the keratinizing epithelial cells produce proteins of the keratin filament–matrix complex, such as profilaggrin, and of the cornified envelope, such as involucrin. Besides synthesizing proteins, the keratins produce special lipids, such as glucosylceramides, which are stored in membrane-coated vesicles and are later released into the intercellular space of the stratified epithelium via exocytosis. All of these processes are coordinated in parallel to the keratin synthesis.

Keratins and keratin filament-associated proteins

The KFAPs are non-filamentous structural proteins that are produced in keratinocytes of the *Stratum granulosum* of stratified keratinizing and cornifying epithelia and stored in keratohyalin granules (Jessen, 1970). In general, two types of KFAPs can be distinguished based on the structures to which the KFAPs contribute. The KFAPs (e.g. involucrin, loricrin) that are stored in a subset of keratohyalin granules (i.e. the L-granules) form the subcytolemmal cornified cell envelope. The KFAPs that are stored in another subset of keratohyalin granules (i.e. the F-granules) combine with keratin filaments to form the filament–matrix complex stabilizing the cytoskeleton of cornifying keratinocytes (Eckert et al. 2005). Different matrix-forming KFAPs are produced in the soft-keratinizing and cornifying keratinocytes of the interfollicular epidermis (e.g. profilaggrin/filaggrin) compared with those produced in the modified soft-keratinizing and cornifying cells of the hair follicle (e.g. trichohyalin, trichoplein) (Mack et al. 1993; Nishizawa et al. 2005). This corroborates that the soft keratinization and cornification processes in the interfollicular vs. those in the follicular epidermis are different.

The onset of the synthesis of KFAPs and keratins is coordinated. For example, the synthesis of the KFAP 'profilaggrin' starts shortly after the expression of K1 and K10 (Dale et al. 1985). This profilaggrin has a calcium-binding region in its N-terminal domain and is cleaved by the proteinase caspase-14 into several filaggrin molecules, which then bind to keratins and keratin filaments (Mack et al. 1993; Denecker et al. 2007). Caspase-14 is activated only in cornifying epithelia of terrestrial mammals but is not expressed in the nail corneocytes of humans (Denecker et al. 2008). The latter is not surprising, as Bragulla (1996) and Bragulla & Homberger (2007) have shown for the equine hoof that a *Stratum granulosum* with profilaggrin containing keratohyalingranula is absent in hard-cornifying stratified epithelia. The expression of caspase-14 may be activated by a transcription factor of the family of activator protein 1 (AP-1). During the embryonic development of the epidermis, the expression of caspase-14 coincides with the appearance of a *Stratum corneum* (Denecker et al. 2008). Hence, the observations so far indicate that profilaggrin/filaggrin (and caspase-14) are produced only in soft-keratinizing and -cornifying stratified epithelia (Bragulla & Budras, 1991). Filaggrin itself is a cationic protein (Mack et al. 1993). It is sandwiched between the rod domains of two keratin filaments with which it establishes ionic bonds (see 'ionic zipper model' of Mack et al. 1993).

Keratins and cell adhesive complexes

Keratin filaments are anchored to cell–cell adhesion complexes, such as desmosomes and hemidesmosomes, on the internal sides of cell membranes, thereby ensuring that keratinocytes remain connected to one another, transfer mechanical forces to neighboring cells and maintain the integrity of their cytoskeleton (Hanakawa et al. 2002). At the desmosomes, keratin filaments bind to the tail domain of desmosomal cadherin molecules, such as plakoglobin (Dusek et al. 2007), as well as plectin, periplakin, envoplakin and desmoplakin (Kazerounian et al. 2002), thereby anchoring the cytoskeleton to the cell membrane. Plakoglobin also acts as a signaling molecule within the 'apoptotic' signaling cascade during cornification, as cells that lacked plakoglobin are not able to undergo cornification (Dusek et al. 2007). The head domains of keratins bind to the C-terminal (i.e. the tail) of desmoplakins (Meng et al. 1998). For example, the head domains of the epidermal basic keratins K1, K2, K5 and K6 bind to the tail domain of desmoplakin 1, whereas the other basic keratins and all acidic keratins are not able to do so (Kouklis et al. 1994). The bonds of desmoplakin to keratins in the stratified-cornified epidermis are stronger than those in simple or non-cornified epithelia (Meng et al. 1998).

Keratins and the cornified envelope

During cornification, the keratinocyte cell membrane between the desmosomes is reinforced with special proteins

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

(e.g. involucrin, loricrin, etc.) to form the subcytolemmal cornified cell envelope (see section 'Keratins and keratin filament-associated proteins'). As a consequence, the keratin filaments can attach now not only to the desmosomes but also anywhere else on the cell wall of corneocytes. The keratin filaments do so via their head domains of basic keratins (Kirfel et al. 2003).

Membrane-coating granules and intercellular cementing substances

Keratinizing cells produce membrane-coating granules in addition to the keratins and KFAPs. These membrane-coating granules (i.e. 'Odland bodies', lamellar bodies) are produced in the cells of the *Stratum spinosum* and *Stratum granulosum* of the keratinizing epidermis (Elias et al. 1998). At the border between the *Stratum granulosum* and *Stratum corneum*, the content of the lamellar bodies (i.e. the intercellular cementing substances) is released prior to cornification into the intercellular spaces through exocytosis, which is accelerated in response to environmental stress, such as a disruption of the permeability barrier of the epidermis (Elias et al. 1998). This response is possible because the membrane-coating granules are aligned in the submembranous compartment of a keratinizing cell under the initially shallow grooves of the cell membrane. As the membrane-coating granules merge with the cell membrane and subsequently release their content through exocytosis into the shallow grooves of the cell membrane, these grooves deepen and form a tubulo-vesicular network (Menon & Elias, 1997).

Keratins and the process of cornification

Although epidermal cornification does occur in fishes, it is widespread among terrestrial vertebrates as an adaptation of the epidermis to withstand mechanical abrasion and trauma. The most mechanically resistant cornified epidermis, however, is also the least flexible. In order to maintain mobility, some segments of the terrestrial vertebrate epidermis form hinge regions of soft-cornified epidermis.

The cornification of the murine epidermis involves the proteolytic cleavage of the keratins in the cornifying cells (Roop et al. 1984). As a consequence, the MW of the keratins extracted from the *Stratum corneum* is about 1–2 kDa lower than that of the keratins in their precursor(s), the keratinizing cells (Woodcock-Mitchell et al. 1982). For example, in the murine epidermis the keratin with a MW of 67 kDa, which is an orthologue to K1 in humans, is proteolytically broken down into two different keratins of 62 and 64 kDa (Roop et al. 1984). This breakdown is likely to affect the head and tail domains of the keratin and can explain the fact that anti-keratin antibodies do not cross-react with the keratin filaments in cornified cells.

Cornification involves the cross-linking of keratin filaments and KFAPs via disulfide bonds. In the cornified cells, i.e. the corneocytes, of the hair, the hard keratins are oxidized and cross-linked via disulfide bonds, whereas these keratins exist in their non-oxidized form in the living trichocytes of hair, where they are not cross-linked (Heid et al. 1986).

Programmed cell death of keratinocytes

The process of cornification involves the programmed death of the keratinocytes (Houben et al. 2007). This epidermal programmed cell death is different from the programmed cell death in apoptosis because the extra- and intracellular signaling cascade that is characteristic of apoptosis is not activated (Lippens et al. 2005; Denecker et al. 2007). The cornification of keratinocytes also differs from apoptosis because the terminally differentiated cells are dead but intact and not cell fragments as in apoptotic bodies (Weil et al. 1999). The only genuine apoptosis in the epidermis occurs in so-called sunburn cells, which are basal cells that are damaged by UVB rays (Denecker et al. 2007). Apoptosis can take place at any stage of cell differentiation but the process of cornification can start only after a cell has already gone through a certain differentiation. Hence, cornification is not a type of apoptosis (Denecker et al. 2008). However, apoptotic enzymes, such as caspase-3, are activated in cornifying cells in the transition zone between the granular layer and the layer of cornified cells (Weil et al. 1999). The enzyme caspase-3 may be involved in the dismantling of the cell nucleus and of the organelles (Weil et al. 1999). The apoptotic protease caspase-14, which is also activated in the cornifying keratinocytes of the epidermis (Demerjian et al. 2008), is not expressed in the keratinizing but not cornifying keratinocytes of the oral epithelium (Lippens et al. 2000). Caspase-14 is not activated in the cornifying cells of the nail matrix (Jäger et al. 2007), indicating differences in the processes of soft vs. hard keratinization and cornification.

Desquamation of corneocytes

The desquamation rate and the *de-novo* production of corneocytes need to be delicately balanced to maintain a constant thickness of the *Stratum corneum* (Egelrud, 2000). The desquamation of individual corneocytes or of small aggregates of corneocytes requires the controlled enzymatic destruction of the cell–cell adhesive structures, such as the intercellular glyco- and sphingolipids, as well as the corneodesmosomes (Houben et al. 2007). The first step in the desquamation process in the *Stratum corneum* involves the endoglycosidase activity of heparanase 1 (Bernard et al. 2001). The activity of the enzyme epidermal ceramidase generates free fatty acids from the ceramides, which are constituents of the intercellular lipids connecting

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

the corneocytes of the *Stratum corneum* (Houben et al. 2008). Other enzymes, such as the serine proteases of the kallikrein family, are also involved in the processes of desquamation (Brattsand et al. 2005). These enzymes, e.g. the *Stratum corneum* tryptic enzyme and the *Stratum* chymotryptic enzyme, are mostly confined to the epidermis. They are produced as inactive pro-enzymes in the living cells of the keratinizing epidermis and are activated in the corneocytes of the *Stratum corneum* (Ekholm et al. 2000). Similarly, the enzyme cathepsin D, which is able to break down keratins, is synthesized as an inactive pro-enzyme in the lysosomes of differentiating keratinocytes in the human epidermis and activated in the corneocytes (Horikoshi et al. 1998). In the acid environment of the *Stratum corneum*, the active form of this enzyme cathepsin D break downs keratins (Horikoshi et al. 1999). The desquamated corneocytes and the keratins therein are broken down in the environment by keratinases (i.e. specific enzymes necessary for the proteolysis of the keratin filament network). These keratinases are enzymes produced by fungi and bacteria (Scott & Untereiner, 2004; Gupta & Ramnani, 2006; Thys & Brandelli, 2006).

Mechanical properties of epithelia

The mechanical properties of the different types of epithelia are determined by the types and amount of keratins produced in the epithelial cells. The amino acid sequence of a keratin influences the properties and functions of the keratin filament that it forms (Roop et al. 1984). Different keratin filaments impart different resilience characteristics to different epithelial tissues, and this stress resilience is crucial for the functions of epithelial cells (Pekny & Lane, 2007). Keratin filaments are not simply rigid entities bearing mechanical stresses but are also remarkably dynamic structures based on their molecular (i.e. amino acid sequence) and supramolecular structure (e.g. heterodimer, tetramer, etc.) (Steinert, 2001). The mechanical properties of keratin filament subunits, such as heterodimers and tetramers, change with the pH and with the ionic strength of the local environment in the cytoplasm (Yamada et al. 2002). The keratin filaments, which are anchored in the cell adhesion complexes, probably provide the ability to sense mechanical stress that initiates mechanical stress-specific signal cascades in epithelial cells subjected to cell deformation, such as stretching (Pekny & Lane, 2007).

The mechanical properties of keratinocytes and corneocytes are influenced not only by the intrinsic properties of their keratin filaments but also by the properties of the KFAPs that are cross-linked with the keratin filaments through disulfide bonds (Fudge & Gosline, 2004). These disulfide bonds are formed by covalently bonding cysteine residues of keratins and of KFAPs in the *Stratum granulosum* of the epidermis (Fukuyama & Epstein, 1969). In addition, the keratins and keratin filaments are cross-linked via disulfide bonds to the proteins of the cellular envelope, such as involucrin, locricrin and periplakin (Ruhrberg et al. 1997). All of these intracellular factors contribute to the mechanical properties of each keratinized or keratinized and cornified cells.

On the tissue level, the mechanical properties of epithelia are determined by the types and numbers of cell-to-cell contacts, such as tight junctions and desmosomes, which interconnect the individual epithelial cells. In addition, the shapes of the interfaces between the epithelial cells (whether smooth, ridged or with finger-like projections) influence the mechanically stable connection of epithelial cells. In particular, the interdigitating finger-like projections of epithelial cells increase the contact surfaces between epithelial cells and, thereby, increase the area for cell-to-cell contacts. For example, the three-dimensional shapes of epithelial cells in mechanically loaded stratified epithelia, such as the epidermis and the mucosa of the oral cavity, are characterized by numerous cell projections and ridges on the cell surface.

The epithelial cells in the upper layers of stratified epithelia are connected by intercellular substances, such as glycosylceramides and sphingolipids, in a similar fashion as bricks are held together by mortar in a brick wall. The type and amount of these intercellular substances influence the mechanical properties of stratified epithelia and establish a semi-permeable barrier, such as that of the cornified epidermis (Lillywhite, 2006).

Keratins as products of a multi-gene family of intermediate filament genes

Keratins are encoded by genes that possess a similar nucleotide sequence (Baden & Goldsmith, 1972; Fuchs et al. 1987). A gene encoding a specific keratin is labeled *KRT*, followed by the number designated to the specific keratin, e.g. *KRT10* is the gene of the human keratin K10 (Hesse et al. 2001, 2004; Schweizer et al. 2006).

Structure and location of keratin genes

In mammals, each of the keratins is encoded by a single gene (Krieg et al. 1985). Hence, the various keratins are the genuine products of gene transcription and translation and not of post-translational proteolysis of precursor molecules (Moll et al. 1982). However, as an exception to this general rule, in humans, bovines and murids, the isoforms of K6 are encoded by three genes (Rogers et al. 2005). The orthologue K6 isoforms have amino acid sequences that are more than 95% identical in these species (Wang et al. 2003). The mRNAs of *KRT6a* and *KRT6h* are translated into different isoforms (Rogers et al. 2005) as a result of either alternative splicing or of different post-translational modifications. K6c and K6d are the

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

products of *KRT6a*, and the keratins K6e and K6f are the products of *KRT6h* (Rogers et al. 2005). The subfamily of genes encoding K6 isoforms has developed from genetic modifications that were independently acquired in various species (Navarro et al. 1995).

In tetrapods, the number of genes encoding acidic keratins is almost equal to that of genes encoding basic keratins, whereas in teleost fishes, the number of genes encoding the acidic keratins is three times the number of genes encoding the basic keratins (Schaffeld et al. 2007). The number of genes encoding keratins varies in mammals, e.g. it is higher in opossums than in humans (Zimek & Weber, 2006).

In humans, keratins are encoded in 54 genes and these genes are clustered on two chromosomes (Bowden, 2005; Schweizer et al. 2006). The acidic type I keratin genes are clustered on chromosome 17 and the basic type II keratin genes are clustered on chromosome 12 (Bowden et al. 1998; Hesse et al. 2001; Coulombe & Omary, 2002). The keratins K25–K28, which are specific for the cornifying cells of the inner root sheath of a hair follicle, are encoded by a cluster of genes on chromosome 17 (Bawden et al. 2001; Langbein et al. 2006). Similarly, the keratins K71–K74, specific for the inner root sheath of the human hair follicle, are encoded by genes arranged in a cluster on chromosome 12 (Langbein et al. 2003).

The gene encoding the acidic type I keratin K18 is an exception, as it is located on chromosome 12 next to the type II keratin genes, including the gene for K8, which is the partner of K18 (Waseem et al. 1990). This fact led to the hypothesis that *KRT8* and *KRT18* are close to ancestral keratin genes (Waseem et al. 1990). Additional support for this hypothesis comes from the fact that the sequences of these two genes are similar (Owens & Lane, 2003). K8 and K18 are found in all gnathostome vertebrates (Schaffeld et al. 2007). In humans, a phylogenetic analysis of the genes of the type I keratins (i.e. acidic soft, acidic hard, acidic inner root sheath and related keratins) suggested that the acidic keratin genes related to the inner root sheath originated earlier during evolution than the genes for epidermal keratin or hair keratin (Bawden et al. 2001). The criteria for establishing relationships among keratin genes are (i) a high sequence similarity (> 70%) of the region encoding the rod domain, (ii) the same orientation of the genes on the chromosome (Bowden, 2005) and (iii) the position of the keratin genes in, for example, the same cluster (Zimek & Weber, 2006).

The genes of the two types of keratins (i.e. the acidic and basic keratins) are also clustered on two chromosomes in other mammals, such as the rat, mouse (on chromosomes 11 and 15), chimpanzee, dog, cow and sheep (Hesse et al. 2004; Lu et al. 2006; Makar et al. 2007). The fact that keratin genes are clustered on only two chromosomes supports the hypothesis that tandem duplications have occurred during the evolution of keratin genes (Molloy et al. 1982; Coulombe & Omary, 2002). The genes encoding β-keratins of feathers are also arranged in clusters (Molloy et al. 1982). The nucleotide sequences of keratin-like genes have been identified in lower eukaryotes (e.g. yeast, species *Saccharomyces*) and these genes are encoding intermediate filament proteins (Fuchs & Marchuk, 1983; Fuchs et al. 1983).

Genes encoding proteins, which form intermediate filaments such as keratin filaments, consist of seven or eight exons (Bader et al. 1986). The basic type II keratin genes consist of seven exons, whereas the acidic type I keratin genes consist of eight exons (Makar et al. 2007), with the exception of the gene encoding the acidic keratin K19 with only five introns (Bader et al. 1986). Coulombe et al. (2004) explained the significance of the large number of keratin genes by the need for adaptable viscoelastic properties of epithelial cells through the production of different keratins forming keratin filaments of varying mechanical properties.

Mutations of keratin genes

Mutations of keratin genes may be responsible for the development of new keratins. For example, in the mouse, a segment deletion in the acidic hair keratin gene affects the amino acid sequence in the H2 subdomain in the tail domain of the keratins (Bertolino et al. 1990). Changes of the amino acid sequence of keratins due to mutations may have severe consequences for the assembly of keratin filaments (Steinert, 2001). Szeverenyi et al. (2008) compiled the information concerning the mutations of keratin genes in humans.

Duplications of keratin genes in combination with frame-shift mutations result in the formation of pseudogenes (Hesse et al. 2004). In humans, 87% of all keratin pseudogenes have similar sequences as *KRT8* and *KRT18* but they are scattered on various chromosomes (Hesse et al. 2001; Coulombe & Omary, 2002), possibly through crossing-overs or translocations. A total of 138 human pseudogenes are related to *KRT8* or *KRT18* (Hesse et al. 2004). According to Hesse et al. (2001), this observation supports the inference that *KRT8* and *KRT18* have a long history, i.e. may have been among the first keratin genes in ancestral vertebrates. In contrast, if these pseudogenes have similar sequences as the functional genes in humans, they may have a recent history, i.e. they may have been duplicated recently in the primate lineage. Pseudogenes related to other keratin genes, such as *KRT14*, *KRT16* and *KRT17*, exist (Hesse et al. 2001).

Mutations of keratin genes are responsible for a number of disorders in the human and murine skin (Pekny & Lane, 2007). In the skin disorder 'epidermis bullosa simplex', which involves a breakdown of basal cells in stratified epithelia, either the *KRT5* or *KRT14* is defect. In case of a mutation of *KRT14*, the type I keratin K15 can

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

compensate for K14 to combine with the type II keratin K5, the usual partner of K14; hence, the symptoms are less severe than when *KRT5* is mutated, for which there is no compensating keratin (Steinert, 2001; Pekny & Lane, 2007). The same principle applies to K6 and its partners K16/17 (Coulombe et al. 2004). *KRT1* and *KRT10*, characteristic of keratinizing suprabasal cells in the stratified epidermis, are mutated in the skin disorder epidermolytic hyperkeratosis (Pekny & Lane, 2007). The disease 'white sponge naevus', which affects the oral and genital stratified epithelia, is caused by mutations of *KRT4* and *KRT13* (Pekny & Lane, 2007).

Signals influencing and regulating keratin gene expression

Little is known about the mechanisms that initiate and regulate the transcription and translation of the keratin genes *in vivo* (Magin et al. 2007). Intracellular signaling pathways for gene transcription and translation are mediated by transcription factors and other signaling molecules. The keratin genes (e.g. *KRT1*, *KRT5*, *KRT6*, *KRT8*, *KRT18* and *KRT19*) and the KFAP genes contain binding sites for transcription factors that are produced in keratinocytes in a spatiotemporally coordinated fashion (Eckert et al. 1997). In general, gene activation and expression can be regulated at various levels such as (first) at the level of the transcription of the genetic information into mRNA followed (second) by mRNA translation into the actual keratin protein. The first level of gene expression requires the coordinated expression of transcription factors, which bind to specific DNA sequence elements within the target genes, such as the promoter region, and initiate the translation of the genetic information into mRNA. The activation of a gene expression may require more than one transcription factor, i.e. a combinatorial regulation of gene expression (Eckert et al. 1997).

Within keratinocytes, general transcription factors belonging to particular transcription factor families [e.g. AP-1 and AP-2, signaling protein-1, ets (nuclear factor binding to DNA produced by the retrovirus 'v-ets' in chickens), POU (acronym derived from the first letter of the three transcription factors pituitary-specific factor 1, octamer transcription factor and transcription factor 'Unc-86'), CCAAT-enhancer binding protein, interferon-γ, nuclear factor kappa B (nuclear factor kappa-light chain of activated B cells) and others] bind to specific DNA sequences (e.g. promoter region, response elements, consensus sequences or transcription factor-binding site) but different transcription factors within a family are expressed in different locations within the keratinocytes and the stratified epidermis (Eckert et al. 1997; Komine et al. 2000). For example, the transcription factor signaling protein-1 activates *KRT3* transcription in corneal epithelial cells, whereas the factor AP-2 suppresses the transcription of this gene (Sun & Lavker, 2004). In the keratinocytes of the human hair follicle in particular, the expression of keratin genes is also regulated by various transcription factors (Cribier et al. 2004). For example, the expression of the homeoprotein 13 (HOXC13, i.e. the transcription factor encoded in the 13th homeobox gene of cluster C) coincides with the expression of the acidic hair-specific K35, and LEF1 binds to the promoter region of the genes encoding the hair-specific acidic K31, K32, K35 and K37 (Cribier et al. 2004; Jave-Suarez et al. 2004). The binding site for the transcription factor LEF1 is a special nucleotide sequence in the promoter region of genes encoding hair-specific keratins (Rogers, 2004). The expression of the hair-specific keratin mHa3 in the mouse is promoted in the suprabasal cells of the matrix of pelage hairs, vibrissae and nails by the transcription factor 'winged-helix nude' (Whn) (Meier et al. 1999). This transcription factor is missing in nude mice and, as a consequence, mHa3 (but not mHa1, mHa2 or mHa4) is absent from brittle pelage hair, whereas it is still expressed in vibrissae and nails (Meier et al. 1999). This means that the same gene encoding a specific hair keratin is activated differently in the epidermal cells forming pelage hairs compared with those that form the vibrissae and nails. Another example of the coordinated action of transcription factors shows that two (or more) transcription factors may act simultaneously to initiate keratin gene transcription. For example, *KRT1* contains binding sites for AP-1 transcription factors and for Vitamin D acting as a transcription factor (Eckert et al. 1997). The principle that keratin genes are activated by transcription factors appears to be of general validity for tetrapods, as it is observed not only in several mammals but also in the lissamphibian *Xenopus laevis* (Eckert et al. 1997). Actually, the binding site for transcription factor AP-2 is present in *KRT5* and *KRT14* in mammals as well as in the gene encoding a keratin of 63 kDa in *Xenopus laevis* (Franz & Franke, 1986). The transcription factor p63 in its isoform Tap63α initiates the stratification of simple epithelia that differentiate into stratified ones (Koster & Roop, 2004).

Keratins themselves can also induce the expression of keratin genes. In fibrocytes, the forced expression of a foreign type II epidermal keratin can induce the expression of an endogenous type I epidermal keratin but the forced expression of a foreign type I keratin does not induce the expression of a type II keratin (Giudice & Fuchs, 1987).

The second level of regulation of keratin gene expression concerns the mRNA, which may or may not be translated into the keratin protein. For example, rising levels of *KRT75* transcripts are not immediately followed by an increase in K75 and hence the expression of the keratin lags behind the increase in mRNA (Roh et al. 2004).

Extracellular signaling pathways inducing keratin gene expression are mediated by growth factors and various chemical and environmental 'factors' by binding to membrane-bound receptors, thereby activating intracellular

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

signaling molecules but the details of the signaling cascade are currently only poorly understood. Extracellular molecules of the family of proteins encoded in the wingless gene and the Int-1 gene ('Wnt' signaling pathway) influence the patterning and differentiation of the epidermis (Troy & Turksen, 2005). The activation of the 'Wnt' signaling pathway prevents the degradation of β-catenin, which interacts with LEF proteins and activates keratin genes (Dusek et al. 2007). This supports the observation that β-catenin stimulates hair follicle proliferation (Dusek et al. 2007). The same signaling molecules (i.e. β-catenin and LEF) promote cell proliferation and differentiation in a co-culture of keratinocyte stem cells from the hair follicle and from fibroblasts of hair papilla (Roh et al. 2004). The lack of hair-inducing signals leads to the 'default' epidermal type of terminal differentiation, i.e. the soft cornification, which involves the production of the keratins K1, K2 and K10 (Roh et al. 2004).

Various extracellular growth factors, such as β-cellulin (Schneider et al. 2007), induce keratin gene expression when attaching to their cell membrane-bound receptors of keratinocytes, such as epidermal growth factor receptor (Schneider et al. 2007) or tumor necrosis factor receptor 1 (Komine et al. 2000). For example, tumor necrosis factor-α induces the expression of the basic K6 (Freedberg et al. 2001). The binding of tumor necrosis factor-α to its receptor induces the translocation of the nuclear factor kappa B from the cytoplasm to the nucleoplasm, which finally induces the differentiation of the keratinocyte (Sayama et al. 2006). Interferon-γ activates the promoter region of *KRT17* (Komine et al. 2000; Freedberg et al. 2001). The transmissible growth factor β induces the synthesis of K5 and K14 (Freedberg et al. 2001). Interleukin-1 activates keratinocytes that express K6, K16 and K17 (Freedberg et al. 2001). The epidermal growth factor and the transforming growth factor α induce the expression of K6 and K16 in the suprabasal cells of interfollicular epidermis (Komine et al. 2000).

Oxygen can also act as an extracellular signal. The epidermis experiences higher oxygen levels than tissues of internal organs and is able to obtain oxygen from the air (Ngo et al. 2007). High oxygen levels induce the differentiation of epidermal cells in culture, whereas an oxygen level below 5% induces cell proliferation. Epithelial tumor cells are more susceptible to low oxygen levels than normal epithelial cells (Ngo et al. 2007).

Nitric oxide regulates the synthesis of gene products involved in keratinocyte differentiation (Gallala et al. 2004). For example, nitric oxide is correlated with an increase of *KRT14* transcripts, and a depletion of nitric oxide is correlated with a decrease of these transcripts and an increase in *KRT1* and *KRT10* transcripts (Gallala et al. 2004).

The extracellular level of calcium also influences keratin gene expression. An increase of calcium in the culture medium for keratinocytes is correlated with an increase of *KRT10* transcripts and transcripts of the gene for the KFAP profilaggrin (see section 'Keratins and keratin filament-associated proteins'), both of which are indicative of keratinization (Gallala et al. 2004). The level of calcium in the intercellular space does not affect the level of *KRT14* transcripts, which is indicative of undifferentiated keratinocytes in the basal layer (Gallala et al. 2004).

Zinc chloride influences the production of K10 in epithelial cell cultures. The production of K10 increases in parallel with the concentration of zinc chloride in the culture medium (Paramio et al. 1999).

Retinoic acid (i.e. a derivative of Vitamin A) has a dose-dependent effect on keratinocytes in cell cultures, indicating keratin gene activation or suppression (Kopan et al. 1987). Under the effect of retinoic acid, the amount of K5, K6, K14 and K17 decreases, and the amount of K13 and K19 increases (Gilfix & Eckert, 1985).

Certain hormones are also able to induce the expression of particular keratin genes. For example, the promoter region of the gene encoding the acidic hair-specific keratin K37 contains androgen receptor-binding sites (Jave-Suarez et al. 2004). In humans, K37 is expressed only in the cornifying cells of the medulla of terminal hairs of the beard, pubis and axilla in both sexes but in chimpanzees the orthologous K37 is found in the cornifying cells of the medulla of all body hairs (Jave-Suarez et al. 2004). The genes of other acidic hair-specific keratins do not have this androgen-binding site (Jave-Suarez et al. 2004).

Water loss, which increases the intracellular osmotic pressure, also indirectly influences keratin gene expression. An increase in the intracellular pressure in the keratinocytes is correlated with an increase in the heat shock proteins hsp27 and hsp70 transcripts, as well as the actual heat shock proteins (Garmyn et al. 2001). This increase of heat shock proteins is needed to maintain the regular function as chaperones, ensuring the appropriate tertiary and quaternary molecular structure of keratins (Garmyn et al. 2001). The function of these heat shock proteins depends on their phosphorylation by the enzyme p38 mitogen-activated protein kinase (Garmyn et al. 2001). Heat shock proteins, such as hsp70, bind to K8 and K18 in hepatocytes (Paramio et al. 1999).

Mechanical forces also act as external signals that can influence keratin gene expression. Keratins and keratin filaments can also act as sensors of mechanical force similar to piezoelectric and pyroelectric elements (Hoath et al. 1990). Different keratin filaments impart different resilience characteristics in different epithelial tissues, and this stress resilience is crucial for the functioning of epithelial cells. It is likely that the ability to sense mechanical stress initiates mechanical stress-specific signaling cascades in epithelial cells subjected to cell deformation, such as stretching (Hutton et al. 1998; Pekny & Lane, 2007). Mechanical stretching of keratinocytes activates mitogen-activated protein kinases, and these enzymes help to shape keratin filaments and filament bundles. Mechanical pressure also

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

influences the synthesis of keratins (Görmar et al. 1990). For example, mechanical pressure triggers the phosphorylation of hsp27, which binds to keratins and changes the affinity of these proteins to the assembled keratin filaments (Hofmann et al. 2004). Further studies on the influences of mechanical forces on simple or stratified epithelia, such as endothelia or epidermis, may explain the effects of blood pressure on the vessel walls or the formation of calluses in the epidermis.

Conclusions and perspectives

Research on keratins, keratin filaments and cornified structures began about 80 years ago. Cornified horse hairs were used to study the molecular structure of keratins by X-ray diffraction because the keratins in the horse hair were assumed to be arranged in a regular orientation similar to that of the ions in salt crystals. The X-ray diffraction pattern of the keratins in the hair is actually quite regular and depends on the orientation of the X-ray beam relative to the longitudinal axis of the hair and its keratin filaments. X-ray diffraction studies revealed the α-helical structure or β-sheet structure of the main part, i.e. the rod domain, of a keratin molecule. According to the structure of the rod domain of keratins, two main types of keratins are distinguished, i.e. the α-keratins and β-keratins. The β-keratins are produced only in the keratinizing and cornifying cells of skin modifications in sauropsids.

As the next step in understanding the structure of keratins, the proteins of cornified organs, such as horns, claws and hairs, have been extracted through the use of various solvents with different pH and ionic strength, and of denaturing agents, such as urea. These studies, using the methods of fractionated extraction, revealed the physicochemical properties of the proteins of cornified organs. Some of the extracted proteins were able to reconstitute filamentous structures, and transmission electron microscopic studies revealed that these reconstructed filaments closely resembled keratin filaments in epithelial cells. The proteins extracted from cornified structures (such as the human epidermis with a MW of about 40–70 kDa, a pI between 5.0 and 7.0, and, most importantly, the ability to spontaneously reconstitute filaments) were summarized as keratins forming keratin filaments. Finally, keratins cannot be dissolved in acidic watery solutions and are quite resistant to proteolytic degradation by proteases.

Based on the extracted keratins, antibodies directed against these proteins were produced by the injection of keratins in the peritoneal cavity of mice or guinea pigs. The polyclonal antibodies produced in these animals were used in immunohistochemistry to study the keratins in various tissues in different mammalian species. These studies revealed that keratins are usually produced only in epithelial tissues. The keratins produced in the living cells of stratified epithelia are the precursors ('prekeratins') of the keratins in the cornified cells of the *Stratum corneum* of the epidermis and of epidermal modifications, such as hairs, nails and horns.

The development of the method of one- and two-dimensional electrophoresis allowed the separation of the extracted keratins of epithelial tissues and of distinct layers in stratified epithelia. The results of these studies revealed two distinct types of keratins, the acidic keratins and basic keratins. The isolated and purified keratin proteins were used for experiments to reconstitute keratin filaments *in vitro*, and these experiments showed that equimolar amounts of acidic and basic keratins are necessary to form filaments. In addition, these experiments, in combination with immunohistochemistry, revealed that certain epithelial cells produce tissue-specific combinations of acidic and basic keratins that form the main constituents of the cytoskeleton in these cells. Furthermore, the basal and suprabasal cells of the stratified epidermis produce different acidic and basic keratins. A first catalog summarizing all keratins known up to that date and their physicochemical properties as well as their tissue distribution was published by Moll et al. (1982).

Based on the specific keratins produced in the suprabasal cells of the epidermis, the technical term of 'keratinization' was coined as a special process of differentiation of stratified epithelia. This keratinization was distinguished from the differentiation processes of 'cornification' in the epithelia of hairs, nails and horn based on the resulting hard, nearly stiff properties of these structures. Meanwhile, the results of the ongoing research concerning keratins, keratinization and cornification have shown that the stratified epidermis as well as the stratified epithelia of various mucosae, such as the oral and vaginal mucosa, cornify and form a *Stratum corneum* that consists of dead cornified cells connected by circumscript cell membrane modifications, such as corneodesmosomes, and by intercellular cementing substances, such as sphingolipids and glucosylceramides. The differences between the cornified epidermis and the cornified epidermal modifications (e.g. the hair cortex, nail plate and the cortex of a horn) are, among others, the types of keratins that are produced in the keratinizing epithelial cells prior to cornification. To distinguish the cornified epidermis and the cornified epidermal modifications, the differentiation of the epidermal cells can be summarized as 'soft keratinization and cornification' and the differentiation of the cells forming special cornified structures can be summarized as 'hard keratinization and cornification'. These terms reflect the different types of keratins produced in the soft-keratinizing and cornifying cells of the epidermis (i.e. the 'soft' keratins K1, K2, K9 and K10) compared with those produced in the hard-keratinizing and cornifying cells of the modified epidermal cells (e.g. the 'hard' keratins K31, K33, K81 and K85). In addition, a special type of soft keratinization and cornification in the cells of the inner root sheath of human, murine and ovine hair follicles is characterized by the

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

production of the specific keratins K25–K28 and K71–K74 in humans or of the orthologue keratins in mouse and sheep.

The terms 'soft keratinization and cornification' and 'hard keratinization and cornification' are also useful for demonstrating that, even in the epidermal modifications (e.g. hairs, fingernails and hooves), parts of these organs (such as the medulla of hairs or the periople of fingernails and hooves) are produced by the processes of soft keratinization and cornification. The processes of soft keratinization and cornification in the epidermis are characterized by the synthesis of KFAPs, such as profilaggrin/filaggrin, in the keratinizing epidermal cells, and these KFAPs are stored in basophilic keratohyalin granules prior to the soft keratinization and cornification. Similarly, special KFAPs, such as trichohyalin, are produced in the modified soft-keratinizing cells of the inner root sheath and of the medulla of hairs prior to the special soft keratinization and cornification. In hard keratinization and cornification, in contrast, different KFAPs are produced, which are not stored in granules prior to the hard cornification of the special epidermal cells forming the cortex of hairs or the plate of the fingernail. The KFAPs in hard-keratinizing and cornifying epithelia of the hair or hoof need to be studied in detail to understand dyskeratotic diseases in these organs. Hard and soft cornification of epithelial cells are based on the processes of keratinization, i.e. the synthesis of specific keratins in the keratinizing suprabasal cells. Keratinized cells of cornifying epithelia, such as the epidermis or the plate of the fingernail, undergo a programmed cell death and become corneocytes, i.e. dead but intact cells, which form the *Stratum corneum* of the cornified epithelium. The programmed cell death in hard-keratinizing and cornifying epithelia differs from that in soft-keratinizing and cornifying epithelia as indicated by the different enzymes that are activated to initiate and promote the programmed cell death (see section 'Programmed cell death of keratinocytes').

Research on keratins also reveals that all stratified epithelia (such as the epithelium of the oral mucosa, cornea or forestomach) keratinize because the suprabasal cells produce special keratins in certain combinations (e.g. K3 and K12 or K4 and K13), which are not produced in simple, non-stratified epithelia. Therefore, we conclude that all stratified epithelia are keratinized in comparison to simple epithelia. However, even in simple epithelia, some specialized cells produce some keratins that are different from those of the non-differentiated cells. The additional keratins in the specialized cells may be related to the functions of these cells, such as active vs. inactive secretory cells. This assumption is corroborated by studies concerning the turnover rate of keratin filaments and of keratins in a dynamic cytoskeleton.

The functions of keratins and keratin filaments have also been studied by transfecting epithelial cells in cell culture. Vectors, such as viruses, have been used to introduce genes encoding keratins and a fluorescent protein that is directly attached to the keratin protein. After the introduced keratin gene is activated, the transfected epithelial cells in culture are green fuorescent. This fluorescence can be destroyed using an argon/krypton laser, i.e. 'photobleaching' (Windoffer et al. 2004), without destroying the cells. The cells then continue to produce fluorescent keratins and therefore these epithelial cells are fluorescent again. These experiments reveal that keratins are produced in the periphery of epithelial cells and transported centripetally along keratin filaments and microfilaments to be incorporated into existing keratin filaments or keratins from degraded keratin filaments are transported centrifugally along microtubules. Furthermore, these studies reveal the processes of reconfiguration and interaction of keratins and keratin filaments with microfilaments and microtubules, such as in the postmitotic reconstruction of the cytoskeleton.

Advances in molecular biology, such as the sequencing of the amino acid chain of keratins and of the nucleotide sequences of the genes encoding these keratins used for polymerase chain reaction and *in-situ* hybridization, provided insight into the structures and functions of keratins and of keratin filaments. Based on the results of these studies, the secondary structure (i.e. the domains and subdomains) of keratins was elucidated, and the formation of subunits of keratin filaments, such as heterodimers and tetramers, has been demonstrated, whereas the definite arrangement of these subunits, which form keratin filaments, is still under debate. These molecular biological studies also revealed other than mechanical functions of keratins and keratin filaments, such as functions in signaling or cell transport. For example, keratins can bind signaling molecules, thereby promoting or interrupting internal or external signaling cascades.

Analyses of the amino acid sequence of keratins, as well as of keratin gene sequences, provided insight into various genetic disorders affecting epithelia, such as white spongy naevus of the oral mucosa or epidermolytic bullosa simplex dyskeratosis, and reveal the involvement of keratin genes. Screening of the human genome has since revealed new keratin genes. In mammals, the keratin genes are clustered on two chromosomes, e.g. human chromosomes 12 and 17, and certain genes encoding a functional group of keratins, such as the keratins of the inner root sheath, are arranged in a subcluster within the cluster of keratin genes. This specific organization of keratin genes in various mammals fostered a better understanding of the evolution of keratin genes.

In the future, similar research efforts on the genes encoding β-keratins in sauropsids are necessary. Because of the interest in curing skin diseases in humans, dermatological research concerning keratins and keratin filaments is far ahead of similar research in veterinary dermatology and basic biology. The cultivation of soft-keratinizing and cornifying epidermal cells for treatment of burned skin is one of the most advanced applications of research on

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

keratins, keratinization and cornification. In contrast, the cultivation of hard-keratinizing and cornifying cells (e.g. of hair cortex) has only just begun. Similarly, the special hard keratins that form the cytoskeleton of the hard-keratinized and cornified skin modification, such as the hair cortex or the fingernail plate, have only been studied in the bovine hoof. Studies in the equine hoof similar to those concerning the hard keratins in the human fingernail, as well as concerning the intra- and extracellular signaling molecules influencing keratin gene expression, could provide insight into the pathologic processes of the equine hoof afflicted by laminitis.

One of the most interesting problems concerns the evolution of the different types of α-keratins in vertebrates as compared with the evolution of the various types of β-keratins in reptiles and birds. Recently, the analyses of genes encoding feather β-keratins in 18 avian species revealed sequence variations in the protein-coding region of the genes within and among avian taxa (Glenn et al. 2008). A better understanding of these processes may shed light on the development and evolution of hairs and feathers.

Acknowledgements

We thank our editors Matthew Vickaryous and Jean-Yves Sire for inviting us to write this review and for their patience, suggestions and support during the preparation of our manuscript. Brooke A. Hopkins, Michelle L. Osborn and Margaret Jensen-Shoats proofread the manuscript and provided suggestions and comments for its improvement. Sigrid N. Hamilton provided logistical support.

References

Achtstätter T, Moll R, Moore B, Franke WW (1985) Cytokeratin polypeptide patterns of different epithelia of the human male urogenital tract: Immunofluorescence and gel electrophoretic studies. *J Histochem Cytochem* **33**, 415–426.

Aebi U, Fowler WE, Rew P, Sun TT (1983) The fibrillar substructure of keratin filaments unraveled. *J Cell Biol* **97**, 1131–1143.

Ahvazi B, Boeshans KM, Idler W, Baxa U, Steinert PM (2003) Roles of calcium ions in the activation and activity of the transglutaminase 3 enzyme. *J Biol Chem* **278**, 23834–23841.

Alibardi L, Toni M (2004) Immuno-cross reactivity of transglutaminase and cornification marker proteins in the epidermis of vertebrates suggests common processes of soft cornification across species. *Mol Dev Evol* **302**, 526–549.

Alibardi L, Maurizil MG, Taddei C (2000) Immunocytochemical and electrophoretic distribution of cytokeratins in the regenerating epidermis of the lizard podarcis muralis. *J Morphol* **246**, 179–191.

Alonso L, Fuchs E (2003) Stem cells of the skin epithelium. *Proc Natl Acad Sci USA* **100**(Suppl. 1), 11830–11835.

Aoki N, Sawada S, Rogers MA, et al. (2001) A novel type II cytokeratin, mK6irs, is expressed in the Huxley and Henle layers of the mouse inner root sheath. *J Invest Dermatol* **116**, 359–365.

Baden HP, Goldsmith LA (1972) The structural protein of epidermis. *J Invest Dermatol* **59**, 66–76.

Baden HP, Lee LD, Kubilus J (1976) The fibrous proteins of stratum corneum. *J Invest Dermatol* **67**, 573–576.

Bader BL, Magin TM, Hatzfeld M, Franke WW (1986) Amino acid sequence and gene organization of cytokeratin no. 19, an exceptional tail-less intermediate filament protein. *EMBO J* **5**, 1865–1875.

Banks WJ (1993) *Applied Veterinary Histology*, 3rd edn. pp. 48–67. St. Louis: Mosby.

Banks-Schlegel SP (1982) Keratin alterations during embryonic epidermal differentiation: a presage of adult epidermal maturation. *J Cell Biol* **93**, 551–559.

Bawden CS, McLaughlan C, Nesci A, Rogers G (2001) A unique type I keratin intermediate filament gene family is abundantly expressed in the inner root sheaths of sheep and human hair follicles. *J Invest Dermatol* **116**, 157–166.

Beck IM, Muller M, Mentlein R, et al. (2007) Matrix metalloproteinase-19 expression in keratinocytes is repressed by transcription factors Tst-1 and Skn-1a: Implications for keratinocyte differentiation. *J Invest Dermatol* **127**, 1107–1114.

Bendit EG (1957) The α-β transformation in keratin. *Nature* **179**, 535.

Bern HA, Harkness DR, Blair SM (1955) Radioautographic studies of keratin formation. *PNAS* **41**, 55–60.

Bernard D, Mehul B, Delattre C, Simonetti L, Thomas-Collignon A, Schmidt R (2001) Purification and characterization of the endoglycosidase heparanase 1 from human plantar stratum corneum: a key enzyme in epidermal physiology? *J Invest Dermatol* **117**, 1266–1273.

Bernot KM, Coulombe PA, McGowan KM (2002) Keratin 16 expression defines a subset of epithelial cells during skin morphogenesis and the hair cycle. *J Invest Dermatol* **119**, 1137–1149.

Bertolino AP, Checkla DM, Heitner S, Freedberg IM, Yu DW (1990) Differential expression of type I hair keratins. *J Invest Dermatol* **94**, 297–303.

Block RJ (1951) Chemical classification of keratins. *Ann NY Acad Sci* **53**, 608–612.

Bloor BK, Tidman N, Leigh IM, et al. (2003) Expression of keratin K2e in cutaneous and oral lesions: association with keratinocyte activation, proliferation, and keratinization. *Am J Pathol* **162**, 963–975.

Boas JEV (1881) Ein Beitrag zur Morphologie der Nägel, Krallen, Hufe und Klauen der Säugetiere. *Morph Jb* **9**, 389–399.

Bonucci E, De Matteis A, Anceschi C (1979) Histochemical and electron microscopical investigations on the calcified keratin in the horn pearls of a glans carcinoma (calcified keratin). *Basic Appl Histochem* **23**, 93–102.

Bosch FX, Leube RE, Achtstatter T, Moll R, Franke WW (1988) Expression of simple epithelial type cytokeratins in stratified epithelia as detected by immunolocalization and hybridization in situ. *J Cell Biol* **106**, 1635–1648.

Bousquet O, Ma L, Yamada S, et al. (2001) The nonhelical tail domain of keratin 14 promotes filament bundling and enhances the mechanical properties of keratin intermediate filaments in vitro. *J Cell Biol* **155**, 747–753.

Bowden PE (2005) The human type II keratin gene cluster on chromosome 12q13.13: final count or hidden secrets? *J Invest Dermatol* **124**, xv–xvii.

Bowden PE, Quinlan RA, Breitkreutz D, Fusenig NE (1984) Proteolytic modification of acidic and basic keratins during terminal differentiation of mouse and human epidermis. *Eur J Biochem* **142**, 29–36.

Bowden PE, Hainey SD, Parker G, et al. (1998) Characterization and chromosomal localization of human hair-specific keratin genes and comparative expression during the hair growth cycle. *J Invest Dermatol* **110**, 158–164.

Bragulla HH (1991) [The deciduous hoof capsule of the equine fetus and new-born foal.] *Anat Histol Embryol* **20**, 66–74.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Bragulla HH (1996) [On the fetal development of the equine hoof.] Habilitation Thesis, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Free University of Berlin, Germany.

Bragulla H, Budras KD (1991) Die Homologisierung des foetal-perinatalen 'Eponychiums' als hinfällige Hufkapsel des Pferdes (Capsula ungulae decidua). *Anat Anz* **170**, 539–540.

Bragulla HH, Hirschberg RM (2002) On the fetal development of the modified epidermis of the porcine, bovine, and equine hoof. 24th Congress of the European Association of Veterinary Anatomists, Brno, Czech Republic, 21–25 July 2002.

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. *Mol Dev Evol* **298**, 140–151.

Bragulla H, Mülling CH (1997) [Changes in the architecture of the corneocyte and corneous layer in hoof diseases.] *Anat Histol Embryol* **26**, 51–52.

Bragulla HH, Homberger DG (2007) The role of the specific, profilaggrin-containing keratohyalin granules in the developing epidermis of the fetal horse hoof. *Pferdeheilkunde* **23**, 5–20.

Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T (2005) A proteolytic cascade of kallikreins in the stratum corneum. *J Invest Dermatol* **124**, 198–203.

Brown CH (1950) Keratins in invertebrates. *Nature* **166**, 439.

Budras KD, Hullinger RL, Sack WO (1989) Light and electron microscopy of keratinization in the laminar epidermis of the equine hoof with reference to laminitis. *Am J Vet Res* **50**, 1150–1160.

Buxman MM, Wuepper KD (1975) Keratin cross-linking and epidermal transglutaminase. A review with observations on the histochemical and immunochemical localization of the enzyme. *J Invest Dermatol* **65**, 107–112.

Byrne C, Tainsky M, Fuchs E (1994) Programming gene expression in developing epidermis. *Development* **120**, 2369–2383.

Candi E, Tarcsa E, Digiovanna JJ, et al. (1998) A highly conserved lysine residue on the head domain of type II keratins is essential for the attachment of keratin intermediate filaments to the cornified cell envelope through isopeptide crosslinking by transglutaminases. *Proc Natl Acad Sci USA* **95**, 2067–2072.

Chandler JS, Calnek D, Quaroni A (1991) Identification and characterization of rat intestinal keratins. Molecular cloning of cDNAs encoding cytokeratins 8, 19, and a new 49-kDa type I cytokeratin (cytokeratin 21) expressed by differentiated intestinal epithelial cells. *J Biol Chem* **266**, 11932–11938.

Chou CF, Smith AJ, Omary MB (1992) Characterization and dynamics of O-linked glycosylation of human cytokeratin 8 and 18. *J Biol Chem* **267**, 3901–3906.

Chuong CM, Homberger DG (2003) Development and evolution of the amniote integument: Current landscape and future horizon. *Mol Dev Evol* **298**, 1–11.

Clayton E, Doupe DP, Klein AM, Winton DJ, Simons BD, Jones PH (2007) A single type of progenitor cell maintains normal epidermis. *Nature* **446**, 185–189.

Collin C, Moll R, Kubicka S, Ouhayoun JP, Franke WW (1992) Characterization of human cytokeratin 2, an epidermal cytoskeletal protein synthesized late during differentiation. *Exp Cell Res* **202**, 132–141.

Cooper D, Sun TT (1986) Monoclonal antibody analysis of bovine epithelial keratins. Specific pairs as defined by coexpression. *J Biol Chem* **261**, 4646–4654.

Coulombe PA, Fuchs E (1990) Elucidating the early stages of keratin filament assembly. *J Cell Biol* **111**, 153–169.

Coulombe PA, Omary MB (2002) 'Hard' and 'soft' principles defining the structure, function and regulation of keratin intermediate filaments. *Curr Opin Cell Biol* **14**, 110–122.

Coulombe PA, Kopan R, Fuchs E (1989) Expression of keratin K14 in the epidermis and hair follicle: Insights into complex programs of differentiation. *J Cell Biol* **109**, 2295–2312.

Coulombe PA, Wawersik M, Paladini RD, Noensie E (1998) Type I keratin 16 forms relatively unstable tetrameric assembly subunits with various type II keratin partners: biochemical basis and functional implications. *Biol Bull* **194**, 364–365.

Coulombe PA, Tong X, Mazzalupo S, Wang Z, Wong P (2004) Great promises yet to be fulfilled: Defining keratin intermediate filament function in vivo. *Eur J Cell Biol* **83**, 735–746.

Cribier B, Peltre B, Grosshans E, Langbein L, Schweizer J (2004) On the regulation of hair keratin expression: lessons from studies in pilomatricomas. *J Invest Dermatol* **122**, 1078–1083.

Crick FHC (1953) The packing of alpha-helices: Simple coiled-coils. *Acta Crystallogr* **6**, 689–697.

Crowe DL (1993) Retinoic acid mediates post-transcriptional regulation of keratin 19 mRNA levels. *J Cell Sci* **106**, 183–188.

Cyr JL, Bell AM, Hudspeth AJ (2000) Identification with a recombinant antibody of an inner-ear cytokeratin, a marker for hair-cell differentiation. *Proc Nat Acad Sci* **97**, 4908–4913.

Dale BA, Holbrook KA, Kimball JR, Hoff M, Sun TT (1985) Expression of epidermal keratins and filaggrin during human fetal skin development. *J Cell Biol* **101**, 1257–1269.

De Berker D, Wojnarowska F, Sviland L, Westgate GE, Dawber RP, Leigh IM (2000) Keratin expression in the normal nail unit: markers of regional differentiation. *Br J Dermatol* **142**, 89–96.

Debus E, Weber K, Osborn M (1982) Monoclonal cytokeratin antibodies that distinguish simple from stratified squamous epithelia: characterization on human tissues. *EMBO J* **1**, 1641–1647.

Demerjian M, Hachem JP, Tschachler E, et al. (2008) Acute modulations in permeability barrier function regulate epidermal cornification: role of caspase-14 and the protease-activated receptor type 2. *Am J Pathol* **172**, 86–97.

Denecker G, Hoste E, Gilbert B, et al. (2007) Caspase-14 protects against epidermal UVB photodamage and water loss. *Nature Cell Biol* **9**, 666–674.

Denecker G, Ovaere P, Vandenabeele P, Declercq W (2008) Caspase-14 reveals its secrets. *J Cell Biol* **180**, 451–458.

Domingues MG, Jaeger MM, Araujo VC, Araujo NS (2000) Expression of cytokeratins in human enamel organ. *Eur J Oral Sci* **108**, 43–47.

Dusek RL, Godsel LM, Chen F, et al. (2007) Plakoglobin deficiency protects keratinocytes from apoptosis. *J Invest Dermatol* **127**, 792–801.

Eckert RL (1988) Sequence of the human 40-kDa keratin reveals an unusual structure with very high sequence identity to the corresponding bovine keratin. *Proc Natl Acad Sci USA* **85**, 1114–1118.

Eckert RL, Crish JF, Robinson NA (1997) The epidermal keratinocyte as a model for the study of gene regulation and cell differentiation. *Physiol Rev* **77**, 397–424.

Eckert RL, Sturniolo MT, Broome AM, Ruse M, Rorke EA (2005) Transglutaminase function in epidermis. *J Invest Dermatol* **124**, 481–492.

Egelrud T (2000) Desquamation in the stratum corneum. *Acta Derm Venereol Suppl (Stockh)* **208**, 44–45.

Eichner R, Kahn M (1990) Differential extraction of keratin subunits and filaments from normal human epidermis. *J Cell Biol* **110**, 1149–1168.

Eichner R, Bonitz P, Sun TT (1984) Classification of epidermal keratins according to their immunoreactivity, isoelectric point, and mode of expression. *J Cell Biol* **98**, 1388–1396.

Eichner R, Sun TT, Aebi U (1986) The role of keratin subfamilies and keratin pairs in the formation of human epidermal intermediate filaments. *J Cell Biol* **102**, 1767–1777.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Ekholm IE, Brattsand M, Egelrud T (2000) Stratum corneum tryptic enzyme in normal epidermis: a missing link in the desquamation process? *J Invest Dermatol* **114**, 56–63.

Elias PM, Friend DS (1975) The permeability barrier in mammalian epidermis. *J Cell Biol* **65**, 180–191.

Elias PM, Cullander C, Mauro T, et al. (1998) The secretory granular cell: The outermost granular cell as a specialized secretory cell. *J Invest Dermatol Symp Proc* **3**, 87–100.

Er Rafik M, Doucet J, Briki F (2004) The intermediate filament architecture as determined by X-ray diffraction modeling of hard alpha-keratin. *Biophys J* **86**, 3893–3904.

Feughelman M, Lyman DJ, Willis BK (2002) The parallel helices of the intermediate filaments of alpha-keratin. *Int J Biol Macromol* **30**, 95–96.

Fradette J, Germain L, Seshaiah P, Coulombe PA (1998) The type I keratin 19 possesses distinct and context-dependent assembly properties. *J Biol Chem* **273**, 35176–35184.

Franke WW, Schiller DL, Hatzfeld M, Winter S (1983) Protein complexes of intermediate-sized filaments: Melting of cytokeratin complexes in urea reveals different polypeptide separation characteristics. *Proc Natl Acad Sci USA* **80**, 7113–7117.

Franz JK, Franke WW (1986) Cloning of cDNA and amino acid sequence of a cytokeratin expressed in oocytes of Xenopus laevis. *Proc Nat Acad Sci USA* **83**, 6475–6479.

Frappier BL (2006) Epithelium. In *Dellmann's Textbook of Veterinary Histology*, 6th edn. (eds Eurell JA, Frappier BL), pp. 17–30. Ames, Iowa, USA: Blackwell Publishing.

Fraser RD, Parry DA (1996) The molecular structure of reptilian keratin. *Int J Biol Macromol* **19**, 207–211.

Fraser RD, Parry DA (2008) Molecular packing in the feather keratin filament. *J Struct Biol* **162**, 1–13.

Fraser RD, MacRae TP, Roger GE (1972) *Keratins: Their Composition, Structure, and Biosynthesis*. Springfield, Illinois, USA: Thomas.

Freedberg IM, Tomic-Canic M, Komine M, Blumenberg M (2001) Keratins and the keratinocyte activation cycle. *J Invest Dermatol* **116**, 633–640.

Fuchs E (1983) Evolution and complexity of the genes encoding the keratins of human epidermal cells. *J Invest Dermatol* **81**, 141s–144s.

Fuchs E, Marchuk D (1983) Type I and type II keratins have evolved from lower eukaryotes to form the epidermal intermediate filaments in mammalian skin. *Proc Natl Acad Sci USA* **80**, 5857–5861.

Fuchs E, Kim KH, Hanukoglu I, Tanese N (1983) The evolution and complexity of the genes encoding the cytoskeletal proteins of human epidermal cells. *Curr Prob Dermatol* **11**, 27–44.

Fuchs E, Tyner AL, Giudice GJ, Marchuk D, RayChaudhury A, Rosenberg M (1987) The human keratin genes and their differential expression. *Curr Top Dev Biol* **22**, 5–34.

Fuchs EV, Coppock SM, Green H, Cleveland DW (1981) Two distinct classes of keratin genes and their evolutionary significance. *Cell* **27**, 75–84.

Fudge DS, Gosline JM (2004) Molecular design of the alpha-keratin composite: insights from a matrix-free model, hagfish slime threads. *Proc R Soc Series B Biol Sci* **271**, 291–299.

Fukuyama K, Epstein WL (1969) Sulfur-containing proteins and epidermal keratinization. *J Cell Biol* **40**, 830–838.

Gallala H, Macheleidt O, Doering T, Schreiner V, Sandhoff K (2004) Nitric oxide regulates synthesis of gene products involved in keratinocyte differentiation and ceramide metabolism. *Eur J Cell Biol* **83**, 667–679.

Garmyn M, Mammone T, Pupe A, Gan D, Declercq L, Maes D (2001) Human keratinocytes respond to osmotic stress by p38 Map kinase regulated induction of HSP70 and HSP27. *J Invest Dermatol* **117**, 1290–1295.

Gigi O, Geiger B, Eshhar Z, et al. (1982) Detection of a cytokeratin determinant common to diverse epithelial cells by a broadly cross-reacting monoclonal antibody. *EMBO J* **1**, 1429–1437.

Gilfix BM, Eckert RL (1985) Coordinate control by vitamin A of keratin gene expression in human keratinocytes. *J Biol Chem* **260**, 14026–14029.

Giordano S, Glasgow E, Tesser P, Schechter N (1989) A type II keratin is expressed in glial cells of the goldfish visual pathway. *Neuron* **2**, 1507–1516.

Giudice GJ, Fuchs E (1987) The transfection of epidermal keratin genes into fibroblasts and simple epithelial cells: evidence for inducing a type I keratin by a type II gene. *Cell* **48**, 453–463.

Glenn TC, French JO, Heincelman TJ, Jones KL, Sawyer RH (2008) Evolutionary relationship among copies of feather beta (β) keratin genes from several avian orders. *Integrat Compar Biol*, epub, July 2008.

Görmar FE, Bernd A, Bereiter-Hahn J, Holzmann H (1990) A new model of epidermal differentiation: Induction by mechanical stimulation. *Arch Dermatol Res* **282**, 22–32.

Groff JM, Naydan DK, Higgins RJ, Zinkl JG (1997) Cytokeratin-filament expression in epithelial and non-epithelial tissues of the common carp (Cyprinus carpio). *Cell Tissue Res* **287**, 375–384.

Gu LH, Coulombe PA (2007) Keratin function in skin epithelia: a broadening palette with surprising shades. *Curr Opin Cell Biol* **19**, 13–23.

Gupta R, Ramnani P (2006) Microbial keratinases and their prospective applications: an overview. *Appl Microbiol Biotechnol* **70**, 21–33.

Hanakawa Y, Amagai M, Shirakata Y, et al. (2002) Differential effects of desmoglein 1 and desmoglein 3 on desmosome formation. *J Invest Dermatol* **119**, 1231–1236.

Hanukoglu I, Fuchs E (1983) The cDNA sequence of a Type II cytoskeletal keratin reveals constant and variable structural domains among keratins. *Cell* **33**, 915–924.

Harley CB (1991) Telomere loss: mitotic clock or genetic time bomb? *Mutat Res* **256**, 271–282.

Hashiguchi K, Hashimoto K (1995) The mineralization of crystalline inorganic components in Japanese serow horn. *Okajimas Folia Anat Jpn* **72**, 235–243.

Hashiguchi K, Hashimoto K, Akao M (2001) Morphological character of crystalline components present in saiga horn. *Okajimas Folia Anat Jpn* **78**, 43–48.

Hashimoto K, Mizuguchi R, Tanaka K, Dorman M (2000) Palmoplantar keratoderma (Voerner) with composite keratohyalin granules: Studies on keratinization parameters and ultrastructures. *J Dermatol* **27**, 1–9.

Hatzfeld M, Burba M (1994) Function of type I and type II keratin head domains: Their role in dimer, tetramer and filament formation. *J Cell Sci* **107**, 1959–1972.

Hatzfeld M, Franke WW (1985) Pair formation and promiscuity of cytokeratins. *J Cell Biol* **101**, 1826–1841.

Heid HW, Moll I, Franke WW (1988a) Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. I. Human and bovine hair follicles. *Differentiation* **37**, 137–157.

Heid HW, Moll I, Franke WW (1988b) Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. II. Concomitant and mutually exclusive synthesis of trichocytic and epithelial cytokeratins in diverse human and bovine tissues (hair follicle, nail bed and matrix, lingual papilla, thymic reticulum). *Differentiation* **37**, 215–230.

Heid HW, Werner E, Franke WW (1986) The complement of native alpha-keratin polypeptides of hair-forming cells: a subset of eight polypeptides that differ from epithelial cytokeratins. *Differentiation* **32**, 101–119.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Hendry KA, MacCallum AJ, Knight CH, Wilde CJ (2001) Synthesis and distribution of cytokeratins in healthy and ulcerated bovine claw epidermis. *J Dairy Res* **68**, 525–537.

Herrmann H, Aebi U (2004) Intermediate filaments: Molecular structure, assembly mechanism, and integration into functionally distinct intracellular scaffolds. *Ann Rev Biochem* **73**, 749–789.

Herrmann H, Strelkov SV, Feja B, et al. (2000) The intermediate filament protein consensus motif of helix 2B: Its atomic structure and contribution to assembly. *J Mol Biol* **296**, 817–832.

Herrmann H, Wedig T, Porter RM, Lane EB, Aebi U (2002) Characterization of early assembly intermediates of recombinant human keratins. *J Struct Biol* **137**, 82–96.

Herrmann H, Kreplak L, Aebi U (2004) Isolation, characterization, and in vitro assembly of intermediate filaments. *Meth Cell Biol* **78**, 3–24.

Herrmann H, Bar H, Kreplak L, Strelkov SV, Aebi U (2007) Intermediate filaments: From cell architecture to nanomechanics. *Nat Rev Mol Cell Biol* **8**, 562–573.

Hesse M, Magin TM, Weber K (2001) Genes for intermediate filament proteins and the draft sequence of the human genome: novel keratin genes and a surprisingly high number of pseudogenes related to keratin genes 8 and 18. *J Cell Sci* **114**, 2569–2575.

Hesse M, Zimek A, Weber K, Magin TM (2004) Comprehensive analysis of keratin gene clusters in humans and rodents. *Eur J Cell Biol* **83**, 19–26.

Hieronymus TL, Witmer LM, Ridgely RC (2006) Structure of white rhinoceros (Ceratotherium simum) horn investigated by X-ray computed tomography and histology with implications for growth and external form. *J Morphol* **267**, 1172–1176.

Hoath SB, Donnelly MM, Boissy RE (1990) Sensory transduction and the mammalian epidermis. *Biosens Bioelectron* **5**, 351–366.

Hofmann M, Zaper J, Bernd A, Bereiter-Hahn J, Kaufmann R, Kippenberger S (2004) Mechanical pressure-induced phosphorylation of p38 mitogen-activated protein kinase in epithelial cells via Src and protein kinase C. *Biochem Biophys Res Commun* **316**, 673–679.

Homberger DG (2001) The case of the cockatoo bill, horse hoof, rhinoceros horn, whale baleen, and turkey beard: The integument as a model system to explore the concepts of homology and non-homology. In *Vertebrate Functional Morphology: Horizon of Research in the 21st Century* (eds Dutta HM, Datta Munshi JS), pp. 317–343. Enfield, New Hampshire: Science Publishers Inc.

Homberger DG, Brush AH (1986) Functional-morphological and biochemical correlations of the keratinized structures in the African Grey Parrot, Psittacus erithacus (Aves). *Zoomorphology* **106**, 103–114.

Homberger DG, Ham K, Ogunbakin T, et al. (2009) The structure of the cornified claw sheath in the domesticated cat (*Felis catus*): Implications for the claw shedding mechanism and the evolution of cornified digital end organs. *J Anat* **214**, 620–643.

Horikoshi T, Arany I, Rajaraman S, et al. (1998) Isoforms of cathepsin D and human epidermal differentiation. *Biochimie* **80**, 605–612.

Horikoshi T, Igarashi S, Uchiwa H, Brysk H, Brysk MM (1999) Role of endogenous cathepsin D-like and chymotrypsin-like proteolysis in human epidermal desquamation. *Br J Dermatol* **141**, 453–459.

Houben E, De Paepe K, Rogiers V (2007) A keratinocyte's course of life. *Skin Pharmacol Physiol* **20**, 122–132.

Houben E, Hachem JP, De Paepe K, Rogiers V (2008) Epidermal ceramidase activity regulates epidermal desquamation via stratum corneum acidification. *Skin Pharmacol Physiol* **21**, 111–118.

Hutton E, Paladini RD, Yu QC, Yen M, Coulombe PA, Fuchs E (1998) Functional differences between keratins of stratified and simple epithelia. *J Cell Biol* **143**, 487–499.

Inada H, Izawa I, Nishizawa M, et al. (2001) Keratin attenuates tumor necrosis factor-induced cytotoxicity through association with TRADD. *J Cell Biol* **155**, 415–426.

Inoue T, Kizawa K, Ito M (2001) Characterization of soluble protein extracts from keratinized tissues: identification of ubiquitin universally distributed in hair, nail, and stratum corneum. *Biosci Biotechnol Biochem* **65**, 895–900.

Izawa I, Nishizawa M, Ohtakara K, Ohtsuka K, Inada H, Inagaki M (2000) Identification of Mrj, a DnaJ/Hsp40 family protein, as a keratin 8/18 filament regulatory protein. *J Biol Chem* **275**, 34521–34527.

Jäger K, Fischer H, Tschachler E, Eckhart L (2007) Terminal differentiation of nail matrix keratinocytes involves up-regulation of DNase1L2 but is independent of caspase-14 expression. *Differentiation* **75**, 939–946.

Jave-Suarez LF, Langbein L, Winter H, Praetzel S, Rogers MA, Schweizer J (2004) Androgen regulation of the human hair follicle: the type I hair keratin hHa7 is a direct target gene in trichocytes. *J Invest Dermatol* **122**, 555–564.

Jessen H (1970) Two types of keratohyalin granules. *J Ultrastruct Res* **33**, 95–115.

Jones PH (1996) Isolation and characterization of human epidermal stem cells. *Clin Sci (Lond)* **91**, 141–146.

Jones LN, Simon M, Watts NR, Booy FP, Steven AC, Parry DA (1997) Intermediate filament structure: Hard alpha-keratin. *Biophys Chem* **68**, 83–93.

Jovanovic I, Tzardi M, Mouzas IA, et al. (2002) Changing pattern of cytokeratin 7 and 20 expression from normal epithelium to intestinal metaplasia of the gastric mucosa and gastroesophageal junction. *Histol Histopathol* **17**, 445–454.

Kammerer RA, Schulthess T, Landwehr R, et al. (1998) An autonomous folding unit mediates the assembly of two-stranded coiled coils. *Proc Natl Acad Sci USA* **95**, 13419–13424.

Kartasova T, Roop DR, Holbrook KA, Yuspa SH (1993) Mouse differentiation-specific keratins 1 and 10 require a preexisting keratin scaffold to form a filament network. *J Cell Biol* **120**, 1251–1261.

Kazerounian S, Uitto J, Aho S (2002) Unique role for the periplakin tail in intermediate filament association: Specific binding to keratin 8 and vimentin. *Exp Dermatol* **11**, 428–438.

Kierszenbaum AL (2002) Keratins: unraveling the coordinated construction of scaffolds in spermatogenic cells. *Mol Reprod Dev* **61**, 1–2.

Kim S, Coulombe PA (2007) Intermediate filament scaffolds fulfill mechanical, organizational, and signaling functions in the cytoplasm. *Genes Dev* **21**, 1581–1597.

Kim S, Wong P, Coulombe PA (2006) A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. *Nature* **441**, 362–365.

King IA (1986) Incorporation of 3H-glucosamin into keratin-related polypetides in pig epidermis. *FEBS* **201**, 114.

King IA, Hounsell EF (1989) Cytokeratin 13 contains O-glycosidically linked N-acetylglucosamine residues. *J Biol Chem* **264**, 14022–14028.

Kirfel J, Magin TM, Reichelt J (2003) Keratins: a structural scaffold with emerging functions. *Cell Mol Life Sci* **60**, 56–71.

Kitahara T, Ogawa H (1991) The extraction and characterization of human nail keratin. *J Dermatol Sci* **2**, 402–406.

Knapp B, Rentrop M, Schweizer J, Winter H (1986) Nonepidermal members of the keratin multigene family: cDNA sequences and in situ localization of the mRNAs. *Nucleic Acids Res* **14**, 751–763.

Koch PJ, Roop DR (2004) The role of keratins in epidermal development and homeostasis – going beyond the obvious. *J Invest Dermatol* **123**, x–xi.

Komine M, Freedberg IM, Blumberg PM (1996) Regulation of epidermal expression of keratin 17 in inflammatory skin diseases. *J Invest Dermatol* **107**, 569–575.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Komine M, Rao LS, Kaneko T, et al. (2000) Inflammatory versus proliferative processes in epidermis. Tumor necrosis factor alpha induces K6b keratin synthesis through a transcriptional complex containing NF-kappa B and C/EBP-beta. *J Biol Chem* **275**, 32077–32088.

Kopan R, Fuchs E (1989) A new look into an old problem: Keratins as tools to investigate determination, morphogenesis, and differentiation in skin. *Genes Dev* **3**, 1–15.

Kopan R, Traska G, Fuchs E (1987) Retinoids as important regulators of terminal differentiation: Examining keratin expression in individual epidermal cells at various stages of keratinization. *J Cell Biol* **105**, 427–440.

Korge BP, Gan SQ, McBride OW, Mischke D, Steinert PM (1992a) Extensive size polymorphism of the human keratin 10 chain resides in the C-terminal V2 subdomain due to variable numbers and sizes of glycine loops. *Proc Natl Acad Sci USA* **89**, 910–914.

Korge BP, Compton JG, Steinert PM, Mischke D (1992b) The two size alleles of human keratin 1 are due to a deletion in the glycine-rich carboxyl-terminal V2 subdomain. *J Invest Dermatol* **99**, 697–702.

Koster MI, Roop DR (2004) Genetic pathways required for epidermal morphogenesis. *Eur J Cell Biol* **83**, 625–629.

Kouklis PD, Hutton E, Fuchs E (1994) Making a connection: Direct binding between keratin intermediate filaments and desmosomal proteins. *J Cell Biol* **127**, 1049–1060.

Kreplak L, Doucet J, Briki F (2001) Unraveling double stranded alpha-helical coiled coils: an x-ray diffraction study on hard alpha-keratin fibers. *Biopolymers* **58**, 526–533.

Kreplak L, Franbourg A, Briki F, Leroy F, Dalle D, Doucet J (2002) A new deformation model of hard alpha-keratin fibers at the nanometer scale: Implications for hard alpha-keratin intermediate filament mechanical properties. *Biophys J* **82**, 2265–2274.

Kreplak L, Doucet J, Dumas P, Briki F (2004) New aspects of the alpha-helix to beta-sheet transition in stretched hard alpha-keratin fibers. *Biophys J* **87**, 640–647.

Krieg TM, Schafer MP, Cheng CK, et al. (1985) Organization of a type I keratin gene. Evidence for evolution of intermediate filaments from a common ancestral gene. *J Biol Chem* **260**, 5867–5870.

Ku NO, Omary MB (2000) Keratins turn over by ubiquitination in a phosphorylation-modulated fashion. *J Cell Biol* **149**, 547–552.

Ku NO, Omary MB (2001) Effect of mutation and phosphorylation of type I keratins on their caspase-mediated degradation. *J Biol Chem* **276**, 26792–26798.

Ku NO, Omary MB (2006) A disease- and phosphorylation-related nonmechanical function for keratin 8. *J Cell Biol* **174**, 115–125.

Ku NO, Liao J, Chou CF, Omary MB (1996) Implications of intermediate filament protein phosphorylation. *Cancer Metastasis Rev* **15**, 429–444.

Ku NO, Michie S, Resurreccion EZ, Broome RL, Omary MB (2002) Keratin binding to 14-3-3 proteins modulates keratin filaments and hepatocyte mitotic progression. *Proc Natl Acad Sci USA* **99**, 4373–4378.

Kubilus J, Baden HP (1983) The role of cross-linking in epidermal differentiation. *Curr Probl Dermatol* **11**, 253–263.

Kubilus J, Waitkus RF, Baden HP (1980) Partial purification and specificity of an arginine-converting enzyme from bovine epidermis. *Biochim Biophys Acta* **615**, 246–251.

Kulesh DA, Cecena G, Darmon YM, Vasseur M, Oshima RG (1989) Posttranslational regulation of keratins: Degradation of mouse and human keratins 18 and 8. *Mol Cell Biol* **9**, 1553–1565.

Langbein L, Schweizer J (2005) Keratins of the human hair follicle. *Int Rev Cytol* **243**, 1–78.

Langbein L, Heid HW, Moll I, Franke WW (1993) Molecular characterization of the body site-specific human epidermal cytokeratin 9: cDNA cloning, amino acid sequence, and tissue specificity of gene expression. *Differentiation* **55**, 57–71.

Langbein L, Rogers MA, Winter H, et al. (1999) The catalog of human hair keratins. I. Expression of the nine type I members in the hair follicle. *J Biol Chem* **274**, 19874–19884.

Langbein L, Rogers MA, Winter H, Praetzel S, Schweizer J (2001) The catalog of human hair keratins. II. Expression of the six type II members in the hair follicle and the combined catalog of human type I and II keratins. *J Biol Chem* **276**, 35123–35132.

Langbein L, Rogers MA, Praetzel S, Aoki N, Winter H, Schweizer J (2002) A novel epithelial keratin, hK6irs1, is expressed differentially in all layers of the inner root sheath, including specialized Huxley-cells (Flugelzellen) of the human hair follicle. *J Invest Dermatol* **118**, 789–799.

Langbein L, Rogers MA, Praetzel S, Winter H, Schweizer J (2003) K6irs1, K6irs2, K6irs3, and K6irs4 represent the inner-root-sheath-specific type II epithelial keratins of the human hair follicle. *J Invest Dermatol* **120**, 512–522.

Langbein L, Spring H, Rogers MA, Praetzel S, Schweizer J (2004) Hair keratins and hair follicle-specific epithelial keratins. *Meth Cell Biol* **78**, 413–451.

Langbein L, Rogers MA, Praetzel S, et al. (2005) Characterization of a novel human type II epithelial keratin K1b, specifically expressed in eccrine sweat glands. *J Invest Dermatol* **125**, 428–444.

Langbein L, Rogers MA, Praetzel-Wunder S, Helmke B, Schirmacher P, Schweizer J (2006) K25 (K25irs1), K26 (K25irs2), K27 (K25irs3), and K28 (K25irs4) represent the type I inner root sheath keratins of the human hair follicle. *J Invest Dermatol* **126**, 2377–2386.

Langbein L, Rogers MA, Praetzel-Wunder S, Bockler D, Schirmacher P, Schweizer J (2007) Novel type I hair keratins K39 and K40 are the last to be expressed in differentiation of the hair: Completion of the human hair keratin catalog. *J Invest Dermatol* **127**, 1532–1535.

Larouche D, Tong X, Fradette J, Coulombe PA, Germain L (2008) Vibrissa hair bulge houses two populations of skin epithelial stem cells distinct by their keratin profile. *FASEB J* **22**, 1404–1415.

Leers MPG, Kölgen W, Björklund V, et al. (1999) Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. *J Pathol* **187**, 567–572.

Liao J, Lowthert LA, Ku NO, Fernandez R, Omary MB (1995) Dynamics of human keratin 18 phosphorylation: Polarized distribution of phosphorylated keratins in simple epithelial tissues. *J Cell Biol* **131**, 1291–1301.

Lillywhite HB (2006) Water relations of tetrapod integument. *J Exp Biol* **209**, 202–226.

Lippens S, Kockx M, Knaapen M, et al. (2000) Epidermal differentiation does not involve the proapoptotic executioner caspases, but is associated with caspase-14 induction and processing. *Cell Death Differ* **7**, 1218–1224.

Lippens S, Denecker G, Vandenabeele P, Declerq W (2005) Death penalty for keratinocytes: Apoptosis versus cornification. *Cell Death Diff* **12**, 1497–1508.

Lloyd C, Yu QC, Cheng J, et al. (1995) The basal keratin network of stratified squamous epithelia: defining K15 function in the absence of K14. *J Cell Biol* **129**, 1329–1344.

Lu H, Hesse M, Peters B, Magin TM (2005) Type II keratins precede type I keratins during early embryonic development. *Eur J Cell Biol* **84**, 709–718.

Lu H, Zimek A, Chen J, et al. (2006) Keratin 5 knockout mice reveal plasticity of keratin expression in the corneal epithelium. *Eur J Cell Biol* **85**, 803–811.

Lumpkin EA, Caterina MJ (2007) Mechanisms of sensory transduction in the skin. *Nature* **445**, 858–865.

Lyle S, Christofidou-Solomidou M, Liu Y, Elder DE, Albelda S, Cotsarelis G (1998) The C8/144B monoclonal antibody recognizes

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

cytokeratin 15 and defines the location of human hair follicle stem cells. *J Cell Sci* **111**, 3179–3188.

Lynch MH, O'Guin WM, Hardy C, Mak L, Sun TT (1986) Acidic and basic hair/nail ('hard') keratins: Their colocalization in upper cortical and cuticle cells of the human hair follicle and their relationship to 'soft' keratins. *J Cell Biol* **103**, 2593–2606.

Lyngholm M, Hoyer PE, Vorum H, Nielsen K, Ehlers N, Mollgard K (2008) Immunohistochemical markers for corneal stem cells in the early developing human eye. *Exp Eye Res* **87**, 115–121.

Mack JW, Steven AC, Steinert PM (1993) The mechanism of interaction of filaggrin with intermediate filaments. The ionic zipper hypothesis. *J Mol Biol* **232**, 50–66.

Magin TM, Vijayaraj P, Leube RE (2007) Structural and regulatory functions of keratins. *Exp Cell Res* **313**, 2021–2032.

Mahler B, Gocken T, Brojan M, Childress S, Spandau DF, Foley J (2004) Keratin 2e: a marker for murine nipple epidermis. *Cells Tissues Organs* **176**, 169–177.

Makar IA, Havryliak VV, Sedilo HM (2007) [Genetic and biochemical aspects of keratin synthesis by hair follicles.] *Tsitol Genet* **41**, 75–79.

Maniotis AJ, Chen CS, Ingber DE (1997) Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc Natl Acad Sci USA* **94**, 849–854.

Markl J, Franke WW (1988) Localization of cytokeratins in tissues of the rainbow trout: Fundamental differences in expression pattern between fish and higher vertebrates. *Differentiation* **39**, 97–122.

Marshall RC (1983) Characterization of the proteins of human hair and nail by electrophoresis. *J Invest Dermatol* **80**, 519–524.

Matsuba S, Suga Y, Ishidoh K, et al. (2002) Sulfhydryl oxidase (SOx) from mouse epidermis: molecular cloning, nucleotide sequence, and expression of recombinant protein in the cultured cells. *J Dermatol Sci* **30**, 50–62.

Mbiene JP, Roberts JD (2003) Distribution of keratin 8-containing cell clusters in mouse embryonic tongue: Evidence for a prepattern for taste bud development. *J Comp Neurol* **457**, 111–122.

McGowan KM, Coulombe PA (1998) Onset of keratin 17 expression coincides with the definition of major epithelial lineages during skin development. *J Cell Biol* **143**, 469–486.

Meier N, Dear TN, Boehm T (1999) Whn and mHa3 are components of the genetic hierarchy controlling hair follicle differentiation. *Mech Dev* **89**, 215–221.

Meng JJ, Bornslaeger E, Green KJ, Ip W (1998) Protein–protein interactions in intermediate filament structure and anchorage to the cell surface. *Biol Bull* **194**, 378–379.

Menon GK, Elias PM (1997) Morphologic basis for a pore-pathway in mammalian stratum corneum. *Skin Pharmacol* **10**, 235–246.

Michel M, Toeroek N, Godbout MJ, et al. (1996) Keratin 19 as a biochemical marker of skin stem cells in vivo and in vitro: keratin 19 expressing cells are differentially localized in function of anatomic sites, and their number varies with donor age and culture stage. *J Cell Sci* **109**, 1017–1028.

Miettinen M, Fetsch JF (2000) Distribution of keratins in normal endothelial cells and a spectrum of vascular tumors: implications in tumor diagnosis. *Hum Pathol* **31**, 1062–1067.

Miller AB, Lowe JK, Ostrander EA, Galibert F, Murphy KE (2001) Cloning sequence analysis and hybrid mapping of a mammalian KRT2p gene. *Funct Integr Genomics* **1**, 305–311.

Mogensen MM, Henderson CG, Mackie JB, Lane EB, Garrod DR, Tucker JB (1998) Keratin filament deployment and cytoskeletal networking in a sensory epithelium that vibrates during hearing. *Cell Motil Cytoskelet* **41**, 138–153.

Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* **31**, 11–24.

Moll I, Heid H, Franke WW, Moll R (1987) Distribution of a special subset of keratinocytes characterized by the expression of cytokeratin 9 in adult and fetal human epidermis of various body sites. *Differentiation* **33**, 254–265.

Moll R, Achtstatter T, Becht E, Balcarova-Stander J, Ittensohn M, Franke WW (1988) Cytokeratins in normal and malignant transitional epithelium. Maintenance of expression of urothelial differentiation features in transitional cell carcinomas and bladder carcinoma cell culture lines. *Am J Pathol* **132**, 123–144.

Moll I, Kuhn C, Moll R (1995) Cytokeratin 20 is a general marker of cutaneous Merkel cells while certain neuronal proteins are absent. *J Invest Dermatol* **104**, 910–915.

Moll R, Moll I, Franke WW (1984) Identification of Merkel cells in human skin by specific cytokeratin antibodies: Changes of cell density and distribution in fetal and adult plantar epidermis. *Differentiation* **28**, 136–154.

Moll R, Zimbelmann R, Goldschmidt MD, et al. (1993) The human gene encoding cytokeratin 20 and its expression during fetal development and in gastrointestinal carcinomas. *Differentiation* **53**, 75–93.

Molloy PL, Powell BC, Gregg K, Barone ED, Rogers GE (1982) Organisation of feather keratin genes in the chick genome. *Nucl Acid Res* **10**, 6007–6021.

Narisawa Y, Hashimoto K, Kohda H (1994) Immunohistochemical demonstration of keratin 19 expression in isolated human hair follicles. *J Invest Dermatol* **103**, 191–195.

Navarro JM, Casatorres J, Jorcano JL (1995) Elements controlling the expression and induction of the skin hyperproliferation-associated keratin K6. *J Biol Chem* **270**, 21362–21367.

Nelson WG, Sun TT (1983) The 50- and 58-kdalton keratin classes as molecular markers for stratified squamous epithelia: Cell culture studies. *J Cell Biol* **97**, 244–251.

Ngo MA, Sinitsyna NN, Qin Q, Rice RH (2007) Oxygen-dependent differentiation of human keratinocytes. *J Invest Dermatol* **127**, 354–361.

Nishizawa M, Izawa I, Inoko A, et al. (2005) Identification of trichoplein, a novel keratin filament-binding protein. *J Cell Sci* **118**, 1081–1090.

Norlen L (2003) Molecular skin barrier models and some central problems for the understanding of skin barrier structure and function. *Skin Pharmacol Appl Skin Physiol* **16**, 203–211.

Norlen L, Al-Amoudi A (2004) Stratum corneum keratin structure, function, and formation: The cubic rod-packing and membrane templating model. *J Invest Dermatol* **123**, 715–732.

Norlen L, Al-Amoudi A, Dubochet J (2003) A cryotransmission electron microscopy study of skin barrier formation. *J Invest Dermatol* **120**, 555–560.

Oakley B (1993) The gustatory competence of the lingual epithelium requires neonatal innervation. *Brain Res* **72**, 259–264.

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* **250**, 4007–4021.

O'Farrell PZ, Goodman HM, O'Farrell PH (1977) High-resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell* **12**, 1133–1142.

Ogawa H, Goldsmith LA (1976) Human epidermal transglutaminase. Preparation and properties. *J Biol Chem* **251**, 7281–7288.

Ohgitani S, Fujita T, Fujii Y, Hayashi C, Nishio H (2005) Nail calcium and magnesium content in relation to age and bone mineral density. *J Bone Miner Metab* **23**, 318–322.

Oriolo AS, Wald FA, Canessa G, Salas PJI (2007) GCP6 binds to intermediate filaments: a novel function of keratins in the organization of microtubules in epithelial cells. *Mol Biol Cell* **18**, 781–794.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Oshima RG (2007) Intermediate filaments: a historical perspective. *Exp Cell Res* **313**, 1981–1994.
Owens DW, Lane EB (2003) The quest for the function of simple epithelial keratins. *Bioessays* **25**, 748–758.
Pang YY, Schermer A, Yu J, Sun TT (1993) Suprabasal change and subsequent formation of disulfide-stabilized homo- and hetero-dimers of keratins during esophageal epithelial differentiation. *J Cell Sci* **104**, 727–740.
Papini S, Cecchetti D, Campani D, et al. (2003) Isolation and clonal analysis of human epidermal keratinocyte stem cells in long-term culture. *Stem Cells* **21**, 481–494.
Paramio JM, Casanova ML, Segrelles C, Mittnacht S, Lane EB, Jorcano JL (1999) Modulation of cell proliferation by cytokeratins K10 and K16. *Mol Cell Biol* **19**, 3086–3094.
Paramio JM, Jorcano JL (2002) Beyond structure: Do intermediate filaments modulate cell signalling? *Bioessays* **24**, 836–844.
Parry DA, Steinert PM (1999) Intermediate filaments: Molecular architecture, assembly, dynamics, and polymorphism. *Quart Rev Biophys* **32**, 99–187.
Parry DA, Marekov LN, Steinert PM (2001) Subfilamentous protofibril structures in fibrous proteins: Cross-linking evidence for protofibrils in intermediate filaments. *J Biol Chem* **276**, 39253–39258.
Parry DA, Marekov LN, Steinert PM, Smith TA (2002) A role for the 1A and L1 rod domain segments in head domain organization and function of intermediate filaments: Structural analysis of trichocyte keratin. *J Struct Biol* **137**, 97–108.
Parry DA, Strelkov SV, Burkhard P, Aebi U, Herrmann H (2007) Towards a molecular description of intermediate filament structure and assembly. *Exp Cell Res* **313**, 2204–2216.
Pauling L, Corey RB (1953) Compound helical configurations of polypeptide chains: Structure of proteins of the α-keratin type. *Nature (Lond)* **171**, 59–61.
Pautard FG (1970) The mineral phase of calcified cartilage, bone and baleen. *Calcif Tissue Res* **4**, 34–36.
Pearce EI, Smillie AC (1973) The mineralization of hair follicle tissue. II. An in vitro study. *Calcif Tissue Res* **11**, 23–38.
Pearce EI, Cousins FB, Smillie AC (1972) The mineralization of hair follicle tissue. I. An in vivo study. *Calcif Tissue Res* **8**, 228–236.
Pekny M, Lane EB (2007) Intermediate filaments and stress. *Exp Cell Res* **313**, 2244–2254.
Perrin C (2007) Expression of follicular sheath keratins in the normal nail with special reference to the morphological analysis of the distal nail unit. *Am J Dermatopathol* **29**, 543–550.
Perrin C, Langbein L, Schweizer J (2004) Expression of hair keratins in the adult nail unit: an immunohistochemical analysis of the onychogenesis in the proximal nail fold, matrix and nail bed. *Br J Dermatol* **151**, 362–371.
Peters T, Sedlmeier R, Buessow H, et al. (2003) Alopecia in a novel mouse model RCO3 is caused by mK6irs1 deficiency. *J Invest Dermatol* **121**, 674–680.
Planko L, Boehse K, Hoehfeld J, et al. (2007) Identification of a keratin-associated protein with a putative role in vesicle transport. *Eur J Cell Biol* **86**, 827–839.
Porter RM, Lane EB (2003) Phenotypes, genotypes and their contribution to understanding keratin function. *Trends Genet* **19**, 278–285.
Porter RM, Lunny DP, Ogden PH, et al. (2000) K15 expression implies lateral differentiation within stratified epithelial basal cells. *Lab Invest* **80**, 1701–1710.
Porter RM, Corden LD, Lunny DP, Smith FJ, Lane EB, McLean WH (2001) Keratin K6irs is specific to the inner root sheath of hair follicles in mice and humans. *Br J Dermatol* **145**, 558–568.
Porter RM, Gandi M, Wilson NJ, Wood P, McLean WH, Lane EB (2004) Functional analysis of keratin components in the mouse hair follicle inner root sheath. *Br J Dermatol* **150**, 195–204.
Presland RB, Dale BA (2000) Epithelial structural proteins of the skin and oral cavity: Function in health and disease. *Crit Rev Oral Biol Med* **11**, 383–408.
Presland RB, Jurevic RJ (2002) Making sense of the epithelial barrier: What molecular biology and genetics tell us about the functions of oral mucosal and epidermal tissues. *J Dental Educ* **66**, 564–574.
Quaroni A, Calnek D, Quaroni E, Chandler JS (1991) Keratin expression in rat intestinal crypt and villus cells. Analysis with a panel of monoclonal antibodies. *J Biol Chem* **266**, 11923–11931.
Reichelt J (2007) Mechanotransduction of keratinocytes in culture and in the epidermis. *Eur J Cell Biol* **86**, 807–816.
Rogers GE (2004) Hair follicle differentiation and regulation. *Int J Dev Biol* **48**, 163–170.
Rogers MA, Nischt R, Korge B, et al. (1995) Sequence data and chromosomal localization of human type I and type II hair keratin genes. *Exp Cell Res* **220**, 357–362.
Rogers MA, Langbein L, Praetzel S, et al. (1997) Sequences and differential expression of three novel human type-II hair keratins. *Differentiation* **61**, 187–194.
Rogers MA, Winter H, Langbein L, Bleiler R, Schweizer J (2004) The human type I keratin gene family: characterization of new hair follicle specific members and evaluation of the chromosome 17q21.2 gene domain. *Differentiation* **72**, 527–540.
Rogers MA, Edler L, Winter H, Langbein L, Beckmann I, Schweizer J (2005) Characterization of new members of the human type II keratin gene family and a general evaluation of the keratin gene domain on chromosome 12q13.13. *J Invest Dermatol* **124**, 536–544.
Roh C, Tao Q, Lyle S (2004) Dermal papilla-induced hair differentiation of adult epithelial stem cells from human skin. *Physiol Genomics* **19**, 207–217.
Roop DR, Cheng CK, Titterington L, et al. (1984) Synthetic peptides corresponding to keratin subunits elicit highly specific antibodies. *J Biol Chem* **259**, 8037–8040.
Ruhrberg C, Hajibagheri MA, Parry DA, Watt FM (1997) Periplakin, a novel component of cornified envelopes and desmosomes, that belongs to the plakin family and forms complexes with envoplakin. *J Cell Biol* **139**, 1835–1849.
Sawyer RH, Salvatore BA, Potylicki TT, French JO, Glenn TC, Knapp LW (2003) Origin of feathers: Feather beta keratins are expressed in discrete epidermal cell populations of embryonic scutate scales. *Mol Dev Evol* **295**, 12–24.
Sayama K, Hanakawa Y, Nagai H, et al. (2006) Transforming growth factor-beta-activated kinase 1 is essential for differentiation and the prevention of apoptosis in epidermis. *J Biol Chem* **281**, 22013–22020.
Sayama K, Yamasaki K, Hanakawa Y, et al. (2002) Phosphatidylinositol 3-kinase is a key regulator of early differentiation in keratinocytes. *J Biol Chem* **277**, 40390–40396.
Schaffeld M, Schultess J (2006) Genes coding for intermediate filament proteins closely related to the hagfish "thread keratins (TK)" [alpha] and [gamma] also exist in lamprey, teleosts and amphibians. *Exp Cell Res* **312**, 1447–1462.
Schaffeld M, Haberkamp M, Braziulis E, Lieb B, Markl J (2002b) Type II keratin cDNAs from the rainbow trout: implications for keratin evolution. *Differentiation* **70**, 292–299.
Schaffeld M, Hoffling S, Haberkamp M, Conrad M, Markl J (2002a) Type I keratin cDNAs from the rainbow trout: independent radiation of keratins in fish. *Differentiation* **70**, 282–291.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

Schaffeld M, Haberkamp M, Schatzlein S, Neumann S, Hunzinger C (2007) A novel and ancient group of type I keratins with members in bichir, sturgeon and gar. *Front Zool* **4**, 1–9.

Schermer A, Jester JV, Hardy C, Milano D, Sun TT (1989) Transient synthesis of K6 and K16 keratins in regenerating rabbit corneal epithelium: keratin markers for an alternative pathway of keratinocyte differentiation. *Differentiation* **42**, 103–110.

Schneider MR, Antsiferova M, Feldmeyer L, et al. (2007) Betacellulin regulates hair follicle development and hair cycle induction and enhances angiogenesis in wounded skin. *J Invest Dermatol* **128**, 1256–1265.

Schön M, Benwood J, O'Connell-Willstaedt T, Rheinwald JG (1999) Human sweat gland myoepithelial cells express a unique set of cytokeratins and reveal the potential for alternative epithelial and mesenchymal differentiation states in culture. *J Cell Sci* **112**, 1925–1936.

Schweizer J, Bowden PE, Coulombe PA, et al. (2006) New consensus nomenclature for mammalian keratins. *J Cell Biol* **174**, 169–174.

Schweizer J, Winter H (1982) Keratin polypeptide analysis in fetal and in terminally differentiating newborn mouse epidermis. *Differentiation* **22**, 19–24.

Scott JA, Untereiner WA (2004) Determination of keratin degradation by fungi using keratin azure. *Med Mycol* **42**, 239–246.

Senshu T, Kan S, Ogawa H, Manabe M, Asaga H (1996) Preferential deimination of keratin K1 and filaggrin during the terminal differentiation of human epidermis. *Biochem Biophys Res Commun* **225**, 712–719.

Shames RB, Knapp LW, Carver WE, Sawyer RH (1991) Region-specific expression of scutate scale type beta keratins in the developing chick beak. *J Exp Zool* **260**, 258–266.

Shames RB, Knapp LW, Carver WE, Washington LD, Sawyer RH (1989) Keratinization of the outer surface of the avian scutate scale: Interrelationship of alpha and beta keratin filaments in a cornifying tissue. *Cell Tiss Res* **257**, 85–92.

Siedamgrotzky O (1871) *Ueber die Structur und das Wachstum der Hornscheiden der Wiederkaeuer und der Krallen der Fleischfresser*. Dresden, Germany: Schoenfeld's Verlagsbuchhandlung.

Smith FJ, Porter RM, Corden LD, Lunny DP, Lane EB, McLean WH (2002) Cloning of human, murine, and marsupial keratin 7 and a survey of K7 expression in the mouse. *Biochem Biophys Res Comm* **297**, 818–827.

Smith TA, Steinert PM, Parry DA (2004) Modeling effects of mutations in coiled-coil structures: Case study using epidermolysis bullosa simplex mutations in segment 1a of K5/K14 intermediate filaments. *Proteins* **55**, 1043–1052.

Smith LT, Underwood RA, McLean WH (1999) Ontogeny and regional variability of keratin 2e (K2e) in developing human fetal skin: a unique spatial and temporal pattern of keratin expression in development. *Br J Dermatol* **140**, 582–591.

Sprecher E, Ishida-Yamamoto A, Becker OM, et al. (2001) Evidence for novel functions of the keratin tail emerging from a mutation causing ichthyosis hystrix. *J Invest Dermatol* **116**, 511–519.

Sprecher E, Itin P, Whittock NV, et al. (2002) Refined mapping of Naegeli-Franceschetti-Jadassohn syndrome to a 6 cm interval on chromosome 17q11.2–q21 and investigation of candidate genes. *J Invest Dermatol* **119**, 692–698.

Stappenbeck TS, Bornslaeger EA, Corcoran CM, Luu HH, Virata MIA, Green KJ (1993) Functional analysis of desmoplakin domains: specification of the interaction with keratin versus vimentin intermediate filament networks. *J Cell Biol* **123**, 691–705.

Stark HJ, Breitkreutz D, Limat A, Bowden P, Fusenig NE (1990) Keratins of the human hair follicle: 'Hyperproliferative' keratins consistently expressed in outer root sheath cells in vivo and in vitro. *Differentiation* **35**, 236–248.

Steinert PM (1975) The extraction and characterization of bovine epidermal alpha-keratin. *Biochem J* **149**, 39–48.

Steinert PM (1990) The two-chain coiled-coil molecule of native epidermal keratin intermediate filaments is a type I-type II heterodimer. *J Biol Chem* **265**, 8766–8774.

Steinert PM (1991) Organization of coiled-coil molecules in native mouse keratin 1/keratin 10 intermediate filaments: Evidence for alternating rows of antiparallel in-register and antiparallel staggered molecules. *J Struct Biol* **107**, 157–174.

Steinert PM (2001) Keratins: dynamic, flexible structural proteins of epithelial cells. *Curr Probl Dermatol* **54**, 193–198.

Steinert PM, Roop DR (1988) Molecular and cellular biology of intermediate filaments. *Ann Rev Biochem* **57**, 593–623.

Steinert PM, Wantz ML, Idler WW (1982) O-phosphoserine content of intermediate filament subunits. *Biochemistry* **21**, 177–183.

Steinert PM, Parry DA, Racoosin EL, et al. (1984) The complete cDNA and deduced amino acid sequence of a type II mouse epidermal keratin of 60 000 Da: analysis of sequence differences between type I and type II keratins. *Proc Natl Acad Sci USA* **81**, 5709–5713.

Steinert PM, Steven AC, Roop DR (1985) The molecular biology of intermediate filaments. *Cell* **42**, 411–420.

Steinert PM, Kartasova T, Marekov LN (1998) Biochemical evidence that small proline-rich proteins and trichohyalin function in epithelia by modulation of the biomechanical properties of their cornified cell envelopes. *J Biol Chem* **273**, 11758–11769.

Steinmetz MO, Stock A, Schulthess T, et al. (1998) A distinct 14 residue site triggers coiled-coil formation in cortexillin I. *EMBO J* **17**, 1883–1891.

Strachan LR, Ghadially R (2008) Tiers of clonal organization in the epidermis: the epidermal proliferation unit revisited. *Stem Cell Rev* **4**, 149–157.

Strnad P, Windoffer R, Leube RE (2002) Induction of rapid and reversible cytokeratin filament network remodeling by inhibition of tyrosine phosphatases. *J Cell Sci* **115**, 4133–4148.

Sun TT (2006) Altered phenotype of cultured urothelial and other stratified epithelial cells: Implications for wound healing. *Am J Physiol Renal Physiol* **291**, 9–21.

Sun TT, Eichner R, Nelson WG, et al. (1983) Keratin classes: Molecular marker for different types of epithelial differentiation. *J Invest Dermatol* **81**, 109s–115s.

Sun TT, Green H (1978) Keratin filaments of cultured human epidermal cells. Formation of intermolecular disulfide bonds during terminal differentiation. *J Biol Chem* **253**, 2053–2060.

Sun TT, Lavker RM (2004) Corneal epithelial stem cells: Past, present, and future. *J Invest Dermatol Symp Proc* **9**, 202–207.

Swensson O, Langbein L, McMillan JR, et al. (1998) Specialized keratin expression pattern in human ridged skin as an adaptation to high physical stress. *Br J Dermatol* **139**, 767–775.

Szeverenyi I, Cassidy AJ, Chung CW, et al. (2008) The human intermediate filament database: comprehensive information on a gene family involved in many human diseases. *Hum Mutat* **29**, 351–360.

Thys RC, Brandelli A (2006) Purification and properties of a keratinolytic metalloprotease from Microbacterium sp. *J Appl Microbiol* **101**, 1259–1268.

Tobin DJ (2006) Biochemistry of human skin – our brain on the outside. *Chem Soc Rev* **35**, 52–67.

Tomlinson DJ, Muelling CM, Fakler TM (2004) Formation of keratins in the bovine claw: Roles of hormones, minerals, and vitamins in functional claw integrity. *J Dairy Sci* **87**, 797–809.

Toto PD, O'Malley JJ, Grandel ER (1967) Similarities of keratinization and amelogenesis. *J Dent Res* **46**, 602–607.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland

المنارة للاستشارات

Troy TC, Turksen K (2005) Commitment of embryonic stem cells to an epidermal cell fate and differentiation in vitro. *Dev Dyn* **232**, 293–300.

Tullberg T (1883) Bau und Entwicklung der Barten bei Balanoptera sibaldii. *Nova Acta Reg Soc Sci (Upsala)* **3**, 1–36.

Ueda M (2000) Telomerase in cutaneous carcinogenesis. *J Dermatol Sci* **23**, S37–S40.

Vaidya MM, Kanojia D (2007) Keratins: markers of cell differentiation or regulators of cell differentiation? *J Biosci* **32**, 629–634.

Vecht-Hart CM, Bode P, Trouerbach WT, Collette HJ (1995) Calcium and magnesium in human toenails do not reflect bone mineral density. *Clin Chim Acta* **236**, 1–6.

Wang JHC (2006) Mechanobiology of tendon. *J Biomech* **39**, 1563–1582.

Wang H, Parry DA, Jones LN, Idler WW, Marekov LN, Steinert PM (2000) In vitro assembly and structure of trichocyte keratin intermediate filaments: a novel role for stabilization by disulfide bonding. *J Cell Biol* **151**, 1459–1468.

Wang Z, Wong P, Langbein L, Schweizer J, Coulombe PA (2003) Type II epithelial keratin 6hf (K6hf) is expressed in the companion layer, matrix, and medulla in anagen-stage hair follicles. *J Invest Dermatol* **121**, 1276–1282.

Waseem A, Alam Y, Dogan B, White KN, Leigh IM, Waseem NH (1998) Isolation, sequence and expression of the gene encoding human keratin 13. *Gene* **215**, 269–279.

Waseem A, Alexander CM, Steel JB, Lane EB (1990) Embryonic simple epithelial keratins 8 and 18: Chromosomal location emphasizes difference from other keratin pairs. *New Biol* **2**, 464–478.

Waseem A, Dogan B, Tidman N, et al. (1999) Keratin 15 expression in stratified epithelia: Downregulation in activated keratinocytes. *J Invest Dermatol* **112**, 362–369.

Watt FM (1998) Epidermal stem cells: Markers, patterning and the control of stem cell fate. *Phil Trans R Soc (Lond) B Biol Sci* **353**, 831–837.

Watts NR, Jones LN, Cheng N, Wall JS, Parry DA, Steven AC (2002) Cryo-electron microscopy of trichocyte (hard α-keratin) intermediate filaments reveals a low-density core. *J Struct Biol* **137**, 109–118.

Wawersik M, Coulombe PA (2000) Forced expression of keratin 16 alters the adhesion, differentiation, and migration of mouse skin keratinocytes. *Mol Biol Cell* **11**, 3315–3327.

Webb A, Li A, Kaur P (2004) Location and phenotype of human adult keratinocyte stem cells of the skin. *Differentiation* **72**, 387–395.

Weil M, Raff MC, Braga VM (1999) Caspase activation in the terminal differentiation of human epidermal keratinocytes. *Curr Biol* **9**, 361–364.

Werlang PO, Brandelli A (2005) Characterization of a novel feather-degrading Bacillus sp. strain. *Appl Biochem Biotechnol* **120**, 71–79.

Whitbread LA, Powell BC (1998) Expression of the intermediate filament keratin gene, K15, in the basal cell layers of epithelia and the hair follicle. *Exp Cell Res* **244**, 448–459.

Wilson AK, Coulombe PA, Fuchs E (1992) The roles of K5 and K14 head, tail, and R/K L L E G E domains in keratin filament assembly in vitro. *J Cell Biol* **119**, 401–414.

Windoffer R, Leube RE (1999) Detection of cytokeratin dynamics by time-lapse fluorescence microscopy in living cells. *J Cell Sci* **112**(24), 4521–4534.

Windoffer R, Woll S, Strnad P, Leube RE (2004) Identification of novel principles of keratin filament network turnover in living cells. *Mol Biol Cell* **15**, 2436–2448.

Windoffer R, Kolsch A, Woll S, Leube RE (2006) Focal adhesions are hotspots for keratin filament precursor formation. *J Cell Biol* **173**, 341–348.

Winter H, Langbein L, Praetzel S, et al. (1998) A novel human type II cytokeratin, K6hf, specifically expressed in the companion layer of the hair follicle. *J Invest Dermatol* **111**, 955–962.

Witt M, Kasper M (1999) Distribution of cytokeratin filaments and vimentin in developing human taste buds. *Anat Embryol* **199**, 291–299.

Wöll S, Windoffer R, Leube RE (2007) p38 MAPK-dependent shaping of the keratin cytoskeleton in cultured cells. *J Cell Biol* **177**, 795–807.

Woodcock-Mitchell J, Eichner R, Nelson WG, Sun TT (1982) Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. *J Cell Biol* **95**, 580–588.

Woodin AM (1956) Structure and composition of soluble feather keratin. *Biochem J* **63**, 576–581.

Wu KC, Bryan JT, Morasso MI, et al. (2000) Coiled-coil trigger motifs in the 1B and 2B rod domain segments are required for the stability of keratin intermediate filaments. *Mol Biol Cell* **11**, 3539–3558.

Wu P, Hou L, Plikus M, et al. (2004) Evo-devo of amniote integuments and appendages. *Int J Dev Biol* **48**, 249–270.

Yamada S, Wirtz D, Coulombe PA (2002) Pairwise assembly determines the intrinsic potential for self-organization and mechanical properties of keratin filaments. *Mol Biol Cell* **13**, 382–391.

Yamaguchi Y, Itami S, Tarutani M, Hosokawa K, Miura H, Yoshikawa K (1999) Regulation of keratin 9 in nonpalmoplantar keratinocytes by palmoplantar fibroblasts through epithelial–mesenchymal interactions. *J Invest Dermatol* **112**, 483–488.

Yu DW, Pang SY, Checkla DM, Freedberg IM, Sun TT, Bertolino AP (1991) Transient expression of mouse hair keratins in transfected HeLa cells: Interactions between 'hard' and 'soft' keratins. *J Invest Dermatol* **97**, 354–363.

Zatloukal K, French SW, Stumptner C, et al. (2007) From Mallory to Mallory-Denk bodies: What, how and why? *Exp Cell Res* **313**, 2033–2049.

Zhang C, Oakley B (1996) The distribution and origin of keratin 20-containing taste buds in rat and human. *Differentiation* **61**, 121–127.

Zhang JS, Wang L, Huang H, Nelson M, Smith DL (2001) Keratin 23 (K23), a novel acidic keratin, is highly induced by histone deacetylase inhibitors during differentiation of pancreatic cancer cells. *Genes Chromosomes Cancer* **30**, 123–135.

Zhou Q, Toivola DM, Feng N, Greenberg HB, Franke WW, Omary MB (2003) Keratin 20 helps maintain intermediate filament organization in intestinal epithelia. *Mol Biol Cell* **14**, 2959–2971.

Zhou Q, Cadrin M, Herrmann H, et al. (2006) Keratin 20 serine 13 phosphorylation is a stress and intestinal goblet cell marker. *J Biol Chem* **281**, 16453–16461.

Zietzschmann O, Krölling O (1955) *Lehrbuch der Entwicklungsgeschichte der Haustiere*, 2. Aufl., pp. 191–199. Berlin, Germany: Paul Parey.

Zimek A, Weber K (2006) The organization of the keratin I and II gene clusters in placental mammals and marsupials show a striking similarity. *Eur J Cell Biol* **85**, 83–89.

© 2009 The Authors
Journal compilation © 2009 Anatomical Society of Great Britain and Ireland