The expanding significance of keratin intermediate filaments in normal and diseased epithelia

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Abstract

Intermediate filaments are assembled from a diverse group of evolutionary conserved proteins and are specified in a tissue-, cell type-, and context-dependent fashion in the body. Genetically-determined mutations in intermediate filament proteins account for a large number of diseases, ranging from skin fragility conditions to cardiomyopathies and premature aging. Keratins, the epithelial-specific intermediate filaments, are now recognized as multi-faceted effectors in their native context. In this review, we emphasize the recent progress made in defining the role of keratins towards the regulation of cytoarchitecture, cell growth and proliferation, apoptosis, and cell motility during embryonic development, in normal adult tissues, and in select diseases such as cancer.

Keratins are the epithelial-specific members of the superfamily of intermediate filament (IF) genes and proteins. As many as 28 type I and 26 type II keratin genes are tightly regulated in a pairwise fashion, reflecting the heteromeric nature of the 10 nm filaments they form, as well as in a tissue-specific and differentiation-dependent manner in body epithelia [1–3] (Box1). Rapid pace progress in recent years has set forth the notion that keratin IFs fulfill two fundamental roles in epithelial cells: 1) structural support, without which incident physical trauma exposes an inherent fragility and leads to loss of integrity, and 2) regulation of metabolic processes and of pathways governing their growth, proliferation, migration and apoptosis. These two roles involve regulated interactions with a diverse group of cellular proteins [4, 5].

Substantive progress has been achieved in spatially mapping, in living epithelial cells, the initiation of keratin assembly, and the growth, maturation and turnover of the keratin IF network. These advances (Figure 1) have been covered in recent reviews [6, 7]. Likewise, the mechanisms responsible for attachment of keratin filaments at sites of cell-cell and cell-matrix adhesion, and at the nuclear surface, are better understood and have been recently

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reviewed (Figure 1; see [8–10]). The role of keratin mutation as causative agents in inherited epithelial disorders continues to receive much attention, and have been commented upon as well [11–14]. For this review, we chose to focus on recent progress made in characterizing the role of keratin proteins in regulating cytoarchitecture, protein synthesis and growth, apoptosis, and epithelial cell motility in a myriad of contexts including embryonic development, normal adult tissues, and in select diseases such as cancer.

BUILDING UPON THE ALREADY KNOWN: STRUCTURAL SUPPORT, RESPONSE TO STRESS, AND CYTOARCHITECTURE

Body surfaces and several internal organs are lined by polarized epithelial sheets. Among their multiple roles, these tissues protect us from environmental stresses that encompass many forms, including mechanical, cytotoxic, oxidative, and metabolic insults. Interference with these protective roles underlies many diseases [11, 15, 16]. Accordingly, significant efforts continue to be devoted to determining when, where and how the protective roles of keratin are manifested.

Structural support

The structural support function of keratins is brought to the fore in skin fragility disorders involving mutations in epidermal keratins [11, 15, 16]. Epidermolysis bullosa simplex (EBS) and epidermolytic hyperkeratosis (EHK) are examples of genetic conditions caused by mutations in K5/K14 and K1/K10, the keratin pairs expressed in the basal and suprabasal layers of epidermis, respectively, and are characterized by cytolysis of keratinocytes and loss of structural integrity in the relevant epidermal layers [11, 13, 14]. Several key aspects of the human EBS phenotype are manifested in transgenic mice expressing dominantly-acting deletion mutants in K14 [17–19] and in mice null for *K14* 20] or *K5* 21]. Likewise, transgenic mice expressing a truncated, dominant negative K10 mutant protein [22] or the K10 R154C mutant [23] exhibit lesions that resemble EHK. By contrast, inactivating *K10* triggers hyperproliferation as is seen in EHK but no obvious cell fragility in the epidermis [24, 25]. This difference is likely related, at least in part, to the upregulation of K5 and K14 proteins, and their co-polymerization with K1, in differentiating suprabasal keratinocytes of *K10* null mouse epidermis [24]. A recent study supports that notion, as it reports that mice doubly null for *K10* and *K1* exhibit a lethal neonatal phenotype along with extensive skin lesions and cytolysis of suprabasal epidermal keratinocytes [26]. The *K1/K10* double-null mouse phenotype also hints at the involvement of keratins in regulating the integrity of the nucleus as well as desmosome-based adhesion, as further discussed below.

Keratin mutation-based fragility phenotypes closely correlate with alteration in the micromechanical properties of the cytoskeleton [11, 12, 27, 28]. A role for keratin filaments in providing structural support [28–32] is promoted by their unique intrinsic properties – for instance, their ability to self-organize into crosslinked networks [33, 34] – as well as by their linkages at cell-cell (desmosomal) and cell-matrix (hemidesmosomal) junctions, and to F-actin and microtubules [8–10] (Figure 1). While the ultrastructural features associated with various keratin deficiencies *in situ* clearly point to their importance for cell and tissue mechanics, understanding this role at a deeper level is a challenging proposition given limitations inherent to the ex vivo cellular models and assays being used [30, 31, 35–38]. Disruption of keratin IF anchorage at adhesive junctions via inherited mutations can also single-handedly result in disorders accompanied by keratinocyte and tissue fragility [39–41]. In addition to fostering mechanical integration at a cellular and supracellular levels, attachment of keratin IFs can also impacts the formation, size and molecular composition of desmosomes [42, 43].

Response to stresses

Keratin IFs afford crucial protection to internal organs, even though these are unlikely to experience mechanical stress to the extent that skin and other surface epithelia do. The liver, for instance, is a metabolism-intensive organ that functions in part to detoxify foreign substances. Previous efforts have clearly established that, in part through site-specific phosphorylation in cis, K8 and K18 are instrumental in the ability of liver hepatocytes to cope with a broad variety of metabolic, oxidative, and chemical stresses [44–46]. A recent study revealed that, in addition to phosphorylation, covalent O-linked N-acetylglucosamine (O-GlcNAc) modification (at serine residues 30/31/49) on K18 enhances the stress-buffering function of liver keratins [47]. Indeed, relative to controls mice that overexpress the human K18 S30/31/49A mutant (which cannot undergo O-GlcNAc modification) show multi-organ failure and increased lethality when treated with streptozotocin, PUGNAc (1, 5-hydroximolactone), or antibodies to Fas. The authors, Ku et al. [47], propose that under normal conditions the site-specific O-glycosylation of K18 serves to positively regulate the activity of the pro-survival Akt and PKC kinases, thereby protecting cells against apoptosis and promoting their adaptation to stresses (Figure 2A).

A role for keratins in regulating the nucleus?

In addition to their roles at cell junctions, the keratin IF network may be important for regulating the size and shape of the nucleus. Plectin, a well-known IF-binding protein [9, 48] was recently found to bind the outer nuclear envelope protein nesprin-3 [49] (Figure 1), providing the long-awaited rationale for the attachment of cytoplasmic IFs to the nucleus [50]. Consistent with this, targeted deletion of nesprin-3 in Zebrafish impairs the perinuclear concentration of keratin filaments [51].

Lee et al. [52] succeeded in crystallizing and solving the first atomic resolution structure of a keratin heterotypic complex consisting of the heptad repeat-containing 2B subdomains of K5 and K14. The interface of the coiled-coil heterodimer features several asymmetric (i.e., unidirectional) bonds that likely guide the intrinsic process of type I-type II heterodimer formation. Surprisingly, however, a symmetry-related contact in the crystal lattice has coiled-coil heterodimers forming a rather astonishing X-shaped object, owing to a trans-dimer homotypic disulfide bond involving cysteine 367 in K14. This disulfide bond is enriched in the perinuclear region of basal keratinocytes of epidermis, where it is poised to stabilize a cage of keratin IFs that may impact the size and shape of the nucleus at an early stage of differentiation [52]. This cysteine residue is conserved in K14 and related type I keratins including K10. Given this, it is intringuing that the *K1/K10* double-null mice show premature loss of nuclei in the suprabasal layers of their epidermis, along with reduced levels of emerin, lamin A/C, and Sun-1, despite a relatively normal differentiation program [26]. While caution is warranted at this time, such findings suggest that as is the case for nuclear lamins [53], the perinuclear cytoplasmic network of keratin IFs might also contribute to define nuclear morphology and participate in nuclear physiology.

KERATINS MODULATE CELLULAR GROWTH, APOPTOSIS AND MOTILITY: IMPLICATIONS FOR EMBRYONIC, ADULT, AND DISEASE SETTINGS

Embryonic development

The role of keratins in embryonic development has been difficult to define because of complicating factors including functional redundancy and genetic background effects [54]. Early mouse models with K8 or combined K18/K19 deficiency nevertheless have clearly pointed to a role for keratins in influencing embryonic development by impacting trophoblast giant cells and placental functions [55–57] (Figure 2B). To get around functional redundancy, Magin and colleagues boldly deleted the entire type II keratin gene cluster on

mouse chromosome 15, thereby generating the *KtyII*$^{-/-}$ model [58]. This deletion proves lethal at E9.5 in mouse embryos (i.e., mid-way through gestation), but their epithelial tissues appear largely intact, with no sign of cell lysis or tissue fragility. Instead, death correlates with other factors including mislocalization of the GLUT-1 and GLUT-3 transporters, increased AMP kinase activity, depressed mTORC1 activity and reduced protein synthesis in the yolk sac [58]. Additionally, *KtyII*$^{-/-}$ embryos exhibit defective adhesion between the endoderm and mesoderm layers, possibly accounting for severe defects in vasculogenesis and hematopoiesis, along with a placental defect (Figure 2B) related to the altered distribution of secondary trophoblast giant cells. The latter could result in hyperoxia in the decidual tissue owing to impaired vasculogenesis and insufficient gas exchange between maternal and embryonic blood [59].

Cells are called upon to migrate during embryogenesis, often as cohesive assemblies, in order to form new structures and tissues. A recent study examining the mechanically-induced collective migration of Xenopus cells sheds new light on the regulation of embryonic migration events by keratins [60]. Weber et. al. observed that polarized protrusion and migration of single mesendoderm cells occurs in the direction opposite from where a pulling force is applied to surface-exposed C-cadherins. Remarkably, this manipulation triggers a reorganization of keratin IFs including their recruitment to cadherin sites, and requires xCK1 (hK8) expression. Silencing the junctional protein plakoglobin (PG) produces similar protrusive defects and affects keratin network reorganization after pulling. Likewise, deleting either xCK1 or xPG randomizes the protrusive activity of mesendodermal cells in actual embryos, presenting compelling evidence for participation of keratins and PG in collective cell migration *in vivo* (Figure 2C). Whether these findings relate to the involvement of K6, PG and other keratin associated proteins in the regulation of keratinocyte motility during adult setting (see next Section) is worth pursuing.

Adult tissue homeostasis: Enacting the proper balance between competing processes

Much has been learned about keratin's role in cellular growth through studies showing the ability of select keratins to positively regulate cell cycle progression, entry into mitosis, and protein synthesis [45, 61] via interaction with key effectors such as 14-3-3 proteins (Figure 2D). Docking sites enabling 14-3-3 binding are generated via site-specific phosphorylation of keratins by growth-promoting kinases [45, 62, 63]. Depending on cell type and context, such keratin-14-3-3 complexes can subsequently impact cell cycle-associated regulators like Cdc25, as in the case of Xenopus eggs [64], and mTOR (Target Of Rapamycin), as in epidermal keratinocytes [65]. The kinase Akt represents another powerful effector that is strongly (and differentially) impacted by various keratins. Akt activity is increased when K8/K18 are genetically depleted in liver hepatocytes [66] but is decreased in K17 null mouse keratinocytes [65] or when K14 is silenced in the HaCaT (human) keratinocyte cell line or in an oral squamous carcinoma cell line [67]. Further, K10 has been reported to bind to and negatively impact Akt activity and cellular proliferation [68] though this has been disputed [69] (Figure 2D). The keratin-Akt relationship, whether in physical or functional terms [25, 69, 70], is worth a thorough re-examination given its ramifications.

Regulation of apoptosis represents an additional mechanism through which keratin IFs are able to coordinate cell and tissue growth [5]. Keratins can accomplish this by "sequestering" death-promoting effector molecules such as tumor necrosis factor receptor 2 (TNFR2) [71] and TNF receptor-associated death domain-containing protein (TRADD) [72–74], by influencing the targeting and density of relevant cell surface death receptors [75, 76], or by acting as "sponges" to absorb excessive stress and pro-apoptotic signals [77, 78] (Figure 2A). A recent study revealed that, in contrast to the evidence accumulated to date [44], K8 can be pro-apoptotic in the colonic epithelia, a phenomenon that depends upon the microflora. Transcriptional profiling in FVB/N K8$^{-/-}$ colons, which show hyperplasia and

colitis, revealed an unexpected upregulation of various pro-survival factors (e.g. survivin) and pathways [79] (Figure 2A). Such findings point (yet again) to the context-dependence of keratin function, and to the impact of partners and processes susceptible to genetic background effects.

The response to injury elicits changes in homeostasis and triggers significant alterations in IF gene expression and/or IF protein regulation in surviving wound-proximal cells in a broad array of tissues and organs, ranging from the central nervous system to skin [80]. The importance of these changes is conveyed by the altered outcomes observed whenever the "IF response to injury" is genetically manipulated. Somewhat paradoxically, genetic ablation of the two K6 isoforms (K6a and K6b), which are normally induced in wound proximal keratinocytes soon after injury to the skin [81], results in enhanced migration of keratinocytes so long as they are not subjected to a mechanically challenging setting, which exposes their inherent fragility [82]. This enhanced migration, it turns out, involves a robust stimulation of Src activity in *K6a/K6b* null keratinocytes [83]. In addition to binding to and regulating Src directly[83], K6a/K6b and associated proteins (e.g., plectin) may partner to sequester signaling molecules such as RACK1 and negatively impact upstream effectors, like protein kinase C (PKC) and integrin and focal adhesion kinase (FAK), to regulate cell migration [84, 85] (Figure 2C). Like *K6a/K6b* null cells, *Plakophilin1*-null, *Plectin*-null, *Plakoglobin*-null, as well as *Epiplakin*-null keratinocytes also migrate faster than their wild-type counterparts, with *Plectin*- and *Plakoglobin*-null keratinocytes exhibiting enhanced Src activity [86–89]. The possibility arises, therefore, that a larger complex comprised of keratins and associated proteins is able to interact with and regulate Src activity [83] (Figure 2C). Whether the relevant molecular events take place in detergent-resistant membrane (lipid rafts), as proposed [83], and how they are regulated in actively migrating cells, awaits further investigation.

Like the *K6* paralogs, *K16* and *K17* exhibit a dual mode of transcriptional regulation, with constitutive expression in specific cellular compartments within epithelial appendages (e.g., hair follicle, nail, various glands, oral papillae, etc.) and inducible expression after tissue injury and in diseases such as psoriasis and cancer [90]. *K6a/K6b* and *K17* null mice have been available for more than a decade, and a fair amount has been learned from their study [91–93]. By contrast the consequences of *K16* ablation, a long-awaited development, were only recently brought to light [94]. As previously seen in *K6a/K6b* null animals, *K16* null mice exhibit oral lesions early after birth, reflecting cell fragility in the filiform papillae of dorsal tongue epithelium [94]. These lesions are considerably less severe, however, likely due to the presence of K17), and a third of the *K16* null mice survive to adulthood. At that stage, the mice develop a severe palmoplantar keratoderma phenotype that exceeds and overshadows the limited amount of cytolysis occurring in footpad epidermis [94]. The *K16* null mice thus provide an opportunity to acquire insight into the genesis of the palmoplantar keratoderma lesions that arise in individuals with pachyonychia congenita, a group of disorders caused by mutations in *K6a, K6b K16* or *K17* 95].

Keratins and cancer: More than mere biomarkers…

Carcinogenesis involves a departure from normal cell differentiation pathways, and is typically accompanied by alterations in the regulation of keratin genes and proteins. Keratin profiling provides biomarkers that are useful for the diagnosis and, increasingly, the prognosis or associated risk for many types of epithelial-based tumors [96, 97]. The issue arises, therefore, as to whether such alterations in keratin expression or sequence impact any aspect of tumor biology.

Basal cell carcinoma is the most frequent tumor in the human population [98] and invariably features an induction of K17 in the context of what is, otherwise, a relatively simple keratin

profile [99, 100]. DePianto et al. [101] reported a delay in the inception of Gli2-induced basal cell carcinoma-like tumors in the absence of K17, correlating with marked reductions in keratinocyte proliferation and skin inflammation. In such Gli2-induced mouse ear tumors, remarkably, the genetic loss of *K17* polarizes the immune response and production of cytokines from a Th1/Th17- to a Th2-dominated profile. In particular, the *K17* status was shown to impact the expression of chemokines shown by others [102] to participate in the pathogenesis of basal cell carcinoma in humans, e.g., CxCl5, CxCl9, CxCl10, and CxCl11, in a keratinocyteautonomous fashion [101]. Interestingly, *K5* deficiency is associated with the misregulation of a distinct group of chemokines, in particular Ccl2, Ccl19, Ccl20, correlating with an increased density of Langerhans (dendritic) cells in epidermis [103]. The emerging notion of an immune modulatory role for keratins is certainly worth paying attention to, going forward. In addition to these findings, a recent genome-wide association study identified SNPs (single nucleotide polymorphisms) in the human *K5* locus that confer an increased lifetime risk of developing basal cell carcinoma [104]. Of note, K5 is the main type II partner for K17 in this tumor [101].

Cancer and metastasis provide yet another very important setting involving altered cell motility and migration. As shown a decade ago by Beil et al. [30], treating human pancreatic cancer cells with SPC (sphingosylphosphorylcholine, a bioactive lipid found in high levels in ovarian cancer patients) induces a perinuclear collapse of keratin filaments coinciding with phosphorylation of K18 at Ser52 and K8 at Ser431, a softening of the cytoplasm, and enhanced cell migration. Transglutaminase-2 and mitogen-activated protein kinase family members (JNK and ERK) have been recently shown to stimulate K8-Ser431 phosphorylation, promote the perinuclear reorganization of keratin IFs, and enhance tumor cell migration [105, 106] (Figure 2C). In the distinct settings of cell culture and oral squamous cell carcinoma, however, overexpression of shRNA-resistant K8 phospho-mutants of S73 or S431 in K8-knockdown cells is associated with increases in cell motility and in tumorigenicity [107]. This apparent incongruity may arise from differences in cell types utilized and methods used to induce cell migration, highlighting yet again the importance of context when studying keratin regulation and function.

Conclusion

Who would have thought, a decade ago, that keratins and associated proteins would be found to regulate protein synthesis, act as immune modulators, and regulate individual as well as collective cell migration in settings ranging from embryonic development to cancer? Because of their diversity, abundance, mode of polymerization, and various other properties, keratin proteins are poised to function in ways that extend beyond a conventional role as intracellular cytoskeletal proteins. Areas of greatest need in this field include an understanding of IF structure with atomic resolution; a better appreciation of the mechanisms regulating the assembly, dynamics and turnover of keratin IFs in various epithelial settings; gaining a sense for the significance and role(s) of the soluble pool of IF subunits within cells; and understanding how keratin IF function may be partitioned to specific subcellular domains, and is otherwise manifested in a context-dependent fashion.

Acknowledgments

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Box 1 Introduction to keratin intermediate filament genes and proteins (Refs. [1–3])

- There are ~70 genes that code for intermediate filament (IF)-forming proteins in the human genome, with an astounding 54 of them coding for keratin proteins that are expressed in epithelia.

- IF proteins represent a very heterogeneous group, with sizes ranging from 40 kDa (keratin 19) to 240 kDa (nestin). This said, they all share a common tripartite domain structure, consisting of a central α-helical rod domain featuring long range, coiled-coil forming heptad repeats and variable end domains located at their N- and C-termini.

- IF genes can be partitioned into six major subtypes based on either gene substructure or sequence homology over the defining rod domain existing in all IF proteins. Keratins comprise the type I (28 members) and type II (26 members) IF genes.

- All 28 type I keratin genes but one are organized as a cluster on the long arm of human chromosome 17 (mouse chromosome 11), whereas all 26 type II keratin genes along with the type I K18 gene are clustered on the long arm of human chromosome 12 (mouse chromosome 15)

- Mature, 10-nm keratin filaments are obligatory heteropolymers, with type I and type II proteins occurring in a 1:1 molar ratio. This requirement explains the coordinated transcription of a least one of each of the two subtype of keratin genes in epithelial cells. Remarkably, many type I genes are co-regulated with specific type II "partner" genes, leading to the notion of pairwise expression of keratin genes.

- The transcriptional regulation of keratin genes and the primary structure of their protein products are highly conserved across mammals, from mice to man, pointing to an intimate relationship between the complement of keratin proteins and the epithelial phenotype.
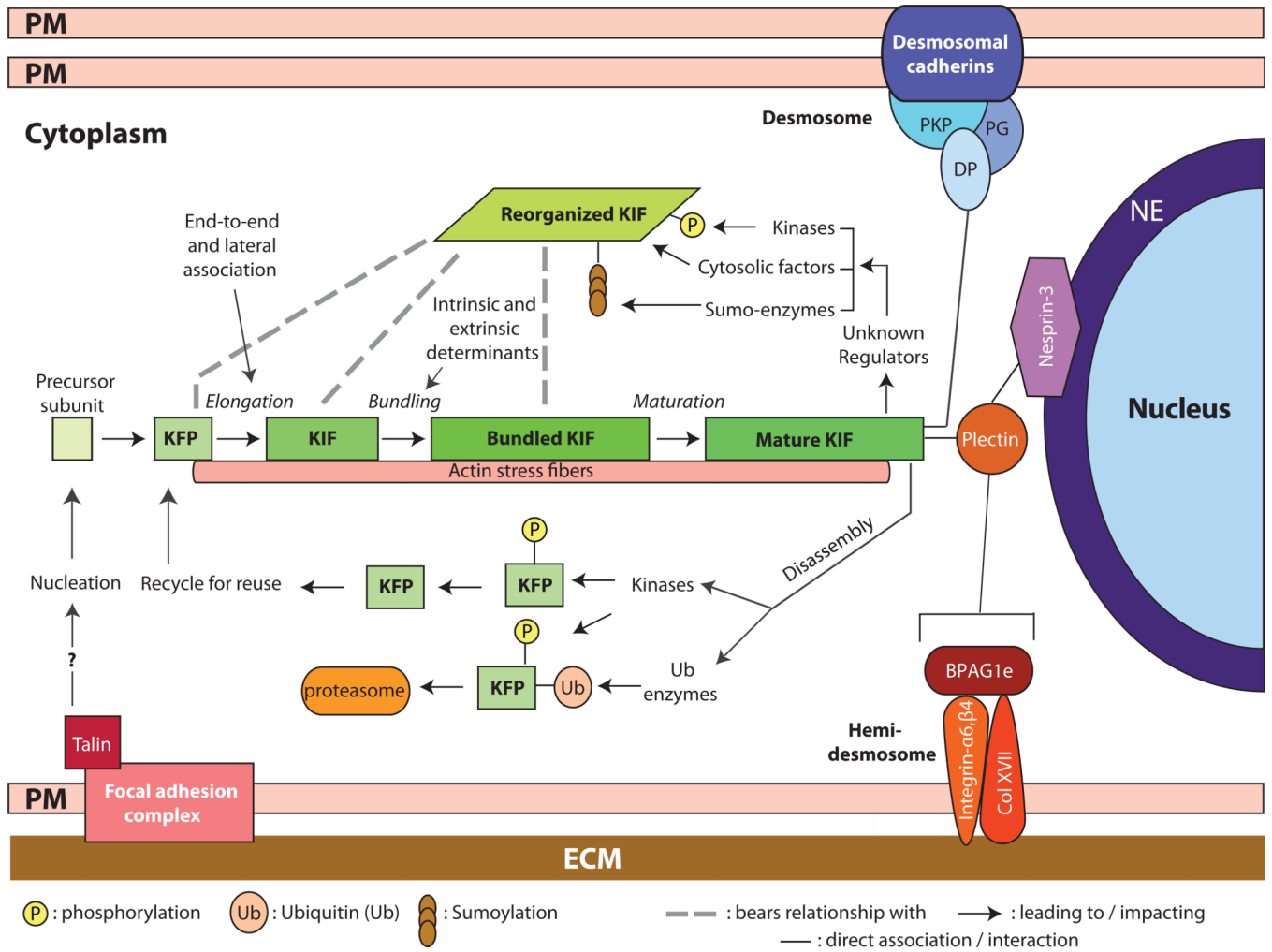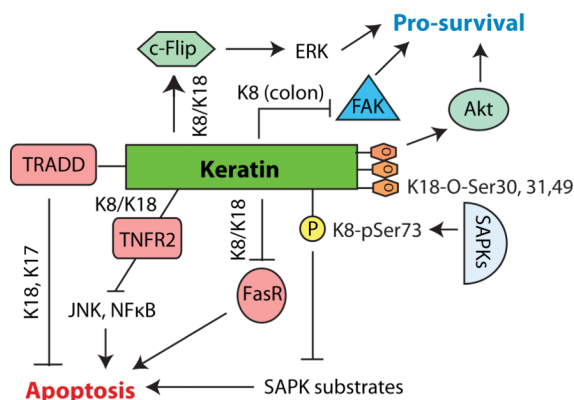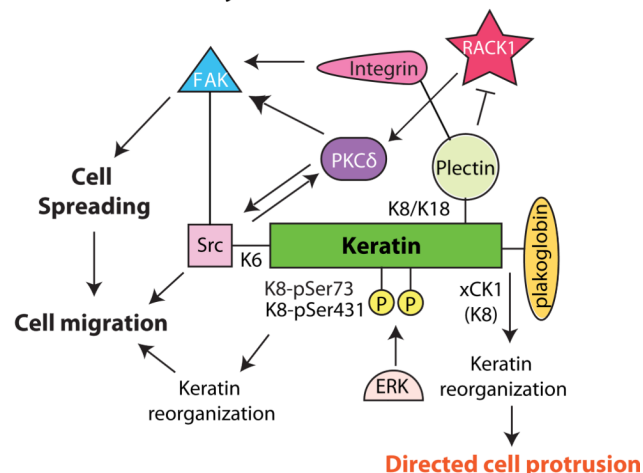
Figure 1.
Assembly, organization, and regulation of keratin intermediate filaments (KIFs). Live imaging studies in epithelial cells in culture show that keratin filament assembly is initiated at the periphery of the cell, near focal adhesions, and that newly formed filaments and their maturation into an organized network takes place in the context of a continuous centripetal flow with disassembly and turnover steps taking place near the nuclear envelope. The resulting "keratin cycle" is highly dependent on interactions with F-actin, with additional proteins, and on several types of post-translational modifications including phosphorylation, ubiquitination, sumoylation and (though not shown here) O-glycosylation. Most likely, the biological context dictates the rate of flow through this cycle. The figure also conveys that KIFs are attached at the surface of the nucleus (via a plectin/Nesprin-3 complex), at desmosome cell-cell adhesion sites, (via desmoplakin (DP), among other proteins), and at hemidemosome cell-matrix adhesions (via plectin and BPAG1e). Not shown here are the interactions with F-actin and microtubules. The structural support role of KIFs depends upon their organization as a crosslinked network that is fully integrated with other structural elements within and between epithelial cells. NE: nuclear envelope; PM: plasma membrane; ECM: extracellular matrix; DP: desmoplakin; PKP: plakophilin; KFP: keratin filament precursor; KIF: keratin intermediate filament.
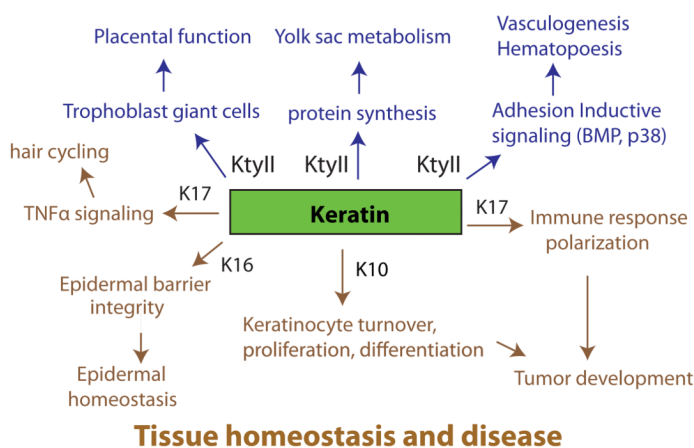
Figure 2.
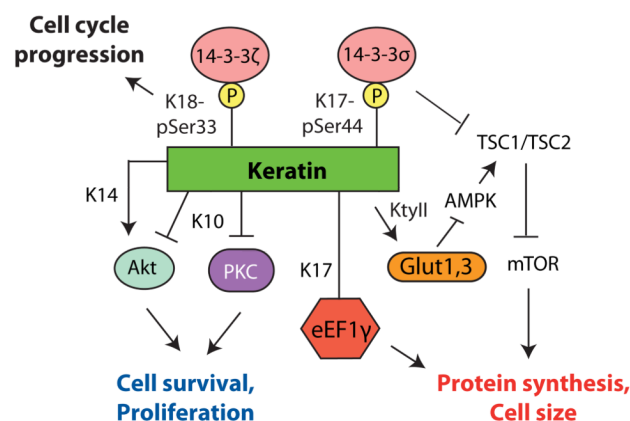Examples of cellular and physiological processes regulated by keratin proteins. A) Cell survival and cell death. B) Embryonic development (top; blue lettering) and tissue homeostasis and disease (bottom; brown lettering). C) Cell motility and related processes. D) Cell growth. In all cases, lines connecting keratins and the molecule(s) of interest convey a physical interaction. Abbreviations: Akt, Protein kinase B; AMPK, AMP-activated protein kinase; eEF1γ, Eukaryotic translation elongation factor 1 gamma; ERK, Extracellular signal-regulated kinase; FAK, Focaladhesion kinase-1; FasR, Fas-ligand receptor; Glut1, 3, glucose transporters 1, 3; K, mammalian keratins; KtyII, Type II mammalian keratins; mTOR, Mammalian target of rapamycin; PKC, protein kinase C; RACK1, Receptor for activated protein kinase C; SAPK, stress activated protein kinases; Src, Src kinase; TNFR2, Tumor necrosis factor receptor 2; TRADD, Tumor necrosis factor receptor type I-associated Death domain protein; TSC1/2, Tuberous Sclerosis Complex (Hamartin/Tuberin); xCK, Xenopus cytokeratin (ortholog to mammalian K18).