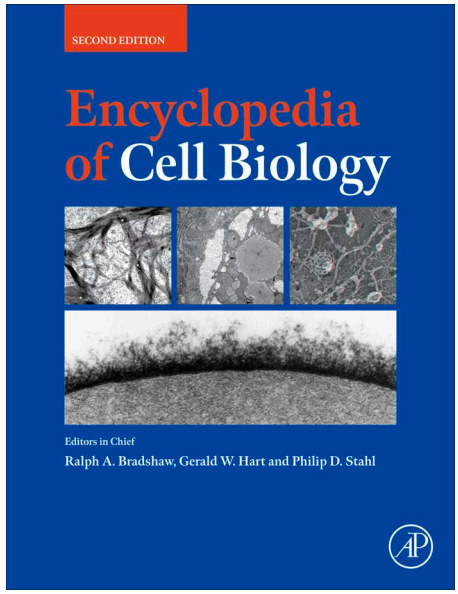

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Intermediate Filaments

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Abstract

Intermediate filaments, together with microtubules and filamentous actin, constitute the cytoskeleton. Cytoplasmic intermediate filaments form complex transcellular networks determining tissue architecture and function, and nuclear intermediate filaments form the nuclear lamina, a filamentous structure supporting the nuclear envelope. The intermediate filament protein family is composed of approximately 70 members. Their tissue- and differentiation-dependent expression patterns are established parameters for tumor classification. Besides providing a resilient scaffold, intermediate filaments are involved in stress response and many other basic cell functions. Mutations in intermediate filament genes are linked to a variety of human diseases ranging from mild skin blistering to life-threatening heart failure.

Key Points

The article highlights features of the intermediate filament cytoskeleton by describing its:

- Compositional and structural diversity.
- Use in tissue and tumor diagnosis.
- Unique assembly mechanisms and biochemical properties.
- Integration into structural and functional cellular networks.
- Regulation by posttranslational modification.
- Contribution to structural stability, subcellular compartmentalization.
- Participation in cell- tissue-specific functions.
- Role as a guardian against physicochemical and microbial stress.
- Contribution to hereditary and acquired human diseases.

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Introduction – Intermediate Filament Networks Have Distinct Properties That Render Them different From the Other Cytoskeletal Systems

Intermediate filaments are the major components of the cytoskeleton. They differ in profound ways from the actin- and tubulin-based cytoskeletal networks (Coulombe and Wong, 2004; Herrmann *et al.*, 2009).

(i) The human intermediate filament polypeptide family is highly diverse encompassing more than 70 polypeptides with multiple splice variants. (ii) Intermediate filaments assemble spontaneously from rod-like subunits in physiological salt solutions without a need for nucleotide triphosphates or proteinaceous co-factors. (iii) Intermediate filaments are subject to 'dynamic subunit exchange' whereby subunits are exchanged along the entire filament length. (iv) Biochemically, intermediate filaments are difficult to solubilize. High concentrations of chaotropic reagents are needed to dissociate the extremely stable coiled-coil dimeric subunits. In addition, assembled intermediate filament networks are very stable and retain their arrangement, even when cells are treated with highly concentrated salt solutions and nonionic detergents. (v) Intermediate filament proteins have a long half-life of up to several months. (vi) The intermediate filament network provides compliance to small deformations on one hand and strengthens cells on the other hand when large deformations are applied. (vii) Intermediate filaments are apolar fibrous structures, which cannot guide motor protein-driven directional transport.

In the following article we will highlight the molecular basis for these unique properties.

The Intermediate Filament Protein Family is Subdivided into 6 Types With Cell Type-Specific Expression Patterns

The members of the mammalian intermediate filament protein family are classified into 6 types based on sequence homology, gene organization, net charge, assembly mechanism, and expression pattern (**Table 1**; (Coulombe and Wong, 2004; Herrmann *et al.*, 2009)).

The more than 50 type I and type II keratins comprise the “soft” cytokeratins that are expressed in epithelial cells and the 'hard' keratins found in hair and nails. They are among the most abundant cellular proteins. Equal amounts of the more acidic type I and more basic type II keratins assemble into intermediate filaments in tissue- and differentiation-specific combinations. For example, the type I keratin 18 together with its type II partner keratin 8 is expressed in simple, one-layered epithelia of the intestinal mucosa, endometrium or exocrine glands. In multi-layered epithelia such as the epidermis other keratin pairs are expressed with specific keratin pairs in the basal cell layers (keratins 14 and 5) and suprabasal cell layers (keratins 10 and 1).

The type III intermediate filament proteins include vimentin, which is typically expressed in cells of mesenchymal origin, such as fibroblasts, leukocytes and endothelial cells, whereas desmin and syncoilin are found in muscle cells. Glial fibrillary acidic protein (GFAP) is produced in glial cells. Peripherin is mainly detected in peripheral neurons and in central nervous system neurons, which have projections towards the peripheral nervous system.

The type IV low-, middle- and high-molecular weight neurofilament proteins NF-L, NF-M and NF-H, whose carboxytermini differ dramatically in length, are highly expressed, together with α-internexin, in axonal processes of central nervous system neurons. Neurofilaments co-assemble in vivo as heteropolymers of NF-L and either NF-M or NF-H. Synemin, which is produced in glial astrocytes and muscle cells, and nestin, which is synthesized in developing and regenerating cells, do not assemble into intermediate filaments on their own but have specific regulatory functions for the assembly and disassembly of other intermediate filaments.

The type V lamin intermediate filament proteins are localized in the nucleus. At least one B-type lamin (B1, B2 and B2 splice variant B3) is expressed in every nucleated cell whereas A-type lamins (lamin splice variants A and C) have a more restricted distribution and are absent during early embryogenesis. Lamins are the major components of the nuclear lamina, a layer subjacent to the inner surface of the nuclear envelope.

The type VI intermediate filament proteins filensin and phakinin make unusual beaded filaments that are solely expressed in lens fiber cells.

Table 1 The intermediate filament protein family is subdivided into 6 types with distinct distribution patterns. Source: available at http://www.interfil.org/ (accessed 14.09.2021)

Type	*Members*	*Distribution*
I	'Acidic' keratins	Epithelial cells
II	'Basic' keratins	Epithelial cells
III	Vimentin	Mesenchymal cells
	Desmin, Syncoilin	Muscle cells
	Glial fibrillary acidic protein (GFAP)	Glial cells
	Peripherin	Neurons
IV	Neurofilament proteins (NF-L, NF-M, NF-H), α-Internexin	Neurons
	Synemin	Astrocytes, muscle cells
	Nestin	Developing and regenerating cells
V	Lamins	Nuclear lamina of all nucleated cells
VI	Filensin, Phakinin	Lens fiber cells

Intermediate Filament Proteins are Reliable Markers of Cellular Differentiation

The cell type-specific expression of intermediate filaments is exploited in tissue and tumor diagnosis (Moll *et al.*, 2008; Bragulla and Homberger, 2009; Karantza, 2011). It is well established that cellular differentiation is reflected by specific intermediate filament proteins. The main tissue types can thus be distinguished on the basis of their intermediate filament composition: epithelia are characterized by keratins, connective tissues by vimentin, muscles by desmin and nervous tissues by neurofilaments in neurons and GFAP in glial cells. Intermediate differentiation states usually go along with defined admixtures of intermediate filament proteins. For example, myoepithelial cells contain desmin together with keratins or myofibroblasts vimentin together with desmin. In addition, further classification of tissue differentiation is possible by analysis of intermediate filament protein isotype combinations. This is especially true for the abundant epithelial keratins. Changes of differentiation, which occur during development or as a reaction to various insults, are reflected by changes in intermediate filament expression. Taken together, the intermediate filament composition of a given cell can be taken as a reliable molecular marker of its differentiation and activity status. Remarkably, the same holds true for tumor cells. Thus, epithelium-derived carcinomas are generally characterized by keratins whereas mesenchymal tumors continue to produce vimentin. Furthermore, specific keratin pairs may provide clues for the origin of a given carcinoma and its differentiation status. Thus, simple epithelium-derived adenocarcinomas produce keratins 8 and 18, whereas multilayered epithelium-derived squamous cell carcinomas synthesize other keratin pairs, which are typical for multilayered epithelia. Changes in differentiation, however, which usually occur during malignant transformation and accompany de-differentiation go along with alterations in intermediate filament protein expression. A prominent example is epithelial to mesenchymal transition, which is paralleled by dramatic shape changes and increased motility of invading cancer cells. These cells start to co-express vimentin together with reduced amounts of keratins.

Intermediate Filament Proteins Share a Common Secondary Structure

Despite the sequence diversity among intermediate filament proteins they all share a common tripartite secondary structure (**Fig. 1**; (Herrmann *et al.*, 2009)): a ~45 nm long α-helical rod domain is flanked by non-helical amino-terminal head and carboxy-terminal tail domains. The central rod domain is highly conserved and consists of four α-helices (coils 1A, 1B, 2A and 2B) with interspersed non-helical linkers (L1, L12 and L2). Lamins carry an additional 42 amino acid insertion in helical subdomain 1B. The head and tail

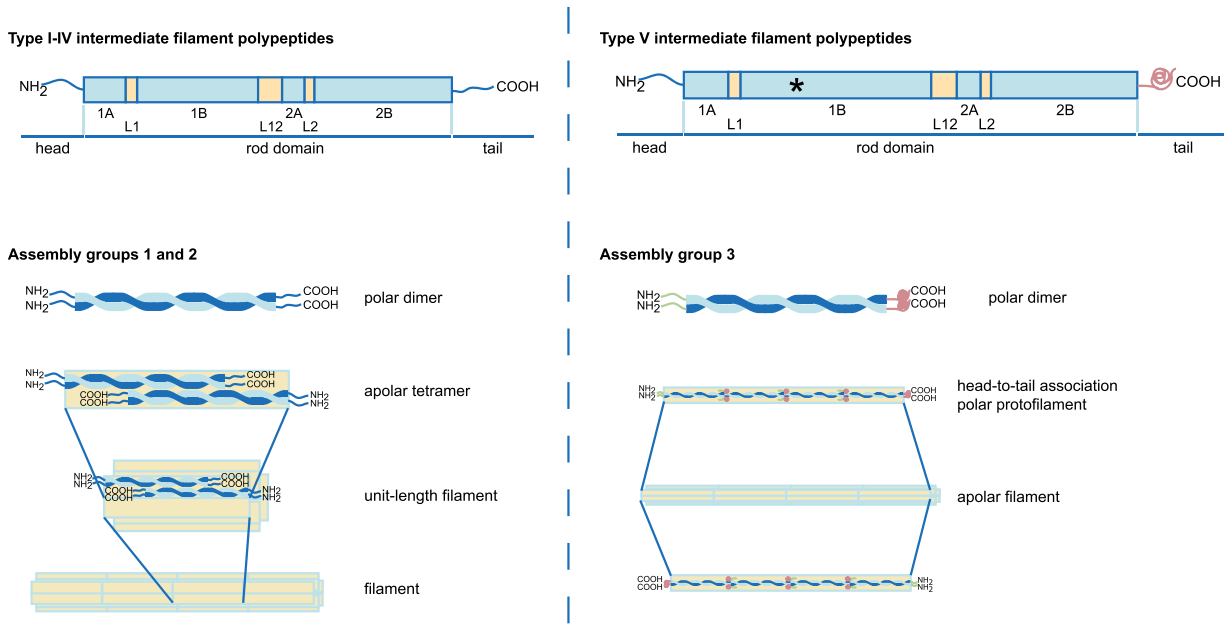

Fig. 1 Schemes of intermediate filament protein structure and major stages of intermediate filament assembly. The top panel depicts the major domains of the cytoplasmic type I-IV (left) and nuclear type V intermediate polypeptides (right). All contain a central α-helical rod domain, which is subdivided into segments 1A, 1B, 2A and 2B by the non-helical linker domains L1, L12 and L2. The rod is flanked by the highly variable amino-terminal head and the carboxy-terminal tail domains. Type V intermediate filament polypeptides differ from types 1-IV by a 42 amino acid insertion (*) in coil-domain 1B, a nuclear localization signal, and an immunoglobulin-like beta fold in the tail domain. The lower panel shows features of the major assembly types. Assembly groups 1 and 2 comprising the cytoplasmic intermediate filaments are characterized by parallel, non-staggered dimers consisting either of obligatory heteropolymers (assembly group 1) or facultative homopolymers (assembly group 2). Two antiparallel dimers associate in a staggered fashion to form a tetramer. 6–10 tetramers associate laterally into ~60 nm-long unit-length filaments, which elongate longitudinally. Nuclear lamins, which are members of assembly group 3, also form polar, non-staggered dimers. However, dimers attach head-to-tail to generate protofilaments. Association of protofilaments occurs antiparallel to generate apolar intermediate filaments. Aminotermini (NH_2) and carboxytermini (COOH) are labelled.

domains differ considerably in size, sequence and secondary structure between different intermediate filament polypeptides. While the tail domain of human keratin 19 contains only 15 amino acids, nestin has more than 1300.

The rod domains interact with each other to form filaments, whereas the head and tail domains bind to various cytoplasmic elements including other cytoskeletal components. The amino-terminal head domain regulates intermediate filament protein assembly, while the carboxy-terminal tail domain is dispensable and only affects filament stability.

The amino acid sequences at the beginning and the end of the rod domain are evolutionary most highly conserved. They are referred to as the helix initiation and helix termination motifs and are genetic hot spots for mutations in many hereditary intermediate filament disorders.

Highly Diverse Intermediate Filament Proteins Occur Throughout the Animal Kingdom

A prominent feature of intermediate filament proteins is their high degree of sequence diversity. Evolutionary pressure is apparently placed on the basic design of the rod domain but neither on its specific amino acid composition nor on the sequences in the highly variable end domains. This diversity enables intermediate filaments to perform isotype-specific functions in different cellular environments. It may explain why large intermediate filament multigene families have evolved and why the individual family members are still rapidly evolving. For example, silencing of a hair keratin gene in humans occurred as recently as 200,000–240,000 years ago (Winter *et al.*, 2001).

Although intermediate filaments are apparently absent in plants and fungi, they are present throughout the animal kingdom (Erber *et al.*, 1998; Peter and Stick, 2012). Comparing intron positions of intermediate filament genes and the amino acid sequences of their encoded polypeptides indicates that they evolved from a lamin-like ancestor. Simple metazoans such as Hydra attenuata express only nuclear intermediate filaments. In the invertebrate nematode Caenorhabditis elegans, however, one nuclear and 11 cytoplasmic intermediate filament genes have been identified (Carberry *et al.*, 2009). The cytoplasmic intermediate filament proteins contain an elongated coil 1B domain as is typical for lamins. They can be subdivided into two expression groups: Group 1 involves IFB-1 co-assembled with 4 IFA variants in a cell-specific manner; group 2 consists of the remaining intermediate filament proteins that are co-expressed in the intestine and require IFB-2 for network assembly. The nuclear intermediate filament encoded by the single *lmn-1* gene is expressed in all nuclei and localizes to the nuclear envelope. The insect Drosophila melanogaster has two nuclear lamin-type intermediate filament proteins but lacks cytoplasmic intermediate filaments. Identification of the cytoplasmic intermediate filament-like protein isomin in the intestine of the basal hexapod Isotomurus maculatus provides an evolutionary link between the different metazoan phyla (Mencarelli *et al.*, 2011).

Intermediate Filaments can be Assigned to Three Different Assembly Groups

The understanding of intermediate filament assembly is still fragmentary. At present, there are no crystallization data of polymerized full length intermediate filament proteins available. Since the entire rod domains could not be crystallized, several rod fragments were analyzed in a 'divide and conquer approach' (Chernyatina *et al.*, 2015). It is known, however, that the initial building block of all intermediate filaments is the parallel, in-register, two-stranded coiled-coil dimer whose alpha-helices are strongly bound to each other by non-covalent hydrophobic forces (**Fig. 1**).

Depending on the subsequent mode of assembly intermediate filaments can be assigned to three different assembly groups (**Fig. 1**; (Herrmann *et al.*, 2009)).

Type I and type II keratins belong to assembly group 1. Stages of assembly are depicted in more detail in **Fig. 2**. Keratins are obligatory type I-type II heterodimers. Two antiparallel-oriented dimers associate laterally into staggered tetramers. The resulting lack of polarity of this and all further assembly intermediates is one of the distinguishing features of cytoplasmic intermediate filaments in comparison to the polar actin- and tubulin-based filament systems. Lateral association of 6 tetramers forms short ~60 nm unit-length filaments (ULFs). ULFs then anneal longitudinally giving rise to longer mature intermediate filaments.

Assembly group 2 includes type III and type IV intermediate filaments, which typically assemble from homodimers. Further assembly intermediates are comparable to those found in assembly group 1 but may differ with respect to the number of subunits per filament cross section and a process termed compaction, which occurs after longitudinal ULF annealing. In addition, the assembly rate is much slower than that observed in group 1.

The type V lamin intermediate filaments belong to assembly group 3. Lamins have a large carboxy-terminal globular domain that contains an immunoglobulin-like beta-fold together with a nuclear localization sequence. Lamin dimers associate head-to-tail into polar protofilaments with protruding globular carboxytermini. Protofilaments then further associate laterally in an antiparallel fashion into extended mature apolar fibrils.

Recent advances in cryo-electron microscopy helped to reveal fundamental differences in the morphology of the different intermediate filament assembly groups in their native microenvironment. For example, lamin filaments are only 3.5 nm thick with globular domains on the outside (Turgay *et al.*, 2017). On the other hand, cytoplasmic filaments formed by keratins 5 and 14 are much thicker with an average diameter of ~10 nm (Weber *et al.*, 2021). They have a hexameric symmetry and contain an electron-

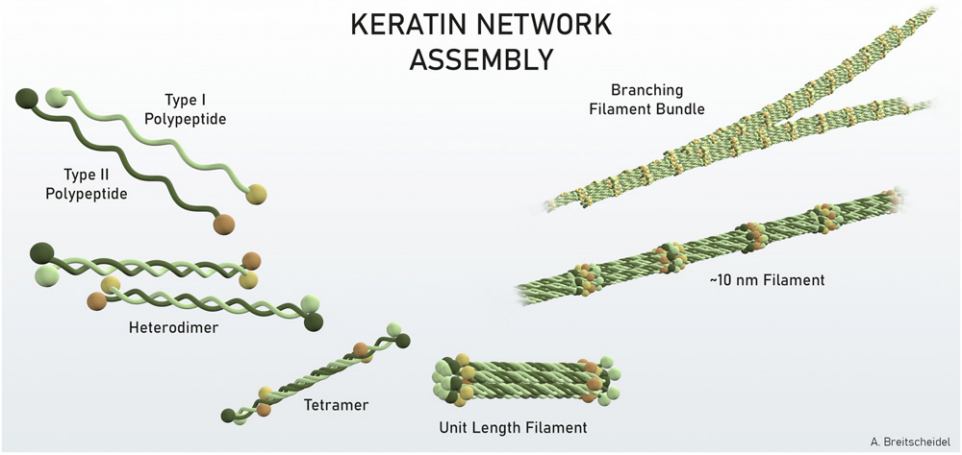

Fig. 2 3D representation of keratin filament network assembly stages. The α-helical rod domains of type I and type II keratin polypeptides associate in register through hydrophobic interactions to form extremely stable coiled-coil heterodimers. Two heterodimers align in opposite orientation with different degrees of stagger. Six tetramers attach laterally to generate unit length filaments, which elongate by end-to-end interdigitation into mature intermediate filaments. Bundling and branching are further features, the extent of which is dependent on keratin isotype, cell type and functional status.

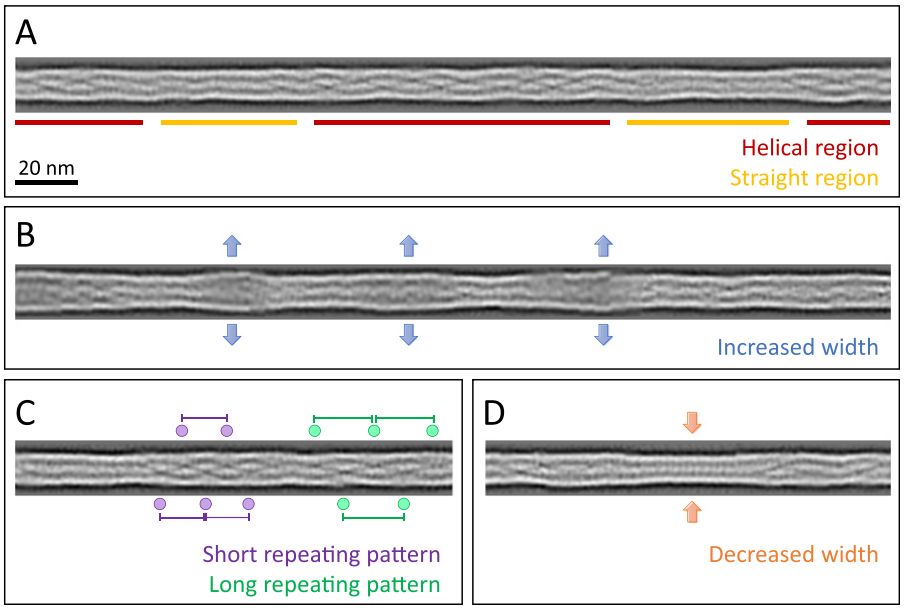

Fig. 3 Ultrastructure of keratin filaments in situ. The images were reconstituted from cryo-electron micrographs of murine keratinocytes producing only keratins 5 and 14. They illustrate the structural polymorphism along individual keratin filaments presenting helical patterns with different pitches and straight-line patterns as well as prominent width fluctuations. The figure is taken from Weber, M.S., Eibauer, M., Sivagurunathan, S., *et al.*, 2021. Structural heterogeneity of cellular K5/K14 filaments as revealed by cryo-electron microscopy. eLife 10, e70307. https://doi.org/10.7554/eLife.70307 and was kindly provided by Miriam Weber and Dr. Ohad Medalia, University of Zurich. (creative common license https://creativecommons.org/licenses/by/4.0/).

dense core. Most remarkable is their intrinsic heterogeneity with alternating twisted and parallel substructures and significant width fluctuations (**Fig. 3**).

In vivo imaging revealed further aspects of intermediate filament assembly. Despite their stability, intermediate filaments have been shown to be dynamic in cultured cells. Based on high-resolution time-lapse imaging of cells transfected with fluorescently tagged keratin a model for the keratin filament turnover cycle was described (Windoffer *et al.*, 2011). Keratin filament precursors are formed in the periphery near the cell membrane where they assemble. They subsequently integrate into the existing filament network by moving inward. Near the nucleus filaments bundle and become either part of the perinuclear cage or dissolve and the disassembled soluble subunits diffuse back towards the periphery where a new cycle is initiated starting with the formation of keratin filament precursors.

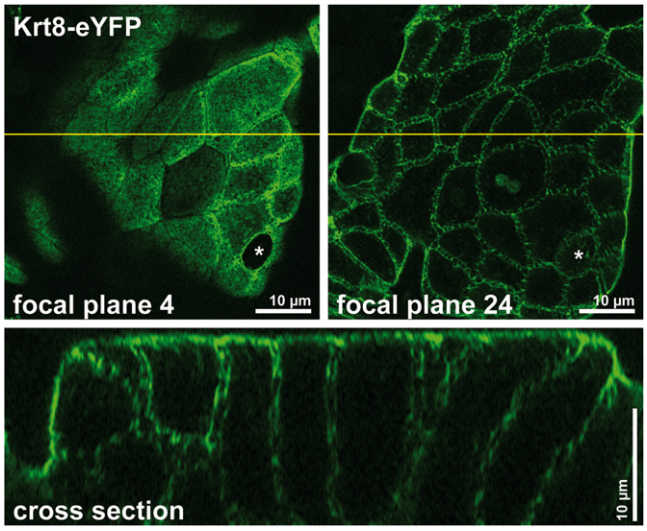

Fig. 4 Intermediate filament network organization in the murine intestinal epithelium. The fluorescence micrographs were recorded in vital intestine of a mouse expressing fluorescence-tagged keratin 8 (Krt8-eYFP). cf. Schwarz *et al.*, 2015). Note the enrichment of keratin 8 below the apical surface of enterocytes in a dense network (upper left). It is interrupted in goblet cells (*) and extends along the basolateral plasma membrane, where it is attached to desmosomal cell-cell adhesions forming a subplasmalemmal rim pattern (upper right). The micrograph in the lower panel is a cross section along the yellow line in the upper panel. This arrangement is also encountered in other polarized epithelia and is evolutionary conserved.

Cytoplasmic Intermediate Filament Network Organization is Determined by Interaction With Major Cellular Components Through Associated Proteins

Intermediate filament networks present many different types of spatial arrangement. They form dense bundles of closely packed filaments in neuronal axons, they are part of a complex anchoring structure of Z-discs to the plasma membrane in striated muscle, they are concentrated in cortical compartments of hepatocytes, they are enriched subapically in enterocytes or make up a 3D-meshwork in epidermal keratinocytes (**Figs. 4** and **5**). The molecular basis of the different organizational forms is still poorly understood. But the linkage of intermediate filaments to each other and to various other determinants of cellular architecture including microtubules, actin filaments, and adhesive complexes at cell membranes, and to cellular organelles such as mitochondria, Golgi apparatus, endoplasmic reticulum, and the nucleus certainly contribute to it. These interactions are mediated by intermediate filament associated proteins many of which contain a plakin domain (Sonnenberg and Liem, 2007; Bouameur *et al.*, 2014; Mohammed *et al.*, 2020). A prominent plakin family member is the 500 kDa protein plectin with its multiple splice variants containing different binding modules (Wiche, 2021). This ubiquitously expressed and multifunctional cytolinker integrates the intermediate filament system into the cytoskeleton and connects it to other cellular components thereby affecting cell shape, tissue stability, and cell migration. The cytolinker function of plakins is particularly evident in epithelial cells, which are attached to each other by desmosomal cell-cell contacts and to the basement membrane by hemidesmosomes. Plectin has been localized to hemidesmosomes whereas the plakin desmoplakin is present in desmosomes. In electron micrographs both structures are characterized by electron-dense plaques at the cell membrane with associated keratin intermediate filament bundles (**Fig. 5(A)**). In this way, forces are evenly distributed throughout the epithelial layer and propagated to the underlying connective tissue providing mechanical coherence and resilience.

Other intermediate filament-associated proteins have been identified with distinct functions for network organization. For example, filaggrins promote keratin filament bundling in epidermal cells (Steinert *et al.*, 1981). In muscles, the intermediate filament type IV protein synemin, which does not form filaments on its own, integrates into existing intermediate filament networks through polymerization with vimentin and desmin type III intermediate filaments. Synemin recruits these mixed intermediate filament networks to extracellular matrix adhesion sites by interaction with focal adhesion components (Bellin *et al.*, 2001). In heart muscle, synemin thereby helps to distribute forces by attaching the contractile apparatus to the surrounding connective tissue.

Lamin Intermediate Filaments Contribute to Nuclear Structure and Organization

The nuclear lamina of most mammalian somatic cells contains A- and B-type lamins, which form separate but interacting meshworks (Dechat *et al.*, 2008; Burke and Stewart, 2013; Zwerger and Medalia, 2013). These meshworks are attached to the inner nuclear membrane through lamina-associated proteins that are either integral or peripheral membrane proteins. The LINC (linker of nucleoskeleton and cytoskeleton)-complex, which traverses the nuclear envelope, further connects the nuclear lamina to the

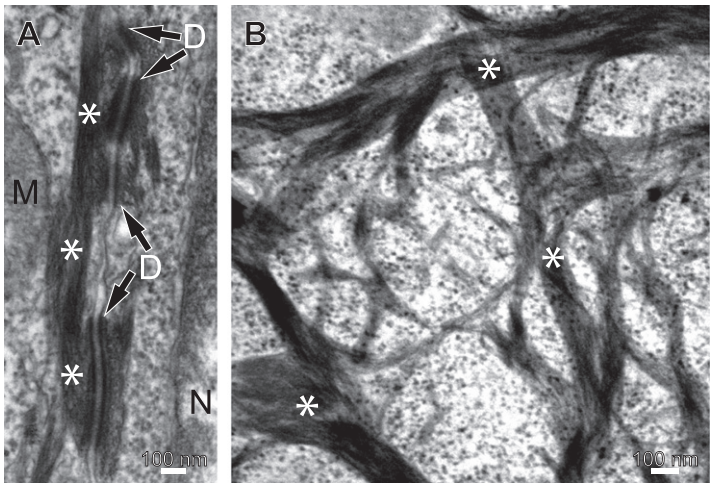

Fig. 5 Electron microscopy of keratin intermediate filaments in epidermal cells. The micrograph in (A) depicts thick bundles of keratin (stars) connecting multiple desmosomes (D). M, mitochondrium; N, nucleus. The image in (B) shows a cytoplasmic region with thick intermediate filament bundles (*) that are part of the highly interlaced three-dimensional cytoskeletal network. Images were kindly provided by Sabine Eisner, RWTH Aachen University.

cytoplasmic cytoskeleton. The outer nuclear membrane components of the LINC-complex are nesprins (nuclear envelope spectrin repeat proteins). Specific nesprin isoforms bind directly to actin or indirectly to intermediate filaments via plectin to determine positioning of the nucleus (Wilhelmsen *et al.*, 2005).

Nuclear deformability depends on the level of lamin expression. High lamin levels correspond to stiffer nuclei. Consequently, lamin A/C-deficient nuclei are more deformable, more fragile and prone to spontaneous rupture, and lamin B-deficient cells show increased extrusions of nuclear material into the cytoplasm that are referred to as nuclear blebs (Lammerding *et al.*, 2006). In addition, lamin A or lamin B1 knock-out leads to increased mesh size of the nuclear lamina in combination with irregularly distributed nuclear pore complexes (Kittisopikul *et al.*, 2021).

Lamins and their associated proteins control positioning of chromosomes (Gruenbaum and Foisner, 2015). Chromatin is bound to lamin through specific DNA sequences, called matrix attachment regions. For example, the Drosophila melanogaster lamin Dm0 has been shown to interact with transcriptionally silent DNA sequences that are often localized to the nuclear periphery (Pickersgill *et al.*, 2006). Furthermore, the lamin tail domain binds to core histones and several lamin-binding proteins such as the inner nuclear membrane protein lamin B receptor (LBR) bind DNA and heterochromatin protein 1. The DNA-bridging protein BAF is another example of a lamin-chromatin linker. It interacts with the inner nuclear transmembrane protein emerin, DNA, histones and A-type lamins thereby contributing to the reorganization of chromatin (Burla *et al.*, 2020).

Intermediate Filament Proteins are Posttranslationally Modified

Intermediate filament proteins are subject to several types of posttranslational modification and are among the most highly phosphorylated cellular proteins (Snider and Omary, 2014).

During mitosis cells need to disrupt their cytoskeleton. While actin filaments and microtubules are re-organized and used for specific functions during cell division, intermediate filaments do not participate in essential mitotic processes. But they need to be redistributed to the daughter cells. Some cells disassemble their cytoplasmic intermediate filament network into aggregates (**Fig. 6**) while others only show restricted disassembly at the cleavage furrow. After cell division, fully extended cytoplasmic intermediate filament networks are re-established. During mitosis an increase in serine and threonine phosphorylation of the head and tail domains occurs, which leads to increased intermediate filament protein solubility and altered intermediate filament network morphology (Sawant and Leube, 2017). Phosphorylation-deficient vimentin mutants have been shown to interfere with cytokinesis resulting in binucleated cells (Goto and Inagaki, 2014). Similarly, an increase in phosphorylation of the nuclear lamins occurs during nuclear lamina disassembly in mitosis. Ablation of the phosphorylated serine residues leads to the inhibition of nuclear lamina disassembly (Heald and McKeon, 1990).

Sumoylation, the covalent and reversible attachment of small ubiquitin-related modifier (SUMO) proteins, is a conserved posttranslational modification of intermediate filaments and regulates filament formation and solubility. Inhibition of sumoylation in Caenorhabditis elegans resulted in aggregate formation of the intermediate filament protein IFB-1 in the epidermis which was accompanied by reduced intermediate filament turnover and abnormal embryonic elongation (Kaminsky *et al.*, 2009).

O-linked glycosylation, the enzymatic addition of beta-D-*N*-acetylglucosamine to serine and threonine residues, is related to nutrient sensing and stress responses. In simple epithelia, glycosylation of keratin 18 promotes the phosphorylation and activation of cell survival kinases in stress situations (Ku *et al.*, 2010).

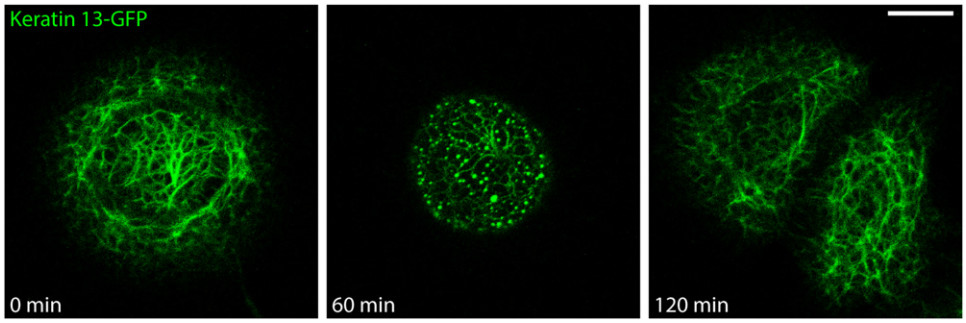

Fig. 6 Intermediate filament network reorganization in a cultured cell during mitosis. The fluorescence micrographs are taken from a time series of a vulva carcinoma-derived A431 cell producing fluorescent protein-tagged keratin 13 (Keratin 13-GFP). Note the drastic network rearrangement during mitosis with transient formation of highly motile cytoplasmic granules (middle). Scale bar = 10 μm. Images were kindly provided by Dr. Marcin Moch, RWTH Aachen University.

Intermediate filaments are cleaved by caspases during apoptosis and are ubiquitinylated to be subjected to proteasomal degradation for maintaining normal cell homeostasis. Recent work in Caenorhabditis elegans has provided evidence for a link between intermediate filament ubiquitination and aging (Koyuncu *et al.*, 2021).

A well worked-out example of successive posttranslational modifications is provided by lamins (Burke and Stewart, 2013). They are initially translated as pre-lamins whose cysteine residue in their carboxyterminal -CAAX motif becomes modified by addition of the 15-carbon isoprenoid farnesyl. Next, the -AAX part is removed. Subsequently, the cysteine residue becomes carboxymethylated generating the mature membrane-anchored B-type lamin. In case of A-type lamins, the carboxyterminal 15 amino acids including the modified cysteine residue are cleaved off producing mature lamin A, which lacks the farnesyl membrane anchor.

Intermediate Filaments Determine Cellular Plasticity

Intermediate filaments are arguably the most plastic cellular components. This plasticity is reflected at multiple levels and involves diverse mechanisms ranging from rapid evolutionary genetic drift of the encoding genes to functional and structural diversity of the respective polypeptides with extensive posttranslational modifications for precise control. The resulting complexity provides a rich repertoire of options for tuning tissue properties and activities at the single cell level.

The vast combinatorial choice of intermediate filament polypeptides is based on the co-assembly of different members of the three major assembly groups, which are encoded by multigene families. Genetic diversity of the more than 70 intermediate filament polypeptide-encoding genes in human is further enhanced by the presence of multiple splice variants (e.g., more than 10 variants for the GFAP-encoding gene (Hol and Capetanaki, 2017)). Genetic polymorphism adds to the complexity. Polymorphism is particularly pronounced in keratin genes (Mischke, 1998). Different regulatory mechanisms determine not only gene transcription but also mRNA stability, translational efficiency and protein stability. As a result, the precise admixture of intermediate filament polypeptides appears to be unique for any given cell reflecting its derivation, functional status and physicochemical microenvironment.

The compositional complexity of the intermediate filament cytoskeleton in each cell leads to structural diversity. The structural diversity becomes evident at the single filament level with different ultrastructural features and morphologies and extends to network organization consisting of intermediate filament bundles with different thickness in combination with unique mesh size and subcellular topologies. As a result, the local intermediate filament network organization is a major player in tissue morphogenesis and its finely-tuned subunit composition.

The structural complexity of the intermediate filament cytoskeleton is linked to functional specificity. Thus, specific cellular properties including biomechanics, subcellular compartmentalization and trafficking are supported by the cell type-specific and context-dependent intermediate filament systems. A tight crosstalk exists between cellular function and cytoskeletal organization. It is subject to regulation affecting not only the above mentioned mechanisms but also posttranslational modifications that are particularly abundant in the intermediate filament cytoskeleton.

Intermediate Filaments Provide Structural Support

The identification of intermediate filament mutations in diseases that are characterized by tissue fragility provides compelling evidence for the structural function of intermediate filaments. This is best exemplified in human skin disease, where single point mutations in keratin genes induce blistering in mechanically-challenged areas. Furthermore, epithelial cells lacking cytoplasmic intermediate filaments are less resistant to mechanical stress evoked by magnetic tweezers or optical stretching (Ramms *et al.*, 2013; Seltmann *et al.*, 2013). Similarly, fibroblasts without vimentin display decreased stiffness and increased instability when subjected

to mechanical challenges (Eckes *et al.*, 1998). Desmin deficiency results in myopathy with impaired force transmission in skeletal and heart muscle (Milner *et al.*, 1996).

In vitro assays have shown that intermediate filaments are much more deformable than the other cytoskeletal filaments and can be stretched several times their initial length before they tear (Kreplak *et al.*, 2005). Upon application of force, the filaments become more viscoelastic, a behavior that is referred to as strain stiffening (Janmey *et al.*, 1991). Interestingly, post-translational modifications have been implicated in regulating biomechanical properties of intermediate filaments in vitro (Kraxner *et al.*, 2021).

The evolving view is that cytoplasmic intermediate filaments are part of transcellular network systems, which connect cells with each other and with fibers of the extracellular matrix (**Fig. 7**). The different fiber-based systems are linked through multiprotein complexes. They include the LINC complex, which connects the nuclear lamins with the cytoplasmic intermediate filament network. Specific desmosomal adhesion sites coordinate the intermediate filaments between cells. Hemidesmosomes and costameres attach cytoplasmic intermediate filaments to the extracellular matrix. In this way, transcellular networks are generated which are the basis for tissue homeostasis.

Evidence for mechanosensitive functions of intermediate filaments was provided in shear stress experiments of lung epithelial cells, which reacted by increasing keratin solubility, aggregation and degradation (Ridge *et al.*, 2005). Vimentin expression in blood vessels is increased when high shear stress is applied strengthening the arterial wall structure and counteracting arterial diameter enlargement (Schnittler *et al.*, 1998). A remarkable observation was that lamin A expression scales closely with tissue stiffness suggesting a link between both (Swift *et al.*, 2013). In support, modulation of lamin A levels was shown to influence mesenchymal stem cell differentiation. Interestingly, lamin A expression was also linked to the integrity of the cytoplasmic keratin cytoskeleton indicating a role of keratins in mechanotransduction (Laly *et al.*, 2021).

Nuclear shape is at least partly determined by lamins. For example, spermatocytes, which have a unique hook-shaped nucleus, specifically express lamin B3. Ectopic expression of lamin B3 in somatic cells, which usually have a spherical nucleus, induced hook-shaped nuclei (Furukawa and Hotta, 1993). Lamin mutations furthermore result in disfigured skeletal and myocardial nuclei and increased susceptibility to nuclear fragility (Davidson and Lammerding, 2014).

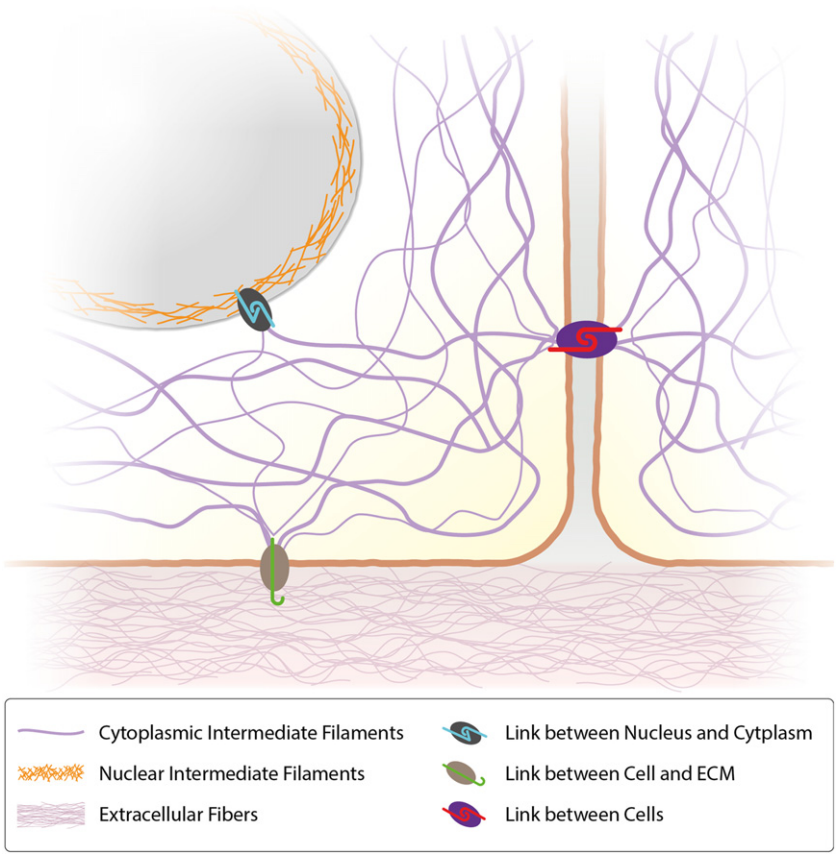

Fig. 7 Scheme highlighting the connectivity between different fiber systems in the nucleus, cytoplasm and extracellular matrix. Lamins form a dense network below the inner nuclear membrane and attach to the LINC complex, which extends through the perinuclear cisterna and outer nuclear membrane into the cytoplasm, where it is connected to the cytoplasmic intermediate filament system. Cytoplasmic keratin intermediate filaments are anchored to symmetrical desmosomal cell-cell adhesions generating transcellular networks and to asymmetrical hemidesmosomal cell-extracellular adhesions, which are associated with extracellular collagen-based fiber systems. Linkage occurs in different contextual modifications and is the basis of tissue coherence.

Intermediate Filaments Support Specialized Tissue Functions

In order to maintain the structure and barrier function of the epidermis, keratinocytes need to differentiate from proliferating cells in the basal layer towards postmitotic, tightly sealed cells in the upper cell layers. The cells eventually die by apoptosis and give rise to the stratum corneum that is made up by the cornified envelope and aggregated keratins. These differences in cellular differentiation are reflected by different keratin pairs with keratins 5 and 14 in the basal cell layer and increasing levels of keratins 1 and 10 in the suprabasal cell layers. Injury induces the expression of keratins 6 and 16 to support wound closure (Redmond and Coulombe, 2021). Disruption of keratin genes therefore results in reduced mechanical stability, altered wound closure and compromised barrier function in the epidermis (Seltmann *et al.*, 2013; Ramms *et al.*, 2013; Kumar *et al.*, 2015).

Keratins have been linked to secretion in endocrine cells of the pancreas. Reduced insulin levels were observed in mice lacking keratin 8 (Alam *et al.*, 2013). In colonic mucosa, keratins are important for proper electrolyte transport (Toivola *et al.*, 2005).

During axonal growth, neurofilament subunits are incorporated throughout the axon in a dynamic process that involves the addition of subunits along the entire filament length as well as at filament ends. The amount and specific admixtures of neurofilaments determine axonal diameter and are therefore responsible for diameter-dependent axonal conduction velocity (Liem and Messing, 2009).

Intermediate Filaments are Guardians of Cells in Stressful Situations

In stress situations, intermediate filaments are usually hyperphosphorylated resulting in changed network morphology and intermediate filament protein solubility (Ridge *et al.*, 2005; Snider and Omary, 2014). Interestingly, heat shock proteins, which are increased during heat stress, bind intermediate filaments (Toivola *et al.*, 2010).

Intermediate filament proteins protect cells from apoptosis. Their hyperphosphorylation acts as a phosphate sink preventing the phosphorylation and therefore activation of pro-apoptotic factors (Lai *et al.*, 1993; Ku and Omary, 2006). Consequently, keratin-deficient mice are more susceptible to apoptosis induced by toxins or other stressors.

Nestin, an intermediate filament protein that is usually expressed during development and downregulated in the adult organism, is re-expressed during pathological situations such as glial scar formation in the central nervous system and regeneration of injured muscle tissue. Relevant in this context may be the interaction of nestin and cyclin-dependent kinase Cdk5 to promote cell survival (Sahlgren *et al.*, 2006).

Intermediate Filaments are Reorganized During Migration, Wound Healing and Tissue Remodeling

Cell migration and wound healing strongly depend on the fundamental re-organization of the intermediate filament cytoskeleton (Chung *et al.*, 2013; Leduc and Etienne-Manneville, 2015; Yoon and Leube, 2019). Thus, induction of usually absent keratin isoforms is observed during wound healing in epidermal keratinocytes to make them more malleable for repopulating the wounded area. Similarly, there is a rapid upregulation of vimentin and nestin during repair of damaged muscle (Vaittinen *et al.*, 2001). Peripheral blood mononuclear cells of mice lacking vimentin have a reduced capacity to home to lymph nodes and spleen because surface receptors involved in homing are aberrantly expressed and distributed in both the blood mononuclear cells and endothelial cells (Nieminen *et al.*, 2006). This indicates an important role of intermediate filaments in anchoring and organizing adhesion molecules.

In the brain, tissue re-organization following insults is also coupled to altered intermediate filament expression. Peripherin is induced in cerebral ischemia and GFAP is upregulated in glial scar formation (Beaulieu *et al.*, 2002; Hol and Capetanaki, 2017).

Intermediate Filaments Contribute to Organelle Localization

Intermediate filaments are involved in the positioning, shaping and function of cellular organelles (Toivola *et al.*, 2005; Schwarz and Leube, 2016). The interplay of mitochondria and intermediate filaments is well established although molecular mechanisms have not been elucidated. In cardiac muscle of desmin knock-out mice mitochondria are mislocalized, fragmented and functionally altered (Milner *et al.*, 2000). This aberrant mitochondrial distribution and morphology was also shown for keratin 8 knock-out hepatocytes as well as in neurons with neurofilament gene mutations (Szebenyi *et al.*, 2002; Tao *et al.*, 2009). Likewise, disease-associated keratin mutations cause fragmentation of the Golgi apparatus (Kumemura *et al.*, 2008).

Vimentin, peripherin and alpha-internexin bind to an adaptor protein that carries vesicles between endosomal and lysosomal compartments and regulates sorting of different vesicles. In vimentin-null cells, the uniform localization of lysosomes throughout the cytoplasm is lost and lysosomes are concentrated near the nucleus (Styers *et al.*, 2004).

Cytoplasmic Intermediate Filament Dysfunctions Cause Tissue-Specific Diseases

Hereditary skin diseases and degenerative diseases of muscles and neurons are characterized by disruption of the intermediate filament network and prominent intermediate filament protein-containing cytoplasmic aggregates (Liem and Messing, 2009; Coulombe *et al.*, 2009; Clemen *et al.*, 2013). The highly conserved helix initiation and termination motifs of the central rod domain contain genetic hotspots for the most severe disease mutations. These mutations interfere with filament assembly and network stability.

The clinical importance of keratin intermediate filaments became first evident when single point mutations in the epidermal keratin 14 were linked to the skin disease epidermolysis bullosa simplex (Coulombe *et al.*, 1991; Bardhan *et al.*, 2020). A hallmark feature of this disease is the formation of blisters in the basal layer of the epidermis because of cytolysis, i.e., cell rupture. Cytoplasmic keratin aggregates are pathognomonic features in electron micrographs. Subsequently, skin disease-related mutations were identified in all other epidermal keratins. The localization of the respective pathologies reflects in each case the distribution of the affected keratin. In addition to blistering, increased proliferation and altered differentiation were observed to different degrees. Similar phenotypes are reproduced in mice lacking keratins. Surprisingly, multilayered epidermis is still established in the absence of all epidermal keratins. In addition to pronounced blistering, however, considerable deficiencies in epidermal differentiation and barrier formation occur in this situation (**Fig. 8**) (Kumar *et al.*, 2015).

Mutations of hard keratins have been linked to human diseases affecting hair and nails (Langbein and Schweizer, 2005). In contrast to cytoplasmic keratins, which possess glycine- and serine-rich head and tail domains, hard keratins possess cysteine- and proline-rich end domains. They form tight cross links with each other and with keratin-associated proteins. The most common hard keratin-related disease is monilethrix. It is characterized by fragile hair and nail deformities. Affected individuals display periodic changes in the width of the hair shaft leading to beaded hair that easily breaks.

In simple keratins, mutations of the helix initiation and termination motifs have not been identified in human disease. This may be due to crucial functions of these keratins during early embryogenesis which rely on properly formed keratin networks. But mutations in their head and tail domains predispose to chronic liver disease. This is apparently related to decreased stress protection. In addition, simple keratin-rich aggregates have long been known as Mallory-Denk bodies typically found in cirrhotic liver disease (Strnad *et al.*, 2008).

Mutations in vimentin, phakinin and filensin have been identified in human cataract patients (Song *et al.*, 2009). Similarly, deletion of filensin or phakinin or expression of mutant vimentin leads to opacification and cataract formation in mice.

Desmin mutations are associated with progressive skeletal myopathy that leads to skeletal muscle weakness in combination with cardiac arrhythmia and heart failure (Clemen *et al.*, 2013). A distinctive histopathological feature is the accumulation of amorphous cytoplasmic deposits containing desmin and other proteins.

Mutations in the GFAP gene cause Alexander disease, a rare degenerative disease of the white matter in the brain which leads to loss of myelin (Liem and Messing, 2009). Again, cytoplasmic protein aggregates occur that contain several ubiquinated stress proteins along with the mutant GFAP. Mutations of neurofilament proteins have been identified in neurodegenerative Charcot-Marie-Tooth disease. Affected individuals progressively loose muscle tissue and touch sensation.

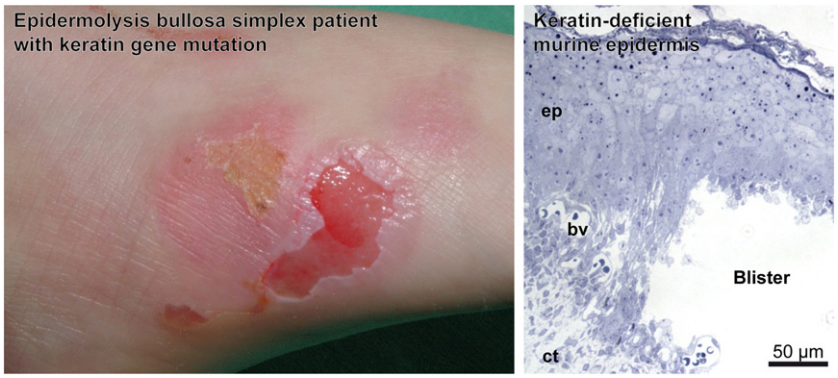

Fig. 8 Epidermal blister formation in an Epidermolysis bullosa simplex patient and a keratin-deficient mouse. The photograph at left shows a torn blister that had formed upon mechanical stress in a patient harboring a keratin mutation. The toluidine blue-stained histological section at right was prepared from murine skin lacking keratin filaments altogether. A multilayered epithelial tissue is still formed but tissue resilience is considerably compromised resulting in blister formation because of cytolysis. ep, epidermis; ct, connective tissue; bv, blood vessel. The photograph at left was provided by Dr. Cord Sunderkötter, Halle University; the murine tissue sample was kindly provided by Dr. Thomas Magin, Leipzig University.

Lamin Intermediate Filament Mutations Cause Complex Syndromes

Progeria infantilis or Hutchinson-Gilford syndrome is one of the most remarkable laminopathies (Worman and Bonne, 2007). It leads to premature aging and cardiac dysfunction. The rare disease has been linked to mutations in the lamin A gene, which cannot be processed into the mature form by preventing cleavage of the farnesylated and methylated carboxyterminus. Restructuring of the nuclear lamina is observed in several cell types. It is characterized by dissociation of heterochromatin from the nuclear periphery and by changes in histone methylation. The lamin A mutations also induce increased nuclear stiffness. In contrast, laminopathies, which are caused by other lamin A mutations and lamin B mutations and lead to muscle dystrophy syndromes, are associated with reduced nuclear stiffness and increased nuclear envelope fragility.

Conclusion

Cell proliferation and viability rely on actin filaments and microtubules whereas depletion of intermediate filaments still allows basic cell functions. While intermediate filaments are not essential, they act as a universal and highly versatile buffer. By absorbing and attenuating potentially adverse stimuli they are involved in all aspects of life and become crucial for survival in constantly changing environments. Their protective capabilities cover different length and time scales. Length scales range from single filaments to complex cytoplasmic networks that form transcellular scaffolds through cell-cell adhesions and are coupled to the fibrous components of the extracellular matrix to support tissue and organ function. Time scales range from immediate responses such as strain stiffening and posttranslational modifications to intermediate responses involving architectural network remodelling to long-term compositional adaptations to withstand chronic wear-and-tear. The versatility of intermediate filaments is afforded by their intrinsic plasticity and complexity, which is reflected by rapidly evolving large multigene families. They are expressed in complex patterns to generate polypeptide assemblies with highly specialized properties. Understanding the contribution of the intermediate filament cytoskeleton to cell, tissue and organismal resilience is therefore of the utmost medical relevance.

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