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## Review Article

## Structural and regulatory functions of keratins

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## ABSTRACT

The diversity of epithelial functions is reflected by the expression of distinct keratin pairs that are responsible to protect epithelial cells against mechanical stress and to act as signaling platforms. The keratin cytoskeleton integrates these functions by forming a supracellular scaffold that connects at desmosomal cell–cell adhesions. Multiple human diseases and murine knockouts in which the integrity of this system is destroyed testify to its importance as a mechanical stabilizer in certain epithelia. Yet, surprisingly little is known about the precise mechanisms responsible for assembly and disease pathology. In addition to these structural aspects of keratin function, experimental evidence accumulating in recent years has led to a much more complex view of the keratin cytoskeleton. Distinct keratins emerge as highly dynamic scaffolds in different settings and contribute to cell size determination, translation control, proliferation, cell type-specific organelle transport, malignant transformation and various stress responses. All of these properties are controlled by highly complex patterns of phosphorylation and molecular associations.

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## Introduction

The intermediate filament (IF) cytoskeleton of all epithelia is built from type I and type II keratins which are encoded by two gene families comprising >50 genes in humans and the mouse, respectively [1,2]. These are expressed in various combinations of at least one type I and one type II keratin from the 2-cell stage mouse embryo stage onwards in a tissue-specific fashion [3,4]. Many epithelial cells in the adult express >10 different keratins [2]. The prevailing principle responsible for tissue-specific keratin function and regulation appears to result from the transcriptional regulation of their genes and the intrinsic property of the >50 different protein sequences. The former gives rise to exquisite, cell-type-specific keratin profiles. The latter has endowed them with distinct micro-mechanical and assembly properties, half-life times and provides unique target sites for a large number of kinases and phosphatases [5]. Keratins act as protein scaffolds with structural and regulatory functions in a cell-type-specific manner, as underscored by keratinopathies [6] and knockout mice [7,8]. The analysis of these model systems carried out so far suggests that keratins have a major impact on cell architecture, cell size and proliferation depending on cell context but do not act as major regulators of epithelial differentiation. Furthermore, the identification of keratin-associated proteins and the analysis of keratin phosphorylation, in combination with the identification of keratin mutations in rare diseases, are beginning to provide insights into the molecular mechanisms by which they act. The current review discusses novel, most recently discovered functions (for additional references, see [5,8-10]).

## Mechanical and structural functions of keratins

In relation to the intrinsically dynamic actin filaments and microtubules, IFs and especially the abundant keratin filaments (KFs) in epidermal keratinocytes have long been considered to be the "bones" of the cellular scaffold. This concept insinuated that keratins confer rigidity to epithelial cells much like the steel frame in a building. Recent advances, however, show that this comparison is much too simplistic and that the keratin cytoskeleton is quite unique by allowing rapid and localized restructuring and that it is involved in an intimate crosstalk with all aspects of cell behavior including migration, differentiation and proliferation.

The *in vitro* micromechanical properties of KFs differ from those of F-actin and microtubules. Thus, KFs as well as other IFs are characterized by high viscoelasticity and flexibility but harden in response to increasing deformation, yielding rapidly without breakage and recovering quickly upon ces-

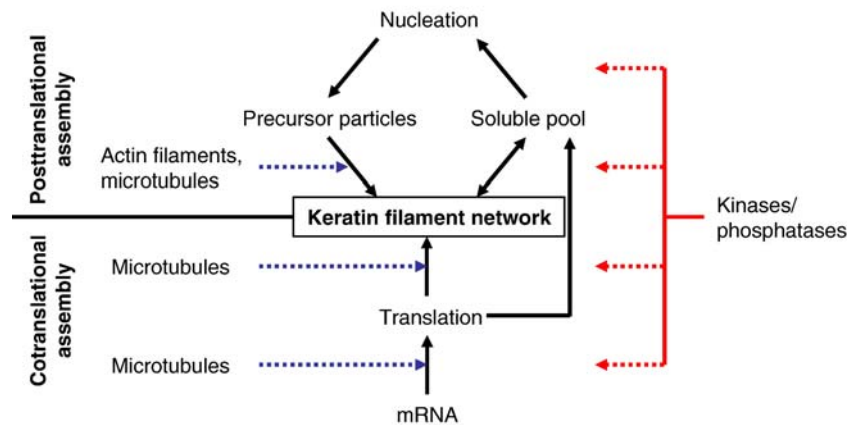
sation of the deformation stress (e.g., [11,12]). An interesting aspect of the unique mechanical properties is the possibility that the constitutive subunits allow axial sliding as suggested for vimentin filaments [13]. Furthermore, self-induced bundling is another unusual feature that is salt and pH dependent and presumably contributes to filament network stiffness (e.g., [14]), a view that is also supported by biophysical examinations of KF networks in cells [15]. In addition, KFs form relatively stiff gels *in vitro*, especially when subjected to small deformations [11,16]. These properties may be significantly altered, however, by various factors as suggested by the observation that KF gels soften significantly in the presence of phospholipids [17].

Analyses of the keratin cytoskeleton in living cells synthesizing fluorescently labelled keratin hybrids revealed an astounding degree of flexibility and intrinsic dynamics even in stationary interphase cells [18-20]. The entire network was shown to be in constant motion irrespective of bundle size and desmosomal anchorage. Most prominent were oscillations [19] including propagated wave-like distortions [18]. It is unknown, however, whether these processes are altered in the presence of mechanical stress which might confer increased tensile strength to the keratin cytoskeleton. In addition, a continuous inward-directed translocation was observed within the keratin network that could be inhibited by nocodazole treatment [19]. Probably the most conspicuous type of dynamic process was noted in the cell periphery, where growing particles were repetitively generated that were in a continuous inward-directed motion [18,20,21].

## Co-translational versus posttranslational keratin assembly

Two different hypotheses have been put forward to explain how the KF network is altered in order to respond to changing mechanical and structural requirements: co-translational integration of keratin particles at multiple sites throughout the entire network *versus* posttranslational integration of keratin particles originating from a juxtamembranous compartment into the peripheral network (Fig. 1). The first, non-vectorial model of KF network modulation was originally proposed on the basis of observations obtained either in epithelial cells that had been microinjected with labelled keratins [22,23] or in non-epithelial cells that were injected with epithelial mRNA thus resulting in *de novo* KF network formation [24]. In both situations, integration and assembly of the exogenous polypeptides were noted at multiple sites. Since similar phenomena were seen for other IF types (e.g., [25-27]), it was assumed to be the major mechanism of network modulation. This notion

## Potential modes of keratin assembly



**Fig. 1 – Modes of keratin assembly.** Co-translational assembly (bottom part): Translation of type I and type II keratin mRNAs can occur throughout the cytoplasm, although microtubules may contribute to the dispersion of translationally inactive mRNA particles along keratin filaments. During or following translation, heterodimeric subunits or higher order precursors form and integrate into the pre-existing keratin cytoskeleton, in analogy to peripherin [28]. Microtubule-mediated transport might also be involved in the transport of precursors to their site(s) of integration. (-) Posttranslational assembly (top part): Soluble and rapidly diffusible keratin subunits assemble in the cell periphery which provides a specific microenvironment favouring keratin polymerization [29]. Regulation of polymerization competence of keratin filament subunits might be accomplished by posttranslational modification(s) and/or association with hsc70 [32]. Progressive assembly occurs during actin-dependent movement of newly-formed keratin particles away from the initial nucleation sites toward the cell centre. In addition to assembly of newly synthesized keratins, there is a constant exchange between their polymeric and oligomeric state. Whether integration of precursors into the cytoskeleton occurs at distinct sites is not known. Numerous kinases and phosphatases control the formation and maintenance of the keratin cytoskeleton at various levels [10].

was further supported by bleaching experiments in cells producing fluorescent keratins [18]. These experiments also demonstrated that kinetic differences exist between the various IF types. The model was recently extended by suggesting that IF subunits are co-translationally integrated and assembled, based on observations concerning the IF polypeptide peripherin [28]. This mechanism may also apply to keratins, considering the co-localization of K8 and K18 mRNA-containing particles [28]. Furthermore, translationally inactive mRNA complexes were shown to be delivered in a microtubule-dependent process to sites of active translation which was initiated upon release from the transport machinery [28]. This mechanism would explain how the rapidly and spontaneously assembling KF polypeptides are targeted to specific sites of integration. Although this model is quite attractive, it does not provide an explanation as to how and where new networks are formed and exactly how polarized network alterations are accomplished. These aspects are better explained by the second scenario that has been worked out for keratins in cells producing fluorescent keratin chimeras. Here, KF precursors were seen to form preferentially in the cell periphery often in close vicinity to focal adhesion sites [20,21,29]. It was suggested that this particular cellular topology provides a microenvironment favouring keratin polymerization by tapping on the rapidly diffusible cytoplasmic pool of soluble keratin oligomers. This could be facilitated by either removing an as yet still elusive solubility factor (see, e.g., [30]) and/or by altering

the phosphorylation status of keratin polypeptides known to affect their polymerization status (review in [31]). hsc70 was recently found to associate with K5 through a motif located in the head domain, but not with K14 *in vitro*. Colocalization between the K5 head domain and hsc70 was also found upon co-transfection into cultured cells. Notably, upon co-translation of K5, K14 and hsc70 in a reticulocyte lysate, followed by co-immunoprecipitation, only K5-hsc70 complexes were found. Possibly, this provides a mechanism to regulate the earliest stages of keratin assembly [32]. The newly formed KF precursors grow continuously, move away from their sites of origin to the peripheral KF network by an actin-dependent mechanism and either integrate into the network end-on or establish a new network that is further extended by a microtubule-dependent process [20,33]. This model allows restructuring of the network in the absence of protein biosynthesis as is the case, e.g., during mitosis. It also assigns specific tasks to actin filaments and microtubules as the other major components of the integrated cytoskeletal network, suggesting a mechanism by which the KF network grows toward the leading edge of the cell, and identifies a compartment that could act as a nucleation centre. It is not clear at present, however, whether these phenomena are valid only with respect to keratins or are of more general relevance. Both models are compatible with continued maintenance of the IF network even in situations of intense restructuring as it occurs in migrating, differentiating or dividing cells and would

thus support uninterrupted mechanical resilience. Future studies will show whether either one or both mechanisms are active in various cell types and how they relate to the *in vivo* situation.

### Keratin deficiency and disease pathology

Nature provided the most compelling evidence for the seminal contribution of keratins to the mechanical stability of epithelia. The multiple blistering diseases caused by single point mutations of keratins testify to this function convincingly and many reviews have covered the occurring diversity in quite some detail (see, e.g., [4,6,8,34,35]). It is apparent that mechanical resilience is compromised in these diseases, most notably in those of the epidermolysis bullosa simplex (EBS)-type, since mechanical trauma exacerbates blistering. Yet, the molecular pathomechanism is still not known, especially given the observation that the mutant polypeptides are capable of forming elongated and perfectly shaped IFs *in vitro* [36]. Thus, higher order assembly states such as bundle formation and network formation *via* associated linker proteins are compromised and/or alterations in signaling pathways contribute to the disease phenotypes. Furthermore, cytolysis in EBS often occurs in a specific zone in the basal part of the cytoplasm just above the hemidesmosomes [37]. It will be interesting to find out whether this region coincides with the peripheral transition zone in which granules containing mutant keratins dissolve in cultured cells and whether it corresponds to the site where KF precursors are usually integrated into the peripheral network [20,38].

Clearly, the mechanical functions of epidermal keratins must be seen in conjunction with their specific anchorage structures, the desmosomes. Again, nature already performed the crucial “experiments”. Most informative is a recent report on patients with compound heterozygote mutations of the desmoplakin gene [39]. In this instance, both alleles were altered such that the resulting desmoplakin polypeptides lacked the keratin binding sites. As a consequence, desmosomes were still formed and KFs were abundant, yet their connection was severed leading to excessive blister formation and causing neonatal lethality. On the other hand, desmosome formation is not dependent on an intact KF network [40] although desmoplakin turnover at cell-cell adhesion sites is altered in keratin network-deficient hepatocytes [41,42] and in epithelial cell lines with downregulated keratins [43]. An interesting aspect of the desmosome-keratin connection is the possibility that both act in concert as mechanosensory devices to elicit specific cellular responses. This view is supported by the observation that wounding-induced protein kinase C $\alpha$  localization in the cell periphery induces alterations in desmosomal adhesion [44] and that antibodies directed against desmosomal cadherins affect p38 and RhoA signaling as well as keratin network organization [45–47].

Further evidence for the prominent mechanical function of keratins was obtained in murine knockout experiments. Given the redundancy of keratin polypeptides in the various epithelial tissues, it is not surprising that the “strongest” phenotypes occur in cells in which compensation by other keratins is not possible. Thus, deletion of K5, the sole type II

keratin polypeptide in basal cells, leads to pronounced cytolysis and neonatal lethality [48] whereas the phenotype of K14<sup>-/-</sup> mice is less pronounced, most likely due to compensation by the related type I keratin 15 [49]. Accordingly, patients lacking K14 exhibit a relatively mild EBS phenotype [50–53], whereas humans lacking K5 altogether have not been identified likely due to associated lethality. The mechanical function of keratins may not be restricted to surface epithelia, the most prominent example being the K8/19 and K18/19 null animals which die early during development caused by a deficiency of giant trophoblast cells [54,55]. The KF-free trophoblast is incapable of withstanding the increasing maternal blood pressure in the developing placental intervillous space. Similarly, hepatocyte fragility was observed in response to stress in a K8<sup>-/-</sup> background [56] and in the presence of dominant-negative K18 R89C mutants [57]. Whether hepatocyte and trophoblast fragility result from structural defects or relate to a function of keratins in modulating apoptosis or stress signaling (see below) remains a challenging issue.

Maintenance of cell shape is of particular importance to epithelial cells resulting in distinct morphologies of the various surface-lining tissues. A particularly striking example of the importance of the keratin cytoskeleton was reported in intestinal cells where keratins are usually concentrated in the terminal web as part of a two-dimensional meshwork that is associated with multiple cell adhesion sites and anchors the microvillar actin filaments. Microvilli of K8<sup>-/-</sup> mice were found to be significantly reduced coincident with a redistribution of microtubules and profound disturbances in apical membrane protein targeting [58,59]. It was further demonstrated that the actin-ezrin scaffold cannot be assembled properly in the absence of K8 [60]. Mechanistically, keratins could contribute to apical polarity by interaction with the  $\gamma$ -tubulin protein GCP6 in a Cdk1 kinase-dependent manner. In cells lacking K8 or expressing a S1397D GCP6 mutant deficient in Cdk1-binding, centrosomes were delocalized. This suggests that GCP6 is one mediator of keratins to the microtubule-organizing centre and thereby contributes to its apical localization in polarized epithelial cells [61].

### Keratin-dependent cell migration and wound healing

In migrating cells, short- and long-term alterations are observed in the KF system that are elicited by and coordinated with altered cell polarity and differentiation programs. The altered KF cytoskeleton may then in turn determine the altered cell behavior. The recently reported link between KF formation and focal adhesions in lamellipodia provides a rapid mechanism to attenuate the KF cytoskeleton in specific cellular subdomains [21,29]. Interestingly, vimentin accumulates at similar adhesion sites that were termed vimentin-associated matrix adhesions [62]. It was further shown that vimentin binds to integrins [63,64] which could thereby recruit it to certain membrane sites. Conversely, vimentin affects integrin recycling in a PKC-phosphorylation-dependent manner [65] thereby possibly affecting adhesion site formation. A similar interdependency is suggested by delayed FAK

autophosphorylation, a target for integrin signaling in K8-deficient hepatocytes [66]. Plectin is another potential linker protein which binds keratins and vimentin [67] and is localized in cell-matrix-adhesion sites [68]. The mutual relationship between IFs and focal adhesions became also apparent in knockdown/knockout experiments in which either the focal adhesion component talin was reduced resulting in inhibition of KF precursor formation [29] or vimentin was depleted leading to reduced focal adhesion size, decreased adhesion and impaired migration [69–71]. In a setting of epidermal wound healing in mid-gestation mouse embryos, no defects were noted in K8-deficient compared to control embryos. In contrast, K8 downregulation was recently shown to inhibit collective epithelial sheet migration and spreading [43,66]. Yet, wound closure was accelerated in the absence of K8 in vimentin-positive, cultured epithelial cells [43] as well as in EBS cell lines producing mutant K14 or K5 [72] and in K6-negative cells [73]. These apparently conflicting data may be explained by context-dependent expression of other keratins or their modification. Reorganization of the K8/18 network by sphingosylphosphorylcholine, for example, elevated migration [15]. A cross-talk between plectin-controlled keratin cytoarchitecture and migration was also recently reported in plectin-deficient cells exhibiting increased migration rates with increased susceptibility of keratin network reduction to osmotic shock and okadaic acid [74]. Evidence was provided that these altered properties are a consequence of plectin-dependent keratin network alterations leading to altered MAP kinase signaling and not merely a result of altered structural properties of the cytoskeleton [74].

Functional consequences of transient alterations in the keratin composition have been investigated in epidermal wounding. In this situation, transcription of the *Krt6/16/17* genes is induced and *Krt1/10* gene expression is reduced, presumably endowing the cell with a more pliable cytoskeleton that favours keratinocyte migration for wound closure [75,76]. Accordingly, loss of K17 compromised wound healing in mouse embryos [77] and loss of K6a led to delayed re-epithelialization upon partial thickness skin wounding [78]. While the transcription of the two major *Krt6* alleles in the mouse, K6a and K6b, is strongly induced in activated keratinocytes of the skin, this is not the case in the mammary gland, highlighting the context-dependent function of keratins. In the mouse mammary gland, K6a and K6b coincide with expression of the steroid receptor in cells that do not cycle actively. The combined deletion of both keratin genes has no obvious effect on mammary gland development but is accompanied by an increase in the number of steroid receptor-positive cells that are in a proliferative state [79]. In addition to the truly inducible keratins K6, K16 and K17, additional keratins are up- or downregulated following tissue injury (for a discussion, see [80]).

### **Keratins and the regulation of cell size and cell proliferation**

The regulation of the cell cycle and of cell size provides the basis for the diversity of mammalian tissues and their enormous adaptability to environmental cues. Although the

mechanisms that coordinate cell size and proliferation remain largely unknown at present, the protein kinase mammalian target of rapamycin (mTOR) and its regulation by 14-3-3 proteins have been shown to play a major role in both [81]. Whether expression of keratin subtypes has an impact on differentiated functions of various epithelial cells is just beginning to be investigated. Recently, mouse genetics and human diseases have provided strong support for the idea that changes in the expression of certain keratins have a profound impact on cell size, cell proliferation and the response to stress [5,8]. A key observation from many studies is that keratin expression changes rapidly during differentiation, tissue injury and metastasis [4]. Thus, during wound healing in the skin, differentiated epidermal keratinocytes rapidly repress keratins 1 and 10, while wound-proximal cells induce keratins K6, K16 and K17. Furthermore, the delayed wound closure in K17 null embryos is accompanied by a decreased size of epithelial cells bordering the wound edge [77]. What could be the molecular mechanism behind these changes? Cultured K17 null keratinocytes are smaller in size and display a reduction of total protein synthesis by ~20 %, accompanied by reduced phosphorylation of the kinases Akt and mTOR [82]. An in-depth analysis of K17-associated proteins revealed a major role for the epithelial-specific 14-3-3 $\sigma$  isoform that binds to one or two consensus motifs in the K17 head domain. 14-3-3 proteins represent a seven member family of highly conserved adapter proteins that regulate the subcellular distribution and activity of > 100 proteins, mostly in a serine/threonine phosphorylation-dependent manner [83]. Of note, 14-3-3 proteins sequester the TSC1/TSC2 protein complex that serves as a negative regulator of mTOR [84]. Hypophosphorylation or absence of K17 permits re-localization of 14-3-3 proteins to the nucleus, preventing mTOR activation. This remarkable phenotype was rescued by re-expression of wild-type K17, but not of a mutant unable to bind 14-3-3 [82].

How general is the interaction between keratins and 14-3-3 proteins, and, most importantly, how is it regulated? Outside the epidermis, the most compelling evidence stems from an increased nuclear accumulation of 14-3-3 $\xi$  in hepatocytes of K8 and K18 null mice. In these, a significant number of hepatocytes were multinucleated giant cells devoid of cortical actin filaments. These cells presented enlarged nuclei with a doubled DNA content. This indicated that the absence of K8 or K18 disturbs the cell cycle, drives cells into the G2-S phase and leads to aberrant cytokinesis [42]. Subsequently, Ku, Omary and coworkers examined a K18 S33A mutation in transgenic mice. Upon partial hepatectomy, a well-established model of liver regeneration, they found anomalous mitotic figures, a partial mitotic arrest and nuclear speckles of 14-3-3 $\xi$  that remained throughout mitosis [85]. However, overall liver regeneration was not impaired and potential changes in protein synthesis were not assessed.

Cell cycle progression requires multiple signal cascades that regulate the progression through mitosis by phosphorylation and dephosphorylation of Cdc2/CyclinB. The phosphatase Cdc25 activates this complex at the G2/M transition by dephosphorylation of two major sites [86]. Cdc25 itself is controlled by phosphorylation and subsequent binding to 14-3-3 $\sigma$  which masks a nuclear localization signal [87]. In a search

for additional proteins regulating Cdc25 activity before entry into mitosis, it was found that phosphorylation of Cdc25 at T138 was not sufficient for 14-3-3 release but required additional phosphorylation of keratins K8 and K18 to provide a surplus of 14-3-3 binding sites [88]. Treatment of cell extracts with okadaic acid, known to induce both hyperphosphorylation and solubilization of keratins, increased the 14-3-3-keratin interaction. Provided the Cdc2/CyclinB complex has the ability to phosphorylate keratins, this could represent a positive feedback activation of Cdc25 by Cdc2 or by kinases active at the entry into mitosis controlled by the former, as suggested by the authors [88]. This exciting study which is the first to link keratins to the cell cycle machinery at the molecular level, raises a number of issues. First, which of the many residues on K8, K18 and K19 that can be phosphorylated serve as binding sites for 14-3-3 [10]? Second, to which extent are keratins phosphorylated and to which extent of the total are 14-3-3 proteins sequestered? Given that there are seven family members that can homo- or heterodimerize, this is a challenging issue. Third, what are the functional consequences of keratin hyperphosphorylation in this particular setting? Phosphorylation is well known to alter the polymer state of keratins in a local or global manner. This should have considerable consequences for cell cycle progression and cytokinesis, as shown for vimentin. Here, mutation of distinct Ser-residues cause a cytokinesis defect [89]. Finally, how is the temporal and spatial phosphorylation of keratins regulated? To that end, searching for scaffold proteins that link keratins to members of the Rho family of GTPases might prove fruitful [90].

### Keratins and vesicle transport

Skin pigmentation depends on the synthesis of melanin by neural crest-derived melanocytes and the transfer of melanosomes into basal epidermal and hair follicle keratinocytes. Melanosomes are lysosome-related organelles which protect the skin against UV light by forming supranuclear caps in keratinocytes. Melanosome biogenesis and transport in melanocytes depend on the regulation of actin and myosin motors by the GTPase Rab27a and Rab effectors [91]. Little is known about transport and distribution of melanosomes in keratinocytes where they exert their major function. Rare skin disorders including EBS with mottled pigmentation, Dowling-Degos disease and Naegeli-Franceschetti-Jadassohn syndrome are caused by non-canonical mutations residing in the head domains of K5 and K14 [92-96]. Unlike most other keratin disorders, the ones above display an intact keratin cytoskeleton but a disorganized distribution of melanosomes in keratinocytes. Further support for a role of keratins in melanosome transport comes from the analysis of mice with chemically induced mutations in K1, K2e and K4 [97-99]. Remarkably, the pigmentation defects seen in Dowling-Degos disease arise from K5 haploinsufficiency, making dominant-negative effects very unlikely [92].

What is the evidence that keratins orchestrate melanosome transport and localization? Both co-localization and protein interaction studies hint towards an interaction of the K5 head domain with dynein light and intermediate chains

[92]. Dynein is also known to play a major role in the retrograde transport of IF proteins. Conversely, the availability of dynein can be regulated by interaction with IF proteins [100]. In support, dynein is involved in the centripetal transport of melanosomes in keratinocytes where they form supranuclear caps [101]. The keratin-related IF proteins vimentin, peripherin and  $\alpha$ -internexin interact directly with the  $\delta$ -subunit of the adapter complex AP-3 involved in clathrin-mediated endocytosis [102]. In a similar fashion, keratins could regulate the availability and positioning of melanosome-resident AP-3 complexes in keratinocytes. Alternatively, keratins could regulate the interaction of AP-3-dependent vesicles with motor proteins and thereby affect organelle transport and membrane protein transport. In a recent proteomic analysis of melanosomes purified from melanocytes, more than 600 resident proteins were identified, among these several 14-3-3 isoforms [103]. The well-established 14-3-3-keratin connection offers additional mechanisms for the regulation of melanosome transport by keratins. A provocative thought is that 14-3-3 proteins, by binding both to melanosomes and to one end of KF precursors might promote unidirectional keratin polymerization and thereby support movement of melanosomes. Alternatively, 14-3-3 activity could help to induce local KF restructuring thereby freeing a passageway for these comparatively large organelles. Given that melanosomes are closely related to lysosomes, a careful analysis of the role of keratins in their transport and distribution holds great promise for the understanding of a general role of IF proteins in vesicle transport.

### Keratins and the stress response

Emerging data have proven beyond doubt the involvement of keratin IFs in resistance towards multiple kinds of stresses and to apoptosis. By using one of the most prevalent keratin mutations in humans that is associated with cirrhosis and fibrosis progression, K8 G61C, it was reported that transgenic mice over-expressing this mutant showed an increased susceptibility to stress-induced liver injury and apoptosis [104]. The mutation inhibits phosphorylation of K8 at S73 by stress-activated protein kinases such as p38, JNK and p42 [104]. Consequently, a similar susceptibility to stress was also observed in the S73A mutant wherein the site for phosphorylation is destroyed. As mentioned above, the authors suggested that keratins, during stress, would act as a "phosphate sponge" absorbing the stress-activated phosphate kinases, thereby reducing their untoward effects and hence protect the cells from injury. In accordance, K18 associates with Raf-1 kinase under basal conditions [105]. Raf-activation during oxidative stress or other toxin exposure disrupts the keratin-Raf-1 association in a phosphorylation-dependent manner. Thereby, keratins can regulate Raf-1 kinase signaling potential by kinase sequestration, activation, inactivation or compartmentalization. Furthermore, in an injury model of lung alveolar epithelial cells, the keratin cytoskeleton disassembles partially in the presence of shear stress coincident with PKC $\delta$ -mediated phosphorylation of K8 at S73, indicating alterations in keratin organization in response to mechanical stimuli [106]. Evidence for such a

non-structural role of KFs was also obtained in hepatocytes isolated from mice synthesizing keratins containing mutated phosphorylation sites including K8 S73 [104] and K18 S33/S52 [85,107]. These cells were mechanically stable following liver perfusion. Furthermore, in the absence of a normal K18 gene, expression of the dominant-negative mutant K18 R89C caused embryo lethality in mice that was fully rescued by one K18 or K19 allele. In adult mice, aggregates co-existed with filaments, cells were mechanically stable and an increased phosphorylation of endogenous K8 and K18 was observed. It was speculated that aggregates may have a protective function as suggested by the “phosphate sponge” model [104,108].

Differential and epitope-specific keratin phosphorylation has been reported for K20 in the small intestine [109]. Phosphorylation of K20 S13 occurred exclusively in mucus-secreting goblet cells but not in the other K20-expressing enterocytes. Furthermore, starvation-induced mucin secretion resulted in K20 hyperphosphorylation. These observations indicated that the spatiotemporal and differential regulation of keratin phosphorylation relates to intricate functional properties of specific epithelial cell types. In colon carcinoma-derived HT29 and Caco cells phosphorylation of K8, K18, K19 and K20 are individually altered in the presence of osmotic stress [109]. Specifically, hypo-osmotic stress alters K8 phosphorylation in a cell-dependent manner and renders K8 S431<sup>P</sup> a physiologic substrate for activated protein phosphatase 2A [109].

Intestinal epithelial keratins play cytoprotective roles by interacting with signaling pathways involved in cell survival. Therefore, K8-deficient and K18-dominant negative hepatocytes are more resistant to Fas-mediated apoptosis when compared to wild-type [110,111]. This resistance is possibly due to K8/18-dependent regulation of Fas density at the plasma membrane together with a c-Flip-induced upregulation of pro-apoptotic caspases and downregulation of anti-apoptotic ERK 1/2 signaling [66,112]. This switch was suggested to be crucial for determining the outcome of death receptor stimulation. Ku et al. [104] have assessed c-Flip expression in livers of K8 and K18 null mice and in transgenic mice that express K18 Arg89Cys or K18 Gly61Cys mutants. They could not detect alterations in c-Flip levels in murine K8 null hepatocytes using two independent antibodies (for a discussion, see [104]). Possibly, following death receptor stimulation, K8 may sequester the pro-apoptotic JNK thereby preventing JNK from phosphorylating pro-apoptotic nuclear transcription factor targets [113].

Apoptosis is induced by death receptor and cell-intrinsic pathways. Keratins have been shown to moderate apoptosis in both settings. K8 and K18 interact with TNF receptor 1 [114]. Furthermore, loss of maternal TNF $\alpha$  increased the survival of K-deficient embryos [115]. In a mechanistic sense, this might be mediated by binding of the pro-apoptotic adapter protein TNFR-1-associated death domain protein TRADD to K18, suggesting a cell-autonomous mechanism [116]. A similar role for K17 in modulating hair follicle cycling was suggested by its association with TRADD [117], although the mechanism underlying the genetic interaction for TNF $\alpha$  and K17 was not identified. The authors were able to partially rescue the apoptotic hair follicle phenotype by depletion of TNF $\alpha$  in K17 null mice, demonstrating that

TRADD sequestration alone does not explain the influence of K17 on TNF $\alpha$  signaling.

Alterations in keratin phosphorylation also occur in other disease conditions. It was proposed that the inhibition of K5 T150 phosphorylation in K5 P152L mutants may contribute to the pathogenesis of EBS [118]. Similarly, a decrease in K8 S431 phosphorylation by ERK 1/2 kinase is seen in the K8 variant G433S [118].

## Keratins and malignant transformation

Keratin typing has become a major tool in tumor and histodiagnosis providing molecular parameters to assess the differentiation status of various types of carcinomas. Some studies have addressed the question as to whether keratin synthesis and, more specifically, whether certain keratin polypeptides affect tumor fate and behavior. Thus, it was reported that high K18 expression either occurring spontaneously or induced by introduction of a transgene is correlated with reduced invasiveness and tumorigenicity of a human breast cancer cell line [119]. These alterations were interpreted as a mesenchymal-epithelial transition. In line, it was found that elevated K18 is a favourable and independent prognostic marker in breast cancer coincident with low metastatic potential [120] while reduced K8, 18 and 19 was shown to be linked to reduced survival in patients with breast cancer [121,122]. A comprehensive study of the keratin profile of colorectal cancers further demonstrated that reduction in K8/20 corresponds to increased aggressiveness, which the authors also interpret as an indication of epithelial-mesenchymal transition [123].

K10, together with K1, forms the keratin cytoskeleton in postmitotic epidermal keratinocytes [124]. Based on forced expression in cultured cells and in transgenic mice, K10 expression was proposed to inhibit cell cycle progression in a retinoblastoma protein-dependent manner through sequestration of Akt and PKC $\zeta$  [125,126]. In K10 null mice, however, no increase in suprabasal keratinocyte proliferation took place [127]. Instead, these animals showed an increased turnover of skin cells *in situ* and developed less papillomas compared to control animals [128]. The increased cell turnover might remove pre-malignant cells. Furthermore, the increased number of sebocytes, in conjunction with an upregulation of c-myc, indicates a feed back-mechanism between postmitotic K10-expressing keratinocytes and the stem cell compartment that needs to be explored [129]. In addition, an increased size of epidermal keratinocytes was observed in K10 null mice. In these, a strong transcriptional induction of 14-3-3 $\sigma$  and of K17 was found in postmitotic cells, supporting the above findings [130]. In an elegant experiment, Chen and coworkers [131] constructed a chimeric keratin that contained the K14 rod flanked by the K10 head and tail domains and introduced it by homologous recombination into the K14 locus. This assured appropriate expression of the chimeric protein in basal keratinocytes by the K14 promoter. Basal keratinocyte turnover was unaffected in these mice but accelerated papilloma formation was noted in a chemical skin carcinogenesis protocol most likely due to suppression of apoptosis. Closer investigation of these mice will be most helpful to reveal

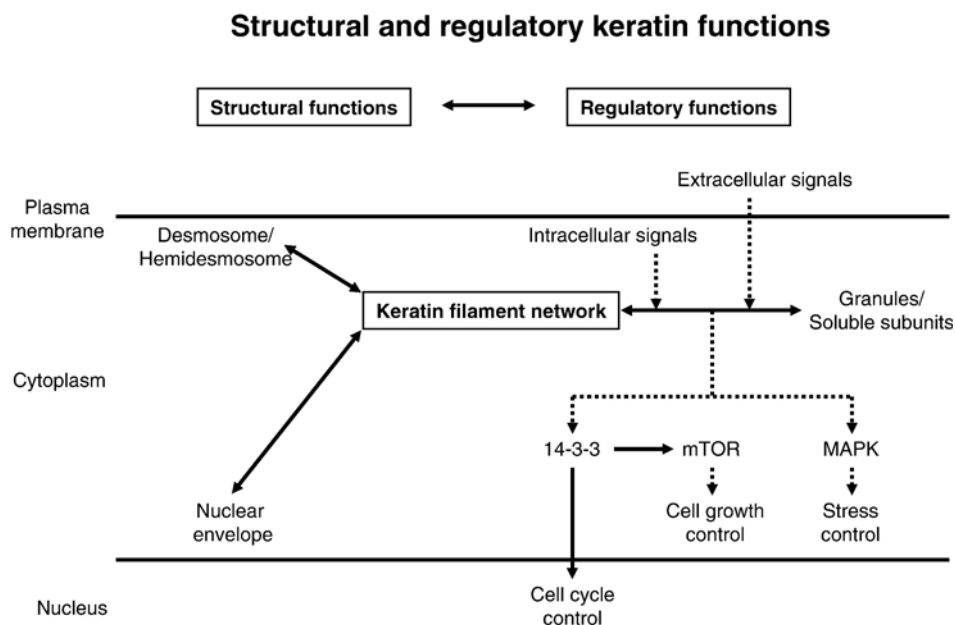
specific functions of keratin head and tail domains. These results are in contrast to earlier findings by another group that observed a suppression of cell proliferation and decreased skin tumorigenesis in transgenic mice ectopically expressing K10 in the basal epidermal cell layer [132] which may be attributable to the K10 overexpression in this instance. In the absence of extensive mechanical damage in K10 null or K10/K14 chimeric mice, it is tempting to speculate that one function of K1/K10 expression is a temporal suppression of cell death, thereby allowing completion of the keratinocyte differentiation program [133]. At present, a causal role in tumorigenesis is not established for any keratin, asking for genetic studies in model organisms.

### Concluding comments

We would like to emphasize that each keratin presents unique structural properties and regulatory functions and that care should be taken not to extrapolate results obtained from one keratin to the other. Clearly, the different and intrinsically heterogeneous epithelia perform divergent functions which are reflected by complex patterns of keratin expression.

However, only very little is known about the underlying molecular determinants. What distinguishes the K5/14 cytoskeleton of basal cells from that of the K1/10 network in suprabasal cells of the epidermis? What is responsible for the different bundling capacity of epidermal versus simple epithelial keratins? Why is the keratin cytoskeleton arranged differently in various epithelial cells? How do the dynamic properties of keratins differ? How do isotype-specific subdomains determine linkages to various binding partners?

The aim of the review was to present an update on current ideas of keratin function contrasting structural and regulatory contributions to tissue homeostasis in health and disease (Fig. 2). Although the structural role of the KF network as a major mechanical stabilizer is widely accepted, it remains a formidable challenge to elucidate the molecular mechanisms mediating this property and to shed light on some of the many unanswered questions, e.g.: Are certain keratins able to sense mechanical stress? If so, how do they react to it? How does the keratin cytoskeleton become reoriented upon the application of mechanical forces? How are local adaptations in network alterations accomplished without loss of mechanical integrity? How is the keratin cytoskeleton coordinated with the other cytoskeletal filament systems? Why do mutant keratins



**Fig. 2 – Structural versus regulatory functions of keratins.** Structural and regulatory keratin functions may not be completely separable. In several epithelia, in particular in basal epidermis, the keratin cytoskeleton protects cells against mechanical stress through formation of a three-dimensional cytoskeleton that associates with proteins of hemidesmosomes and desmosomes. While these interactions at the cell periphery are well documented and essential for epidermal integrity, keratin associations with the nuclear envelope are not well understood but are clearly non-essential for nuclear positioning [68,133,134].

Reorganization of keratin IFs in response to extra- or intracellular signals predominantly involves phosphorylation at several Ser residues leading to generation of granules and an increase of the soluble subunit pool. At the same time, Ser phosphorylation creates binding sites for 14-3-3 family adapter proteins, as demonstrated for K18 [107].

Phosphorylation-dependent recruitment of 14-3-3 to K17 has been shown to regulate protein biosynthesis via mTOR signaling, whereas the interaction of 14-3-3 with K8, K18 and K19 is a prerequisite for cell cycle progression [82,88]. MAPK are known binding partners of K8 and K18 that might be activated and released from their keratin binding sites under conditions of stress that lead to increased keratin phosphorylation. It has not yet been determined which fraction of the total MAPK are bound by keratins; furthermore, it is not known whether the interaction of keratins with kinases occurs between the soluble and/or the cytoskeletal fraction.

form “perfect” IFs *in vitro*? Why are stress-related signaling pathways elevated in cells producing mutant keratins? Probably, the greatest challenge at present is to correlate the spontaneous *in vitro* assembly mechanism to the *in vivo* situation that requires regulated assembly and disassembly processes and leads to complex networks ranging from finely dispersed three-dimensional networks in cultured epithelial cells to thickly bundled filament structures in keratinocytes. The understanding of the underlying molecular mechanisms will allow formulating dynamic models of keratin network plasticity, especially in relation to phosphorylation/dephosphorylation and isotype-specific functions. It will be of great value to understand the growing list of regulatory functions of keratins which cannot be separated from the structural aspect of the keratin cytoskeleton, its specific three-dimensional arrangement and particular structural changes in response to various molecular interactions.

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