Plasticity of cytoplasmic intermediate filament architecture determines cellular functions
Nicole Schwarz and Rudolf E. Leube

Abstract
Cytoplasmic intermediate filaments endow cells with mechanical stability. They are subject to changes in morphology and composition if needed. This remodeling encompasses entire cells but can also be restricted to specific intracellular regions. Intermediate filaments thereby support spatially and temporally defined cell type-specific functions. This review focuses on recent advances in our understanding of how intermediate filament dynamics affect the underlying regulatory pathways. We will elaborate on the role of intermediate filaments for the formation and maintenance of surface specializations, cell migration, contractility, organelle positioning, nucleus protection, stress responses and axonal conduction velocity. Together, the selected examples highlight the modulatory role of intermediate filament plasticity for multiple cellular functions.

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Introduction
Intermediate filaments are major components of the cytoskeleton. They are composed of a diverse group of polypeptides with more than 70 members. Based on their gene structure, sequence homologies, and assembly characteristics they are grouped into 6 types with cell type-specific expression patterns (Table 1). The intermediate filament polypeptides share a common tripartite structure consisting of a defined central rod and highly divergent, polypeptide-specific head and tail domains. The α-helical rod domains pair with each other and self-assemble subsequently via consecutive intermediates into ~10 nm filaments, which bundle, branch and form complex 3D networks. Unlike actin filaments and microtubules, intermediate filaments are non-polar, highly flexible, and are able to withstand high mechanical stresses. The intermediate filament cytoskeleton preserves not only the mechanical integrity of cells but also supports many other cellular processes including organelle function and positioning, directed migration, metabolism and stress responses (e.g., Refs. [1,2]).

The different functions are linked to unique intermediate filament network compositions and arrangements. This requires stability on the one hand but also remodeling on the other hand to adjust to the respective cell type- and differentiation-dependent local functions. The necessary dynamic filament and network architectures are achieved by interlaced processes, notably (Figure 1):

i) controlled cycles of assembly and disassembly: Assembly of intermediate filaments (nucleation, elongation, branching, bundling) is counterbalanced by filament disassembly at homeostasis. Shifting the equilibrium by either increasing assembly or disassembly allows rapid remodeling without the need of protein biosynthesis.

ii) local turnover: Two mechanisms govern local filament turnover. First, lateral subunit exchange can occur throughout the network albeit at different rates. Mechanistically, this is achieved by release and recruitment of soluble subunits resulting in heterogeneity of filament diameter. Second, filament severing allows excision or insertion of filamentous particles resulting in filament shortening and elongation, respectively.

iii) filament modification: Filament modification is achieved by interaction with proteinaceous regulators and post-translational modification. Intermediate filament-associated proteins modulate the morphology and spatial arrangement of intermediate filaments and their turnover resulting in cell type-specific arrangements with specific dynamic properties. Posttranslational modifications fine-tune network morphology, dynamics and function at subcellular precision and multi-scale temporal resolution.

The regulation and kinetics of the listed mechanisms are specific for each intermediate filament polypeptide type. The resulting range of network...
plasticity facilitates cell type-specific compartmentalization for localizing functions in dedicated niches. For this review, we selected examples from the last two years which focus on advances in the understanding of how the morphology and dynamics of cytoplasmic intermediate filaments affect cellular functions (Figure 2).

### Intermediate filament assembly supports subcellular domain specification

Nucleation is the assembly of filaments from soluble oligomeric subunits, i.e. dimers or tetramers. While nucleation of intermediate filaments occurs in vitro without the addition of nucleoside triphosphates or proteinaceous factors, its in vivo regulation is poorly understood. Evidence for controlled nucleation in living cells has been obtained through time-lapse recordings of fluorescently labelled intermediate filament polypeptides detecting the appearance of small particles in certain cellular subdomains [3–6]. It does not rely on de novo protein synthesis but re-uses soluble intermediate filament subunits [7]. In the case of vimentin and keratin, nucleating particles were most frequently observed in the cell periphery [3,8]. It has been suggested that focal adhesions serve as important regulators of vimentin nucleation acting either through direct physical binding or signaling [9,10]. Nucleation of keratin filament precursors has been documented next to nascent hemidesmosomes and desmosomes that appear in the periphery of growing epithelial cell colonies [11,12]. More importantly, keratin filament nucleation was also recorded in developing murine blastocysts at a time, when the first cytoplasmic

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**Table 1**

<table>
<thead>
<tr>
<th>Type</th>
<th>Members</th>
<th>Distribution</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>‘Acidic’ keratins</td>
<td>Epithelial cells</td>
</tr>
<tr>
<td>II</td>
<td>‘Basic’ keratins</td>
<td>Epithelial cells</td>
</tr>
<tr>
<td>III</td>
<td>Vimentin, Desmin, Syncoilin, Glial fibrillary acidic protein (GFAP), Peripherin</td>
<td>Mesenchymal cells, Muscle cells, Glial cells, Neurons</td>
</tr>
<tr>
<td>IV</td>
<td>Neurofilament proteins (NF-L, NF-M, NF-H), α-Internexin, Synemin, Nestin</td>
<td>Neurons, Astrocytes, muscle cells, Developing and regenerating cells, Nuclear lamina of all nucleated cells, Lens fiber cells</td>
</tr>
<tr>
<td>V</td>
<td>Lamin</td>
<td>Mesenchymal cells</td>
</tr>
<tr>
<td>VI</td>
<td>Filensin, Phakinin</td>
<td>Lens fiber cells</td>
</tr>
</tbody>
</table>

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Figure 1

The scheme illustrates how a dynamic intermediate filament network architecture is achieved. Intermediate filament networks undergo a constant cycle of assembly (nucleation, elongation, branching and bundling) and disassembly. Filaments are modified locally by severing and insertion as well as lateral exchange of subunits. Binding of intermediate filament-associated proteins and posttranslational modifications control and fine-tune these processes.
Intermediate filament network is generated [11]. A potential nucleating factor is Ndel1, which was recently shown to promote keratin assembly near desmosomes [13]. The nucleated particles elongate and integrate into the pre-existing intermediate filament networks as has been well documented for keratins, vimentin and neurofilament polypeptides [8,14]. These particles are highly motile and are transported in a microtubule and actin filament dependent fashion until they are integrated into the network. The overall net effect is that network architecture is non-polarized, when nucleation occurs without directional preference. This is the case in cells that are exposed to the same environment on all sides, e.g., in suprabasal cells of the epidermis that are completely surrounded by and bound to similar neighboring suprabasal cells. If nucleation occurs only in certain peripheral regions, which are defined, for example, by restricted contacts to neighboring cells or the extracellular matrix, it will lead to asymmetric, i.e., polarized network remodeling.

**Intermediate filaments affect cellular contractility**

A consequence of differential assembly is the generation of cell type-specific network topologies. Among them, the rim-and-spokes model has received increasing attention [11,15–17]. It describes keratin intermediate filament networks in confluent epithelial cells consisting of a layer of filaments below the plasma membrane, i.e., the circumferential rim interconnecting desmosomes, and radial filament bundles, i.e., the spokes that connect desmosomes to stable filaments forming the perinuclear cage [16]. The keratin rim is adjacent to the submembranous actin cortex. It may be physically attached to it via the cytolinker plectin. In accordance, plectin knock-out hinders the integration of cortical actin and keratin and consequently results in loss of the keratin rim, which leads to an increase of F-actin stress fibers [17].

Even more, vimentin intermediate filaments and F-actin form interpenetrating networks [18]. An interesting study reported on the interdependency of cortical actin...
and vimentin. Treating cells with electrophiles led to a reduction of cortical vimentin and redistribution of cortical actin into actin stress fibers. When cells produced vimentin mutants with a reduced sensitivity to electrophiles, vimentin redistribution was less affected and actin stress fiber formation was prevented [19].

Another example of intermediate filament-actin interdependency was recently described in smooth muscle cells. These cells co-express nestin and vimentin, which form heteropolymers. Increased contractility went along with increased recruitment of polo-like kinase 1 (Plk1) to nestin leading to activation of Plk1 and phosphorylation of nestin and vimentin. Vimentin phosphorylation presumably releases vimentin from the cortex resulting in increased plasma membrane localization of the actin-regulatory proteins cortactin and profilin. Cortactin and profilin are known to promote F-actin stress fiber formation and cell contractility. In accordance, down-regulation of nestin led to a reduced activation of Plk1 and reduced F-actin stress fiber formation and cell contractility [20]. This nicely fits with the observation that vimentin knock-out cells are less contractile [21].

Intermediate filaments contribute to the formation and stabilization of cellular protrusions

Dynamic membrane protrusions such as microvilli and cilia rely on juxtamembraneous actin filament- and microtubule-based-scaffolds [22,23]. The role of intermediate filaments in these structures is more restricted and mostly relevant for their maintenance and stabilization. They may serve as a flexible, yet resilient counter bearing between the rather rigid brush border/ciliated surface and the comparatively soft cytoplasm. Viscoelasticity mapping of this interface in the intestinal brush border of C. elegans by Brillouin microscopy confirmed this notion [24]. Recent publications furthermore emphasized the role of keratin intermediate filaments in the formation and maintenance of microridges [25–27]. Microridges are rigid, actin-based apical surface protrusions that have been described in zebrafish epidermis cells but also occur in multiple human epithelia. The interaction of keratins with the plakin family members periplakin and envoplakin is a prominent feature of mature microridges. This interaction is important to integrate actin and intermediate filaments and determines microridge length.

Lamellipodia are another type of cellular protrusion that are formed at the leading edge of migrating cells. In this instance, the cytoskeleton needs to adapt using the guidance cues provided by newly-formed focal adhesion-based attachment sites to the extracellular matrix. Responses of the intermediate filament system have been the subject of many studies revealing modulatory functions depending on intermediate filament isotype and cell type [28]. Vimentin expression is a hallmark feature of migratory cells. The tight crosstalk of vimentin and focal adhesions and the high degree of malleability of vimentin expressing cells may contribute to it [29,30]. It was recently shown, that migration speed and directionality were reduced by treatment of cells with the small chemical compound R491 which interferes with vimentin dynamics [31]. Nestin knock-down in smooth muscle cells also resulted in a reduced migration capacity and focal adhesion size due to reduced Plk1 activation and subsequent vimentin phosphorylation [32]. Overall, intermediate filaments may have a stabilizing function because of their lower turnover in comparison to the other cytoskeletal filament systems enhancing persistence of cell migration [33–36].

Intermediate filaments contribute to the peripheral localization and function of the endoplasmic reticulum

Besides structuring cell shape and surface specializations, intermediate filaments influence the localization and function of organelles by cytoplasmic compartmentalization [37]. Recently, high resolution microscopy revealed that the endoplasmic reticulum is associated with desmosome-anchored keratin filaments [38]. The tubules of the endoplasmic reticulum were oriented along keratin filaments towards desmosomes. Disruption of the keratin network also disrupted the morphology and dynamics of the endoplasmic reticulum and induced the expression of endoplasmic reticulum stress-related markers.

A function of intermediate filaments for the sarcoplasmic reticulum, which is important for cellular calcium handling in skeletal muscle cells, was recently reported [39]. It was shown that the muscle cell-specific intermediate filament polypeptide desmin interacts with the stromal interaction molecule 1 (STIM1), which is a sarcoplasmic reticulum transmembrane protein that controls Ca$^{2+}$ homeostasis. The interaction occurred specifically in regions, where the sarcoplasmic/ endoplasmic reticulum calcium ATPase (SERCA) pumps and plasma membrane Orai1 store-operated calcium channels reside. SERCA and Orai1 are known to coordinate Ca$^{2+}$ uptake into the sarcoplasmic reticulum. Since Ca$^{2+}$ handling is also linked to contact sites between mitochondria and the endoplasmic reticulum, it is interesting to note, that loss of the intermediate filament polypeptide nestin in senescent Leydig stem cells leads to a reduction of these contacts [40]. Similarly, mutations in keratin 6 led to reduced mitochondria-endoplasmic reticulum contact sites in keratinocytes [41].

Intermediate filaments protect the nucleus

A cytoplasmic intermediate filament-based “nuclear cage” has been described in multiple cell types. It is a
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Intermediate filament network dynamics are needed for cellular homeostasis

A current research challenge is to understand the different intermediate filament assemblies not only in different cells but even in the same cell signifying specialized intermediate filament properties and functions. A recent paper on the differential antibody accessibility of vimentin’s tail domain illustrates this aspect [48]. The authors found that a panel of antibodies recognizing different parts of the tail were able to differentiate between an extended conformation of vimentin that was preferentially detected in loose filaments in the cell periphery and a packed conformation of vimentin that was preferentially detected in more compact filaments in the cell center. The future will show, how this is regulated and how this affects local functionalities in the context of cellular homeostasis.

The cytoplasmic intermediate filament network furthermore unites two apparently contradicting properties: It confers stability supporting the maintenance of cell type- and function-dependent shape while also retaining the ability for remodeling during development, differentiation and stress response. This is achieved by constant network turnover, which can be reversibly regulated at high spatial and temporal precision [49]. The turnover is coupled to multiple mechanisms involving not only local subunit exchange (see above) but also biosynthesis, degradation, and transport. An instructive example of the combined action of degradation and transport was reported for the E3 ligase adaptor protein gigaxonin, which targets intermediate filament polypeptides for proteosomal degradation [50]. Mutations in gigaxonin have been identified in giant axonal neuropathy, which is characterized by large intermediate filament-containing aggregates in neurons that lead to extreme axonal swelling and subsequent cell death. While the phenotype was initially solely linked to reduced intermediate filament degradation, it was recently further shown to be linked to perturbed kinesin-1 dependent transport of intermediate filaments along microtubules. The authors propose that in the absence of gigaxonin, the increase in soluble intermediate filaments sequesters a yet unknown kinesin-1 adaptor and thereby prevents transport and proper distribution of insoluble intermediate filaments, which subsequently form the pathognomonic aggregates.

Intermediate filaments determine axonal conduction velocity

A unique structure-function relationship has been worked out in neurons. Neuronal intermediate filament polypeptides are synthesized in the perikaryon and are subsequently transported into the axon. The polypeptide composition and amounts of neuronal intermediate filaments determine axonal diameter which directly correlates with axonal conduction velocity. Knock-out experiments in mice confirmed this notion [51].

Elongated neurofilament particles have been detected in axons of cultured neurons. They were shown to move bidirectionally along microtubules with an anterograde bias and intermittent stops [52]. The bidirectional transport appears to be important for the longitudinal alignment and straightening of neurofilaments to allow unobstructed transport of other microtubule-motor protein cargoes [53]. With the help of transgenic mice expressing neurofilament protein M fused to a photo-activatable fluorophore, the direction and speed of neurofilament transport could be measured in vivo in myelinated axons of the tibial nerve [53,54]. It was further observed that the majority of the neurofilament pool is mobile [55]. The frequency of active neurofilament transport was dependent on phosphorylation [56]. Furthermore, it was observed that neurofilament transport accelerates locally at nodes of Ranvier [54]. The local constriction is coupled to reduced neurofilaments and increased approximation to microtubules which increases the probability of neurofilament particles to latch onto microtubules.

Conclusions

Taken together, we conclude that the divergent architectural configurations and dynamic properties of
intermediate filament networks support specialized functions depending on the cell type and the subcellular localization. The architectural changes are brought about by posttranslational modifications, interactions with intermediate filament-associated proteins and changes in intermediate filament polypeptide expression which occur at different time and length scales. This allows environmental conditioning of the intermediate filament cytoskeleton with its multifaceted modulatory roles.

**Author contribution**
All authors contributed to the conceptualization and writing of the manuscript.

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**References**
Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

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This study demonstrates that the cytolinker plectin contributes to the arrangement of keratin filaments into a circumferential rim and radial spokes and its linkage to desmosomes. The absence of plectin results in tensional disequilibrium and leads to reduced epithelial sheet cohesion.


This work beautifully shows a surprising intertwining of filamentous actin and vimentin intermediate filaments.


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The study uncovers GFAP splice variant-specific regulation of glioma cell invasion in brain slices and in vivo. The findings are in agreement with the correlation of GFAP splice variant ratio and glioma grading.


The beautiful publication employs high-resolution multidimensional light and electron microscopy to uncover the functional interdependency of desmosomes, keratins and the endoplasmic reticulum in a unique spatial arrangement.


The authors report on the cross-talk between the nuclear lamina and the keratin cytoskeleton finding a correlation between lamin and keratin 8 expression in colonic enterocytes, which is accompanied by changes in the LINC complex and mechanical resilience of the nucleus.


The authors work out how the keratin cytoskeleton responds to extracellular matrix rigidity and transmits mechanical signals to the nucleus.


The authors very carefully dissect the accessibility of segments of vimentin’s tail domain in different subcellular regions and during lipoxidative stress. It is proposed that the observed versatility in filament subdomain arrangement enhances the capability to fine-tune intermediate filament dynamics.


The study presents evidence for a function of the E3 ligase adaptor gigaxonin in kinesin-1-mediated intermediate filament transport.


