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Review

Keratins: a structural scaffold with emerging functions

J. Kirfel[a], T. M. Magin[b] and J. Reichelt[b,*]

[a] Institute of Pathology, Bonn Medical School, University of Bonn, Sigmund-Freud-Str. 25, 53127 Bonn (Germany)
[b] Institute of Physiological Chemistry and Bonner Forum Biomedizin, Division of Cellular Biochemistry, University of Bonn, Nussallee 11, 53115 Bonn (Germany), Fax +49 228 73 4558, e-mail: j.reichelt@uni-bonn.de

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Abstract. Intermediate filament proteins form an essential part of the cytoskeleton and provide topological order to cells and tissues. These features result from their intrinsic property of self-organization and their response to extrinsic cues. Keratins represent the largest subgroup among all intermediate filament proteins and are differentially expressed as pairs of type I and type II intermediate filament proteins in epithelia. Their primary function is to impart mechanical strength to cells. This function is illustrated by patients with keratin mutations and by gene-deficient mice. Additional functions include their participation in the response to stress, cell signalling and apoptosis, and thus the keratin cytoskeleton appears far more dynamic than previously anticipated. This may result from hyperphosphorylation and possibly from interaction with associated proteins. How signalling networks affect keratin organization, turnover and function and vice versa will be a major challenge for future investigations.

Key words. Keratin; intermediate filament; disease; keratin-associated protein.

Introduction

Keratins are the major structural proteins in epithelial cells and form a cytoplasmic network of 10–12-nm-wide intermediate filaments (IFs). Keratins are encoded by a large multigene family of more than 50 individual members which are classified into two major sequence types [1, 2]. Type I keratins include K9–K23, and the hair keratins Ha1–Ha8. Type II keratins include K1–K8, and the hair keratins, Hb1–Hb6. Pairs of type I and type II keratins are expressed differentially in epithelial cells.
Since 1991, mutations in several keratin genes have been found to cause a variety of human diseases affecting the epidermis and other epithelial tissues [3–6]. In addition, transgenic mice, expressing mutant keratins, carrying constitutive or, more recently, inducible null alleles have been generated to serve as mouse models for keratin diseases, often reflecting the phenotypes observed in patients [6, 7].

* Corresponding author.

The aim of the present review is to summarize progress in the field of keratin research in understanding the assembly, structure, function and regulation of the keratin cytoskeleton. We discuss the evidence for the role of keratins in providing stability against mechanical stress, and in providing topological order for associated proteins.

Novel keratin genes

The majority of keratins were identified by biochemical, immunological and cDNA cloning methods. Gene mapping studies established that all keratins with the exception of a few polymorphic variants [8–10] are encoded by single-copy genes [11]. The genes for human type I keratin genes, except for K18 [12], are clustered on chromosome 17q21 and all human type II genes and the gene for K18 are clustered on 12q13 [2].
Recently, we examined the complement of IF genes published in the NCBI and the Celera genome databases and revealed the presence of 208 keratin-related sequences [2].

Table 1. Classification and chromosomal localization of intermediate filament genes and pseudogenes [adapted from ref. 2].

IF gene	Chromosome	Number of pseudogenes	Number of gene fragments
Type I			
K9	17	–	–
K10	17	–	–
K10b[1]	17	–	–
K10c[1]	17	–	–
K10d[1]	17	–	–
K12	17	–	–
K12b[1]	17	–	–
K13	17	–	–
K14	17	1 (17)	1 (17)
K15	17	–	–
K16a	17	2 (17)	–
K17	17	2 (17)	2 (17)
K18	12	62	15
K19	17	3 (6, 15, 12)	1 (4)
K20	17	–	–
K23	17	–	–
*	17	–	2
Hair type I			
KRTHA1	17	–	–
KRTHA2	17	–	–
KRTHA3a	17	–	–
KRTHA3b	17	–	–
KRTHA4	17	–	–
KRTHA5	17	–	–
KRTHA6	17	–	–
KRTHA7	17	–	–
KRTHA8	17	–	–
ψKRTHaA	17	–	–
Type II			
K1	12	–	–
K2e	12	–	–
k2p	12	–	–
K3	12	–	–
K4	12	–	–
K5	12	–	–
K6a	12	–	–
K6b	12	–	–
K6hf	12	1 (12)	–
K7	12	–	–
K8	12	35	26
*	12	–	1
Hair type II			
Hb1	12	–	–
Hb2	12	–	–
Hb3	12	–	–
Hb4	12	–	–
Hb5	12	–	–
Hb6	12	–	–
ψhHbA	12	–	–
ψhHbB	12	–	–
ψhHbC	12	–	–
ψhHbD	12	–	–
Type III			
Vimentin	10	–	1 (6)
Desmin	2	–	–
GFAP	17	–	–
Peripherin	12	–	–
Nestin	1	–	–
Synemin	15	–	–
Type IV			
NF-L	8	–	2 (Y)
NF-M	8	–	1 (10)
NF-H	22	2 (20, 1)	–
α-Internexin	10	–	–
Syncoilin	1	–	–
Type V			
lamin A/C	1	–	–
lamin B1	5	–	–
lamin B2	19	–	–
Others			
Filensin	20	–	–
Phakinin	3	–	–
Novel type II keratins			
K1b	12	–	–
K5b	12	–	–
K5c	12	–	–
K6h	12	–	–
K6i	12	–	–
K6k	12	–	–
K6l	12	–	–

Keratin genes 8 and 18 which give rise to 62 and 35 processed pseudogenes, respectively, are *italicized*. Potential novel keratin genes/gene fragments in the type I and II clusters are indicated by an asterisk. Chromosomal localization of pseudogenes is indicated by numbers in parentheses. Pseudogenes related to hair keratin genes are denoted by ψ. The *superscript 1* indicates type I keratin genes recently identified by Bawden et al. [26]. These are most closely related to K10.

Of these, 49 represented single-copy genes for type I and II keratins. The type I keratin cluster contains at least 25 functional and 2 pseudogenes spread over nearly 1 Mb of DNA; the corresponding type II gene array harbours at least 24 functional genes and 5 pseudogenes distributed over 1.2–1.3 Mb. Among these are the recently described K23 [13] and 7 newly discovered type II keratin genes [2]. Of the latter, 6 displayed homology to K6a, K6b and K5, while 1 was most closely related to K1 [2]. One of the new members of the K6 family had 99% protein sequence identity to K6b; however, its genomic organization was distinctly different since it contained a unique intron 3. Among the newly identified genes, only K23 has been positively identified as an active gene, at least at the transcriptional level [13].

The gene density in the two keratin clusters is approximately 35 kb per gene and, therefore, much higher than estimated for the overall genome [14]. In addition to protein-coding keratin genes, there are 111 pseudogenes. While intron-containing pseudogenes are mostly located within

the two keratin clusters, those with features of processed pseudogenes have invaded other chromosomes, often at several positions. A few earlier analyses have identified pseudogenes for K8, K14, K16, K17, K18, K19 and hair keratins [15–23]. The pseudogenes coding for K14, K16 and K17, which arose by gene duplication, are located outside the type I keratin cluster [18].

Unexpectedly, most of the processed pseudogenes relate to keratin genes 8 and 18, which map side by side on chromosome 12q13 within the type II gene cluster. K8 and K18 are typical of internal epithelia and represent the earliest IF expression pair in embryogenesis. There are 62 processed pseudogenes plus 15 gene fragments for the keratin 18 gene and 35 processed pseudogenes plus 26 gene fragments for the keratin 8 gene [12, 15]. These processed pseudogenes are dispersed over all chromosomes. None of these pseudogenes contained an intact open reading frame. Other keratin genes are either single-copy genes or are additionally accompanied by 1–4 pseudogenes. In the draft for the human genome, genes for keratin 11 [1] which may represent a polymorphic variant of K10 [8–10] and for K6c–f [24] were not found. The status of the latter may have to await complete sequencing of the human genome.

The total number of keratin genes amounts to at least 49. Of these, 22 are expressed in various epithelia, 15 are trichocyte specific and 5 represent inner root sheath keratins. The expression sites of the 7 novel keratins described by Hesse et al. [2] have yet to be determined (table 1).

In addition to our work, Langbein et al. [25] and Bawden et al. [26] have described the existence of 9 previously unknown human type I keratin genes, expressed in hair, nail and oral filiform papillae. They are organized as a cluster along with other type I genes on chromosome 17q12–21. Recently, Langbein and co-workers [27] provided a complete expression catalogue of the type II hair keratins, describing the existence of 6 newly identified functional type II hair keratin genes, clustered within the type II keratin gene domain on chromosome 12q13.

IFs share a common structure

Keratins have a basic molecular structure common to all cytoplasmic IF proteins (fig. 1). All carry an α-helical 'rod' domain consisting of four consecutive domains of highly conserved length: segment 1A accounting for 35, segment 1B for 101, segment 2A for 19 and segment 2B for 121 amino acids. The non-α-helical parts between these segments, also called 'linkers' (L1, L12, L2), are variable in length (8–22 amino acids). The α-helical segments exhibit a heptad substructure (abcdefg), where the a and d positions are commonly occupied by apolar amino acids [28]. These hydrophobic amino acids generate a surface that is wound around the axis of a single right-handed α-helix in a left-handed manner, ultimately leading to superhelix, i.e. coiled-coil formation of two such molecules. The phasing of the heptads is broken in the middle of segment 2B giving rise to a 'stutter' [29]. This break in the continuous heptad pattern is due to a deletion of three amino acids (or the insertion of four residues) and is strictly conserved in all IF proteins. The stutter represents a helical segment which is not engaged in coiled-coil formation. Moreover, atomic structure analysis showed that the end of segment 2B, representing the evolutionarily conserved IF consensus motif (TYRKLLEGEE), is not entirely part of the coiled-coil structure, but bends away from the coiled-coil axis [30, 31].

The formation of heterodimers in which compatible type I and type II polypeptide chains align in parallel and in exact axial register [32] is the first step of keratin filament assembly. Two heterodimers associate, forming tetramer units aligned in an antiparallel manner [33]. The exact alignment of heterodimer units remains controversial: antiparallel molecules can assemble in a half-staggered mode with their 1B segments (A11) or with their 2B segments largely overlapped (A22); alternatively, the molecules may be almost completely overlapped (A12). Pairs of unstaggered antiparallel dimers were found to be the major tetrameric subunit in IFs; and Steinert showed that the A12 mode was consequent upon the formation of one of the half-staggered forms [34–37].

Molecular details of the further steps in the process of elongation and compaction of IFs are much less well understood. At least in vitro, the next steps of assembly consist of a lateral aggregation of eight tetramers into so-called unit-length filaments (ULFs) and the longitudinal annealing of ULFs to successively growing fibres [38].

Recently, rheological assays and digital interference contrast light microscopy have been applied to examine the influence of filament-filament interaction on the micromechanical properties of distinct keratin polymers in vitro. For both the K8/K18 and the K5/K14 polymers, which represent the keratins of simple and complex (stratified) epithelia, respectively, extensive bundling can be promoted by minor changes in the assembly conditions. In contrast, copolymers between K8/K14 and K5/K18 have different properties, underlining the importance of pairwise assembly [39]. In the case of the K5/K14 polymers, the non-helical tail domain of K14 has been proposed to contribute to the ability of filaments to self-organize into bundles [40]. The extent to which this occurs in vivo and applies to other keratins remains the subject of future experiments and has to include the identification of the corresponding protease(s). Rheological studies were also applied by Coulombe and co-workers to determine the impact of mutations in keratin disorders on IF assembly. The severity of the disease is usually correlated with the exact position of the point mutation in the affected keratin,

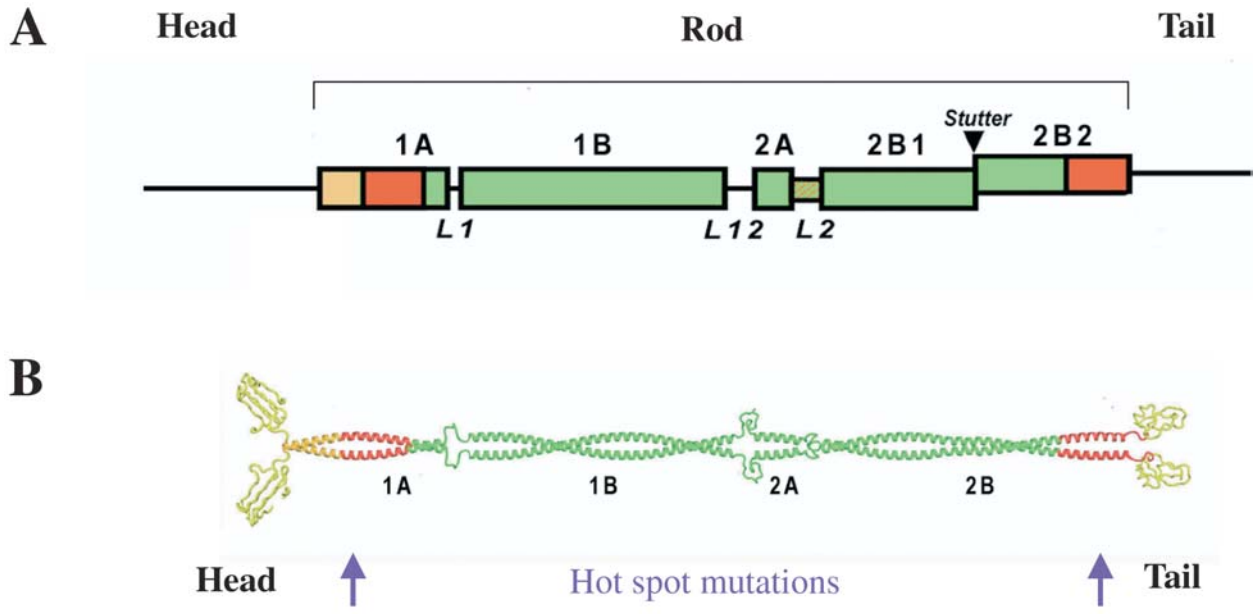

Figure 1. (*A*, *B*) The tripartite domain structure of all keratin proteins, with an α-helical central rod domain dominated by subsegments (1A, 1B, 2A and 2B) and separated by short linker regions (L1, L12 and L2). The stutter represents a helical segment not engaged in coiled-coil formation. Non-helical head and tail domains at the N and C termini flank the rod domain, respectively. At the beginning and end of the rod domain are the highly conserved helix boundary sequence motives, also known as the helix initiation peptide (HIP) in the 1A domain and the helix termination peptide (HTP) at the end of helix 2B. Mutations in the boundaries cause aggregation of IFs and generally lead to more severe phenotypes than those in other regions. For example, the mutation R125H in K14, which is located in the helix initiation motif, leads to epidermolysis bullosa simplex Dowling-Meara type. This arginine codon in the 1A domain is conserved in most type I keratins and represents a mutation 'hotspot' in these proteins. (*C*) Electron microscopic image illustrating the organization of keratin filaments at desmosomes. The filaments are decorated with gold particles.

with the most severe mutations residing in the highly conserved motif at either end of the α-helical rod domain. In several epidermolysis bullosa simplex (EBS) patients, a highly conserved arginine (R125) in K14 has been found to be mutated to either cysteine or histidine [41, 42]. In a rheological assay, the 'hotspot' R125C mutation in K14, which causes EBS of the Dowling-Meara type (EBS-DM), decreased the mechanical resilience of keratin IFs, meaning that in this assay, the bundles of the mutant K5/K14R125C filaments were fewer and smaller than those which arose from K5/K14 filaments [43]. In a different in vitro assay, Herrmann et al. [44] compared filament assembly of wild-type K14 and mutant K14R125H with K5. Quite unexpectedly, the mutant keratin pair formed typical IFs with normal kinetics and were longer and more regular than the corresponding wild-type filaments. Therefore, filament assembly itself is not inhibited by the R125H mutation in K14. This was a rather unexpected result because earlier experiments suggested that keratin mutations in either the helix initiation or the helix termination motif were detrimental to filament assembly [45]. The contrasting results obtained by the different groups may have to do with the distinct experimental conditions they used.

The pathogenic mechanisms that cause skin disorders like EBS are still not well understood. Rather than interfering

with filament assembly, some of the EBS mutations may affect filament bundling [43] and/or inhibit interaction of IFs with other cytoskeletal components [46, 47].

Lessons from human disorders and transgenic animal studies

Human disorders

The consequence of keratin mutations at least for those expressed in stratified epithelia is well established and the corresponding diseases are genetically well defined (table 2). In general, disease severity correlates with the location and the nature of the mutation within the protein although variations of this rule exist [48]. Mutations affecting the conserved initiation and termination sequences of the rod alter the organization of keratin IFs causing epidermal fragility by decreasing mechanical resilience.

In basal keratinocytes, where K5, K14 and K15 are co-expressed [1], keratin IFs are organized in loose filament bundles (see fig. 2). Mutations in K5 and K14 genes cause a number of inherited human skin disorders such as EBS [4, 41, 49, 50]. The characteristic feature of EBS is epidermal blistering resulting from cytolysis of basal keratinocytes. There are three main forms of EBS of which EBS-DM is the most severe. Blisters can be found at any body site, often resulting from mild physical trauma. Keratin filaments collapse and form electron-dense aggregates within the cell cytoplasm [51]. The Koebner variant (EBS-K) is associated with milder widespread blistering and the Weber-Cockayne form (EBS-WC) is the mildest manifestation, where blistering is restricted to hands and feet. In addition to these forms of EBS which represent dominant disorders, a few patients suffering from recessive EBS have been reported [references in refs. 4, 42]. The first EBS-DM mutation was discovered in the highly conserved helix initiation motif at the beginning of helix 1 A of K14 (R125H) [41]. The affected arginine codon of the 1 A domain is conserved in most type I keratins and represents a mutation 'hotspot' in these proteins. A corresponding mutation in the conserved helix termination motif of K5 was reported in a large EBS-DM family [50].

Upon induction of epidermal terminal differentiation, transcription of K5, K14 and K15 is switched off and that of K1 and K10 is switched on [52]. K1/K10-containing filaments are strongly bundled and are typically oriented parallel to the surface in the flattened keratinocytes of the upper epidermis. Upon further differentiation, keratinocytes loose their organelles and differentiate into corneocytes which are eventually shed from the epidermis. Instead of having a cell membrane, corneocytes are coated with the cornified envelope to which keratins become covalently cross-linked via cornified envelope proteins including involucrin [36, 53–55, for reviews, see refs. 4, 56]. Mutations in K1 and K10 cause bullous congenital ichthyosiform erythroderma (BCIE) [57–59]. In histological sections of affected skin, basal epidermal cells appear normal, whereas suprabasal keratinocytes show cytolysis. Within suprabasal cells of BCIE patients aggregates were observed, which labelled with K1 and K10 antibodies [60]. Since the initial reports, several more

Table 2. Expression patterns of keratins and keratin disorders.

Keratin	Expression site	Disease	References
K5, K14, (K15)	basal keratinocytes of epidermis and stratified epithelia	epidermolysis bullosa simplex (EBS)	3, 49, 50
K1, K10	suprabasal cells of epidermis and other stratified epithelia	bullous congenital ichthyosiform erythroderma (BCIE)	60
K1		diffuse non-epidermolytic palmoplantar keratoderma (DNEPPK)	61
		ichthyosis hystrix Curth-Macklin (IHCM)	62
K2e	upper interfollicular and palmoplantar epidermis	ichthyosis bullosa of Siemens (IBS)	68, 69
K9	upper interfollicular and palmoplantar epidermis	epidermolytic palmoplantar keratoderma (EPPK)	71
K6a,	palmoplantar, mucosa, epidermal appendages, wound healing	pachyonychia congenita type 1 (PC-1)	79
K6b	epidermal appendages	pachyonychia congenita type 2 (PC-2)	85, 86
K16	palmoplantar, mucosa, epidermal appendages, wound healing	PC-1 and focal non-epidermolytic palmoplantar keratoderma (FNEPPK)	88
K17	epidermal appendages	steatocystoma multiplex or PC-2	89
K4, K13	mucosa, stratified non-cornified epithelia	white sponge nevus (WSN)	90, 91
K3, K12	cornea	Meesmann's corneal dystrophy (MCD)	94
K8, K18	simple epithelia	cryptogenic liver disease	96, 97

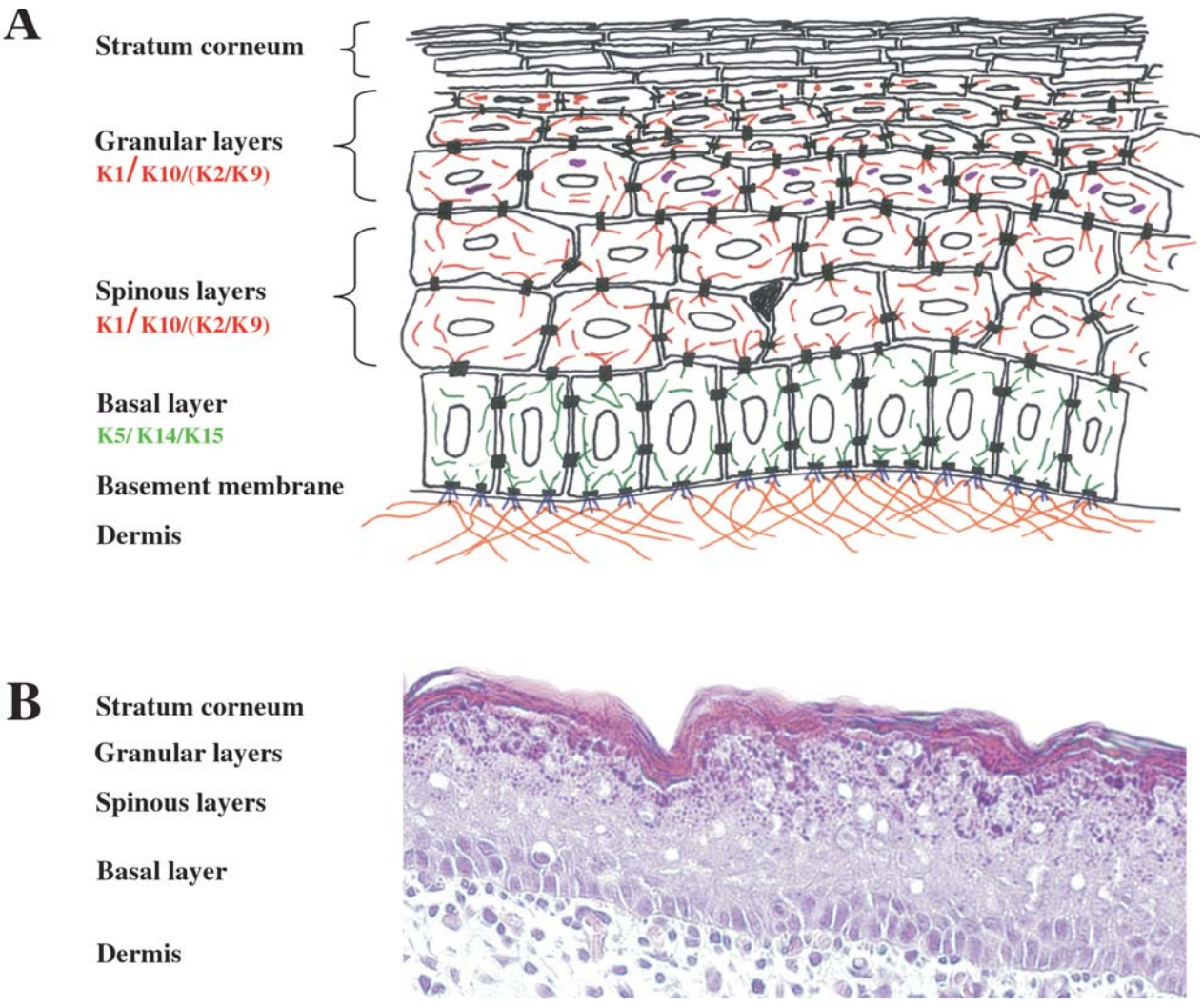

Figure 2. Keratin expression in the epidermis. (*A*) Basal epidermal cells express K5, K14 and K15. As basal cells commit to terminal differentiation, they switch off the expression of K5, K14 and K15 and induce the expression of K1 and K10. As epidermal cells move up through the spinous layers, they express K2e, which can pair with K10. Some keratins are expressed in the epidermis under special circumstances: during wound healing, keratinocytes express K6, K16 and K17. K9 is unique to the suprabasal layers of the palms and soles. (*B*) Cross-section of mouse paw skin stained with haematoxylin and eosin.

mutations have been described in BCIE, clustering at the ends of the central α-helical rod domains in a manner analogous to EBS-DM.

A missense mutation outside the rod domain in the head of K1 was first reported in a single family with diffuse non-epidermolytic palmoplantar keratoderma (DNEPPK) [61]. This mutation was not accompanied by cell lysis, supporting the concept that the primary function of the head domain is not to confer stability to IF but may, rather, involve interaction with other proteins. Sprecher et al. [62] discovered a frameshift mutation in the tail domain of K1 leading to the loss of the majority of its characteristic glycine loops. The patients who carried the mutation suffered from a severe form of BCIE known as ichthyosis hystrix Curth-Macklin. In addition to a distinct cytoskeletal defect, the authors found a mislocalization of the major cornified envelope protein loricrin which normally translocates to desmosomes upon terminal differentiation. Their findings suggested an additional function for K1 in terminal differentiation which depends on its glycine-rich tail region. Whether this involves direct interaction between the K1 tail and the glycine-loop motif in loricrin according to the 'velcro' hypothesis [63] or rather relies on an indirect mechanism is not yet clear. A recent study by Whittock et al. [64] reported a similar frameshift mutation in K1 which surprisingly induced a much milder phenotype of striate palmoplantar keratoderma restricted to the palms and soles in the inspected family. The authors speculated that the reason for the difference between the two patient groups may be the pres-

ence of additional predisposing factors in the severely affected patients.

The epidermal keratin expression pattern described above is modulated by additional keratins which are either constitutively present or induced after tissue injury. The former group is represented by K2e and K9, apparently serving as 'reinforcement' keratins in the upper interfollicular and palmoplantar epidermis [65, 66] and by K19. K19 is restricted to a small group of keratinocytes located in the bulge of the hair follicle [67] and in the deep ridges of the basal cell layer of palmoplantar epidermis [67]. Mutations in the K2e gene were detected in families with ichthyosis bullosa of Siemens (IBS) [68, 69]. Filament aggregation and cytolysis were seen in the upper spinous and granular layers [70]. K9 mutations lead to epidermolytic palmoplantar keratoderma (EPPK), an autosomal dominant skin disease, characterized by thickening of the epidermis of palms and soles [71; and references in ref. 4].

The second group of keratins, induced after disturbances in epidermal homeostasis, comprises K6 isoforms [2, 72–75], K16 and K17 [for a review, see ref. 6]. These three keratins have been suggested to confer particular properties to an activated keratinocyte such as those required in wound healing [76 for review; 77, 78]. K6, K16 and K17 are constitutively present in the hair follicle, tongue and oral epithelia, nail bed and palmoplantar epidermis [73–75]. Mutations in K6a and K16 have been detected in pachyonychia congenita type 1 (PC-1) which is characterized by malformations of nails, palmoplantar and tongue epithelia [20, 79–83]. Analogous mutations in K6b and K17 lead to PC-2 which shows similar symptoms but no oral lesions [80, 84–87]. In addition, K16 mutations lead to focal non-epidermolytic palmoplantar keratoderma (FNEPPK) [88], whereas mutations in K17 cause either steatocystoma multiplex or PC-2 [89]. K4 and K13 mutations cause white sponge nevus (WSN) [90, 91], a benign disorder that affects non-cornifying stratified squamous epithelia (McKusick, MIM 123940 and 148065, respectively). Patients show white plaques in the oral cavity and to a lesser extent in the oesophagus and anogenital mucosa [92].

Meesmann's corneal dystrophy (MCD), an autosomal dominant disorder of the corneal epithelium, is characterized by cytoplasmic inclusions within corneal keratinocytes [93]. This disease is caused by mutations of the corneal keratins K3 and K12 [94, 95]. All K3 and K12 mutations reported to date lie within the helix boundaries.

While the majority of keratin mutations have been detected in epidermis and its appendages, only a few have so far been identified in internal epithelia of the body. These express the type II keratins K7 and K8 and the type I keratins K18, K19, K20 and K23. Many studies on keratins of internal epithelia have focused on the liver. The pattern of keratin expression in hepatocytes consists of K8 and K18, and that of the bile duct epithelium of K7, K8, K18 and K19. The first study identifying a mutation in K18 described a patient who suffered from a cryptogenic liver disease [96]. In a recent publication, mutations were discovered in the K8 gene in 5 patients from a group of 55 patients with cryptogenic liver cirrhosis [97]. These mutations were not conserved compared to those detected in epidermal keratins and how they contributed to liver disease is not clear. Given that the reported mutations represent individual and not familial cases, establishing a genotype-phenotype correlation will be challenging.

Whereas mutations in epidermal keratins clearly interfere with the mechanical function of IF, the mutations in keratins of simple epithelia may, rather, modulate the response of affected cells to external stimuli. Recent studies have provided evidence that K8 and K18 may inhibit apoptosis by modulating the response to tumour necrosis factor (TNF). Caulin et al. [98] demonstrated that epithelial cells lacking K8 and K18 were significantly more sensitive to TNF-mediated apoptosis. These findings were accompanied by an increase in JNK and NF-κB activation, both being downstream targets of the TNF signalling pathway. From the results of an in vitro protein interaction assay, the authors deduced a direct interaction between TNF receptor type 2 (TNFR2) and K8 and K18. In addition, Inada et al. [99] demonstrated that TRADD (TNFR1-associated death domain protein) binds to K18 and K14. TRADD is an adaptor protein for TNFR1 and is involved in TNFR1-mediated apoptosis. TRADD-keratin binding compromised the interaction of TRADD with TNFR1 and subsequent caspase-8 activation and induction of apoptosis. Similar findings resulted from the analysis of primary hepatocytes derived from K8$^{-/-}$ mice [100]. These hepatocytes were more sensitive to Fas-mediated apoptosis. Taken together, these observations suggest that the interaction with receptors and the sequestration of corresponding adaptor molecules may represent a mechanism by which keratins affect cellular signalling. Hence, they may act as mechanotransducers.

Transgenic animal studies

Genetic studies of IF have mostly been confined to human and mouse. Mutation analysis in the former [5] has laid the groundwork for experimental approaches in the mouse in which, to date, approximately half of the known non-hair keratin genes have been inactivated by gene targeting [101; for reviews, see refs. 6, 7]. Keratin 8 was the first keratin gene to be inactivated by gene targeting using homologous recombination [102]. A number of additional keratin genes have been inactivated since then (table 3).

The knockout of K8 [102, 103] has provided evidence for the importance of keratins during embryonic development and in gut epithelia. In the C57Bl/6 strain of mice, embryonic death around E12.5 was observed in 94% of all

Table 3. Keratins inactivated by gene targeting.

Gene(s)	Mutant phenotype	Human pathology	References
K4	mild lysis in oral mucosa	WSN	128
K5	extensive skin blistering, cytolysis of basal cells and death	EBS	111
K6a	none apparent		75, 130, 131
K6a/K6b (double)	extensive blistering in oral mucosa and death	PC	131
K8	phenotype is strain dependent: embryo lethal (C57Bl/6) or colorectal hyperplasia, colitis and liver injury (FVB)		102, 103
K10T	homozygous neonates: lethal; heterozygotes show hyperkeratosis	BCIE	118
K10	none obvious in non-adult mice, compensation via K14 stabilization and hyperproliferation		120, 134
K10 R156C (inducible)	blisters in suprabasal keratinocytes after RU486 treatment	BCIE	126
K12	cell fragility in cornea	MCD	129
K14	skin blistering, cytolysis of basal cells	EBS	109
K14 R131C (inducible)	skin blistering, cytolysis of basal cells after RU486 application	EBS	110
K18	giant hepatocytes, Mallory bodies in ageing mice		105
K19	none apparent		107
K18/K19 (double)	embryonic lethal		101
K8/K19	embryonic lethal		107

K8-deficient embryos [102]. Originally, this was interpreted to result from hepatocyte fragility, as indicated by bleeding in the fetal liver. According to recent experiments, $K8^{-/-}$ mice most likely died from placental malfunction. This was concluded from the embryo aggregation experiments of $K8^{-/-}$ cells with wild-type tetraploid embryos, the latter forming the extraembryonic lineage [104].

K18-deficient mice [105] were viable and fertile, had a normal life span, and showed normal embryonic development. The analysis of several internal epithelia showed that K18 was replaced by K19, which demonstrates that some keratins can be replaced by another one. An unexpected phenotype, however, developed in livers of adult $K18^{-/-}$ mice: about 20% of their hepatocytes showed K8-positive cytoplasmic aggregates, which were identified as Mallory body-like structures. Mallory bodies occur in human diseases like alcoholic hepatitis, biliary cirrhosis and benign and malignant liver tumours [106].

The inactivation of the K19 gene in mice did not produce an obvious phenotype [107]. K19 expression overlaps with that of K18 in many simple epithelia, where K19 may provide a normal cytoskeleton together with K8 in the absence of K18.

The recent combined gene ablations of K8 and K19 [107] and of K18 and K19 [108] have been useful to dissect the role of keratins in mouse development and to eliminate a keratin-based compensation mechanism. Embryos from both lines of double-deficient mice died between E9.5 and E10 but were reported to display different phenotypes. In K18/19 double-knockout mice, no keratin IFs could form at all as these two proteins represent the only type I keratins until late midgestation in the mouse [Hesse and Magin, unpublished data]. The remaining type II proteins were either degraded or formed aggregates [108]. In these mice, embryonic lethality at E9.5 resulted from the fragility of giant trophoblast cells that normally express large quantities of keratins. Rupture of these cells induced the formation of large haematomas and consequently disturbed the nutritional function of the early yolk sac [for a comparison, see ref. 107].

Following the discovery of point mutations in K5 or K14 which lead to EBS [for reviews, see refs. 4, 56], three mouse models were established by gene targeting. We and others have shown that the targeted deletion of K5 or K14 or the introduction of a K14 point mutation in mice provide good animal models for EBS [109–111]. Comparison of these three mouse models revealed a few notable differences. $K5^{-/-}$ mice were most severely affected, as the complete absence of a basal keratin cytoskeleton resulted in devastating cytolysis and in the death of the K5 null mice immediately after birth [111].

The reported phenotype of the $K14^{-/-}$ mice which exhibited generalized blistering of the skin accompanied by increased mortality was similar to that of severe cases of EBS-DM; however, some mice survived the first 3 months of life, possibly due to partial compensation of the K14 loss by endogenous K15 [109].

In contrast to $K14^{-/-}$ mice, the $K5^{-/-}$ mice showed a strong induction of the wound-healing keratin K6 in the suprabasal epidermis of cytolysed areas. In addition, K5- and K14-deficient mice differed with respect to tongue lesions. Unlike $K14^{-/-}$ animals, which displayed basal cell damage along the dorsal tongue [109], $K5^{-/-}$ animals showed a comparable defect along the ventral tongue.

Roop and co-workers utilized the bacteriophage-derived Cre-lox recombination system to produce a mouse model for EBS in which the expression of the targeted gene is

spatially and temporally controlled [110]. To generate a mouse model that mimics EBS-DM at the genetic level, which could provide a good model for therapy approaches, they introduced a point mutation in codon 131 (equivalent to the 'hotspot' mutation of R125 in human K14) in the mouse K14 gene. Upon treatment with the inducer RU486, the mutant keratin was expressed, leading to the formation of blisters. Histological analysis revealed that blisters occurred within the basal layer of the epidermis, as expected. If treatment with RU486 was stopped, blistered areas healed and a normal epidermis continued to form. According to the authors, this was due to a migration of normal keratinocytes into the blister sites where they gave rise to a new epithelium [110]. These mice further showed that a twofold lower expression of the mutant K14 in basal keratinocytes did not lead to a recognizable phenotype. While this observation might be useful for gene therapy approaches, it seems inconsistent with the observation that as little as 2% of a mutant IF subunit is sufficient to lead to the collapse of the cytoskeleton and cause a disease phenotype [112].

How do mutations and deletions of basal keratin genes in the mouse relate to EBS? In humans, the majority of mutations act dominant negatively and lead to perinuclear keratin aggregation and cytolysis. Additionally, a few patients with autosomal recessive EBS have been analysed. The latter were reported to represent 'knockouts' of K14 [113–117] and displayed a phenotype generally less severe than in patients suffering from dominant mutations. While expression of smaller-sized keratin fragments has not been ruled out in any of the recessive patients, all of them clearly have some normal IF in their basal keratinocytes. These seem to provide sufficient overall stability to allow regeneration of stratified epithelia.

K1 and K10 are the major keratin genes expressed in the differentiating layers of the epidermis, representing about 60% of its total protein [52]. Targeted expression of dominant negative forms of K10 produced skin lesions resembling severe forms of BCIE [118, 119]. However, the recent generation of K10$^{-/-}$ mice revealed that K10 was not essential for the stability of the epidermis in mice. In these mice, K5/K14 IFs persisted suprabasally and were able to form IFs and maintain epidermal stability and integrity in the absence of K10. Interestingly, in adult animals, the lack of K10 led to a novel phenotype, characterized by a more than fivefold increase in basal cell proliferation, the induction of K6 and K16 and a mild hyperkeratosis [120]. Most remarkably, an induction of cyclin D1 and of c-Myc in basal and in a restricted number of suprabasal cells, and of the cell cycle regulator 14-3-3σ [121] in postmitotic keratinocytes was noted [120]. Similar changes were recently described in transgenic mice overexpressing c-Myc in their basal epidermis [122, 123]. These data point to an involvement of K10 in the regulation of epidermal cell proliferation. While the molecular mechanisms underlying the increase in basal cell proliferation need to be worked out in K10$^{-/-}$ mice, Paramio et al. [124] have proposed a direct role for K10. They have reported an inhibition of keratinocyte proliferation mediated by the tail domains of K10. Based on more recent experiments [125], the head domain of K10 is now proposed to sequester Akt and atypical PKCζ kinases, thereby acting as a negative regulator of the epidermal cell cycle. The authors argue that interaction of K10 with either of the two kinases inhibits their translocation to the plasma membrane, which is a necessary step in their activation and participation in the PI-3K pathway and results in decreased mitotic activity of K10-expressing cells.

Recently, an inducible model of BCIE was presented by introducing the K10 mutation R154C (equivalent to the 'hotspot' mutation R156C in BCIE patients) in an inducible fashion [126]. In these mice, blisters developed in the suprabasal layers, although basal cells, including stem cells, remained normal. Induced lesions persisted for several months after RU486 application. Because the normal turnover time for mouse epidermis is 8–10 days [127], this suggested that cre-mediated deletion had occurred in epidermal stem cells. This could be verified in the mouse model, using laser capture microscopy of appropriate keratinocytes.

K4 and K13 form the intermediate filaments in the suprabasal cells of stratified, non-cornified epithelia like oesophagus. Minor phenotypic changes have been reported in K4 knockout mice [128]. The lack of K4 was accompanied by cell hyperplasia and cell degenerations in oesophagus and a variety of other tissues. But in general, the tissue architecture remained intact. Induction of K6 has been suggested to compensate for the loss of K4.

K12 is together with K3 expressed in the epithelium of the cornea. K12-deficient mice displayed a mild corneal phenotype with a thinned corneal epithelium [129]. Increased cell fragility was described in the uppermost cell layer, which was depleted of a keratin cytoskeleton. The mild phenotype was explained by the presence of K5/14 IFs, which are normally expressed in cornea [129].

K6 exists as multiple highly related isoforms in the mammalian genome, each encoded by a distinct gene. In mice, K6 genes (K6a and K6b) are constitutively expressed in all major epithelial appendages and are strongly induced in wound healing [73]. Following the inactivation of K6a, which is normally induced in basal and suprabasal cells of stratified epithelia and in the outer root sheath of hair follicles after wounding, no obvious pathological phenotype was reported. In addition, repair of full-thickness wounds was unaffected in these mice [130]. Inactivation of both genes, K6a and K6b, resulted in fragility of the oral mucosa in neonates that interfered with feeding and the homozygous pups died within a week after birth. Interestingly, there was an apparent influence of the genetic

background on the K6a/K6b phenotype. Whereas death was a fully penetrant trait in the 129SvJ-DBA2-C57Bl/6 mixed-strain background [131], approximately 25% of the double-knockout mice exhibited a milder oral phenotype and survived to adulthood in the 129SvJ-C57Bl/6 strain. In the latter case, survival was explained by compensation by K6hf, a type II keratin that is related to K5 in sequence and to K6 isoforms in its expression pattern [132].

Several important conclusions can be made from the genetically engineered mice. First, keratins clearly provide a cytoskeletal backbone not only to stratified but also to certain simple epithelia during embryonic development and in adult life. At first glance, the lack of keratin IFs as in $K5^{-/-}$ [111] and $K18^{-/-}/19^{-/-}$ mice [107, 108], and the expression of mutations in K10 and K14 mice [110, 133] lead to a similar histopathology. Nearly all these phenotypes include fragility of specific populations of epithelial cells. This reflects the general essential role of keratins in maintaining the structural integrity of epithelial cells and tissues. The mechanisms leading to cell and tissue fragility, however, appear to differ. The comparative analysis of K10 mutant [118, 126] and $K10^{-/-}$mice [134] demonstrated that the absence of the protein is without detrimental effects whereas the presence of a mutation in the same protein has devastating consequences. Second, gene deletions of a type I or II keratin normally co-expressed may have a different outcome. The main differences between K5 and K14 null mice [109, 111] resides in the severity of the blistering observed, which may largely depend on the presence of K15 [109] or K17 [135]. The severity of the $K5^{-/-}$ phenotype suggests that the loss of K5 may lead to early death in humans. Another example is illustrated by the distinct liver phenotypes in K8- and K18-deficient mice [103, 105]. Third, a null mutation in the mouse does not necessarily reflect the phenotype of a disease that arises from, e.g. point mutations in the orthologous human gene. Most disease-causing alterations in keratin genes consist of (missense) mutations or small deletions that act dominantly and may represent gain-of-function mutations.

Finally, the generation of inducible mouse models, in which gene targeting is spatially and temporally controlled [110, 133], offers the possibility to test gene therapy approaches for a given disease.

Keratin modifications, keratin-associated proteins and signalling

Phosphorylation is a dynamic process that appears to be involved in regulating keratin solubility and filament organization and function. Zhou et al. [136] characterized the major phosphorylation site of human K19 and its role in keratin filament organization. The cell-cycle-dependent phosphorylation of K18 at S33 allows binding of 14-3-3 proteins which play a role in keratin organization and subcellular distribution [137]. The mutation of this phosphorylation site predisposes to hepatotoxic injury in transgenic mice [138] and leads to a mislocalization of IFs in pancreatic acinar cells [139]. The functional significance of the interaction between K18 and 14-3-3 has not yet been determined. On the one hand, there is evidence for an active role of 14-3-3 in the organization of keratin IFs as well as a role for keratins in the sequestration of 14-3-3, thereby modulating cell cycle progression [140]. Keratin may function as an adapter to bring other molecules in close proximity or it may displace other 14-3-3-binding proteins. For the latter, there is evidence from studies on vimentin [141]. The absence of keratin IFs in K8 and K18 knockout mice leads to a relocalization of 14-3-3ζ from the cytoplasm to the nucleus. Analysis of K8 and K18 knockout mice revealed alterations in the architecture of their liver parenchyma and the formation of giant, multinucleated cells in both mice [142]. By influencing the activity of cdc25, a cell-cycle-regulating phosphatase, 14-3-3ζ may cause a disturbance in mitosis and an S/G2 phase stop, thereby leading to the observed abnormal tissue architecture.

Keratin hyperphosphorylation also occurs in response to injury. The stress-associated kinases p38 and Jun kinase (JNK) are important for these phosphorylation events. In K8, amino acid S73 is a phosphorylation site for p38 kinase, and its phosphorylation may play a significant role in keratin filament reorganization [139]. He et al. [143] demonstrated that K8 was also phosphorylated at S73 by JNK in vitro and that JNK was associated with K8 in vivo, correlating with the decreased ability of JNK to phosphorylate the endogenous c-Jun. The effect of phosphorylation is not understood, but possibly includes modulation of filament organization and distribution or interaction with keratin-associated proteins, which may affect disease pathogenesis.

Until recently, proteases involved in the turnover of keratins were not known. Caspase-mediated keratin degradation has been reported for type I keratins [144–147]. Caulin et al. [144] described for the first time the caspase cleavage of K18 and the reorganization of IFs during apoptosis. More recently, Ku and Omary [148] demonstrated that all type I keratins are likely to be substrates for caspases. Keratin hyperphosphorylation occurs upon an apoptotic signal and provides significant protection from caspase-mediated degradation. The authors argued that mutations within the caspase recognition site might affect proteolysis.

Ku and Omary [149] demonstrated that keratin ubiquitination leads to proteasome-mediated turnover of keratins, at least in cultured cells. In addition they showed that phosphorylation of K8 but not of K18 can protect from ubiquitination.

Transglutamylation occurs for example in the cornified cell envelope and links several type II keratins (K1, K2e, K5 and K6) e. g. to loricrin, involucrin and envoplakin to provide a compact protective structure. Candi et al. [55] demonstrated that a highly conserved lysine residue in the head domain was essential for the attachment of these keratin IFs to the cornified cell envelope. Interestingly, this residue in K1 is mutated in a family with non-epidermolytic palmo-plantar keratoderma, which suggests that the fragility of the palms and soles of these patients may be due to a defective cross-linking of keratin filaments to the cornified envelope [55].

The interaction of keratins with anchoring junctions at the cell membrane is mediated by a family of cytoskeletal-binding proteins, named plakins [for review, see refs. 12, 150]. The three founding members of the plakin family are desmoplakin, plectin and bullous pemphigoid antigen 1 (BPAG1); other plakins include envoplakin and periplakin [151; reviewed in refs. 152, 153]. Periplakin polypeptides are capable of associating with IFs in transfected cells [154] whereas the potential involvement of envoplakin has yet to be determined. Desmoplakin is a major structural protein of the cytoplasmic plaque of desmosomes which are common in epithelial tissues. Desmoplakin plays an important role in anchoring cytoplasmic IF to the cell membrane thereby providing a three-dimensional supracellular scaffolding that provides tissues with mechanical stability [155, 156]. The C-terminus of desmoplakin is responsible for the interaction with IFs [157]. For epidermal type II keratins, a short subdomain of their head has been invoked to mediate binding to desmoplakin [157–159]. This interaction may be inhibited by phosphorylation of a serine in the C-terminus of desmoplakin which may represent a mechanism for modulation and dynamics [160].

At the hemidesmosome which mediates the adhesion of epithelial cells to the extracellular matrix in an $\alpha_6\beta_4$-integrin-dependent fashion, BPAG-1 [161] and plectin [162] appear to represent the major attachment sites for keratin IFs [163]. In addition, plectin is found in the cytoplasm, where it is often associated with IFs [46].

Polycystin-1 is a ubiquitously expressed multispan transmembrane protein which is distributed with IFs at the desmosomal plaque. It supposedly interacts via its C-terminus with K8, K18 as well as with vimentin and desmin, at least in a yeast two-hybrid screen [164]. The localization of polycystin-1 at desmosomes may be crucial for signalling, as it has been shown to bind directly to G proteins [165].

As keratinocytes differentiate, they synthesize profilaggrin, a granular layer protein, which is processed to filaggrin and then promotes the bundling of keratin filaments into large macrofibrils [166, 167]. Interestingly, there are a number of disorders in human epidermis in which the expression and processing of profilaggrin and/or function of filaggrin appear to be abnormal, so that this system also appears to represent an example of an IF-associated protein involved in disease [42]. Trichohyalin which was discovered as a keratin-binding partner and cross-linking protein in the inner root sheath of the hair follicle [168] has also been shown to be involved in skin disorders [169].

Future perspectives

The past decade has seen major breakthroughs in IF research in unravelling the role of keratins in human disease and understanding their structure at the atomic level. Given the power of inducible mouse models [133], advances in green fluorescence protein technology [170] and the breakthrough in structure determination [31], many tools are now at hand to focus on some of the most pressing questions old and new. With respect to the complex keratin expression pattern, we can now address the question of its functional significance, by gene switching experiments in mice. The recently reported interaction of keratins – both as polymers and as soluble oligomers – with key players of cell cycle regulation like 14-3-3, Akt and of the apoptotic machinery has put keratins on the map of cell signalling. Keeping in mind the rapid changes in keratin expression seen in wound healing and metastasis which partially repeat developmental processes, it is surprising how little we know about whether members of the keratin family are instructed by key instructive processes like wnt signalling. If one considers the shape and differentiated properties of cells and tissues as the result of a network of protein interactions, it would not come as a surprise if keratins, equipped with the power of self-organization and protein interaction, are an important part of such signalling cascades. Taking this view, the participation of keratin mutations in cryptogenic liver disease and in other multifactorial diseases can be analyzed in a different way. To that end, we must answer the following questions: How are keratins assembled in vivo? What determines their specific subcellular localization? How dynamic are they in cultured cells and in vivo? What is the significance of their association with proteins known and yet to come?

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