Cytoplasmic Intermediate Filaments

Subjects: Cell & Tissue Engineering

Contributor: Sandrine Etienne-Manneville

Cytoplasmic intermediate filaments (IFs), which together with actin and microtubules form the cytoskeleton, are composed of a large and diverse family of proteins. Efforts to elucidate the molecular mechanisms responsible for IF-associated diseases increasingly point towards a major contribution of IFs to the cell's ability to adapt, resist and respond to mechanical challenges. From these observations, which echo the impressive resilience of IFs in vitro, we here discuss the role of IFs as master integrators of cell and tissue mechanics.

Keywords: cytoskeleton ; mechanics ; resilience ; rigidity ; stiffness ; elasticity ; viscosity ; rod domains ; coiled-coil region

1. Introduction

Tissue integrity, which is necessary for all metazoan life, relies on the ability of cells to adapt their morphology, their interactions, and their function to the conditions of their environment. The cytoskeleton, including actin microfilaments, microtubules, and intermediate filaments (IFs), form essential intracellular networks which support cell shape, cell adhesions and are indispensable for most cellular functions. While the role of actin in cell morphology, motility, and contractility has been extensively studied and the contribution of microtubules to intracellular trafficking, cell polarity, and adhesion dynamics is now well understood, the role of IFs in cell functions and tissue integrity remains unclear. This is partly because, in contrast to the ubiquitously expressed actin and tubulin, IF protein expression varies between cell types and tissues, and IF protein levels can represent anything from 0.3 to 85% of total protein levels in the cell [1][2]. Despite a high level of shared structural features between cytoplasmic IFs such as a head, rod, and tail domain, the more than 70 known IF genes create highly specialized, cell-type-specific networks of polymeric filaments. IFs are subdivided into five subtypes depending on small structural differences, their modes of assembly, and their expression pattern [3]. GFAP, vimentin, synemin, and nestin form the IF network in glia, neurofilaments in neurons, desmin and syncoilin in muscles, keratins in skin, and vimentin in mesenchymal cells. Consequently, the depletion of single IF genes does not always lead to severe phenotypes. However, in humans, mutations in IF genes give rise to a large diversity of diseases commonly characterized by the altered integrity of specific tissues [4]. The lack of associated molecular motors and well-characterized regulators of the assembly/disassembly dynamics further distinguishes IFs from actin and microtubules. These characteristics are probably responsible for our late understanding of IF functions at the cellular and tissue level. Only the painstaking studies of each type of IF's structural and mechanical properties and their integration at the network, cellular and tissue levels are slowly unraveling the contribution of IFs to the physiology and pathology of multicellular organisms.

2. Intermediate Filaments as Key Players in Tissue and Cellular Mechanics

At the cellular level, studies have progressively confirmed that IF's function in tissue integrity relies on their contribution to cell resilience under both mechanical stretching and compression. Indeed, the depletion of keratin or vimentin increases the deformability of stretched cells [5][6] (**Figure 1** B,C). Importantly, the loss of vimentin also decreases the viability of cells submitted to stretching [7] (**Figure 1** C). The role of IFs in cellular resistance to deformation by compressive forces appears to be cell-type specific. Under compression, both the depletion of vimentin in human mesenchymal stem cells [8] and the overexpression of vimentin in amoeboid cancer cells [9] reduce cell deformation. This discrepancy may be due to a different initial level of IF protein expression optimized in a cell-type-specific manner to provide cells with different mechanical properties. Alternatively, the difference may result from a cell-type-specific composition of the cytoskeletal network. For example, the depletion of vimentin in compressed highly contractile cells might have a different effect on their deformability compared to depletion in cells with lower contractility. Taken together, these studies show how IFs contribute to cell resilience by limiting cell deformation under mechanical stress and allowing stretched or compressed cells to recover their initial shape without any damage. However, they also suggest that the mechanical resilience of a given cell type in a specific situation may require an optimal level of expression of a specific IF type.

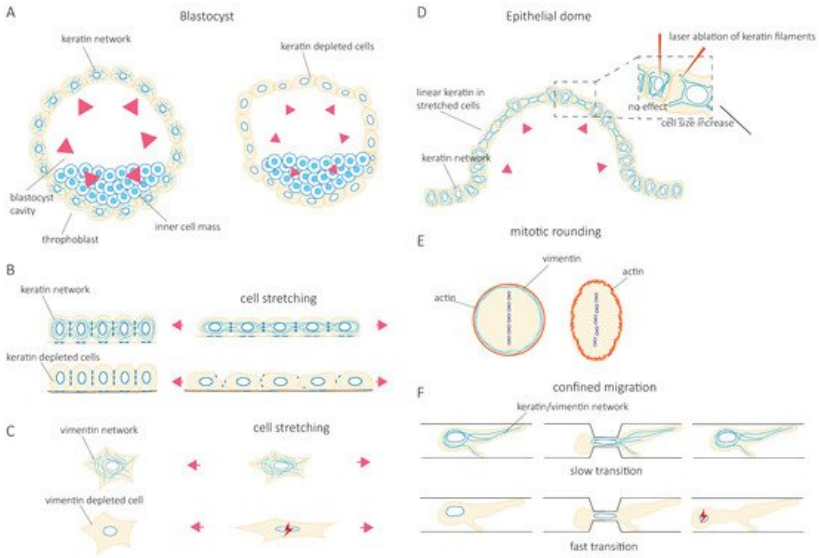

Figure 1. Intermediate filaments, guardians of tissue and cell integrity, adapt cell mechanics to cell behavior. (**A**). During embryo cavitation, keratins are essential to generate apical tension (pink arrowheads) against the increasing internal pressure in the blastocyst. This tension is lost in the absence of keratin 8 and 18, which leads to a decrease in volume and increased surface curvature. (**B**,**C**). Depletion of keratin in a collective cell sheet and vimentin in single cells increases cell deformation upon stretching. In vimentin-depleted cells, this leads to an increase in cell death. (**D**). Expanded epithelial domes consist of stretched and unstretched cells. Stretched cells contain unusually straight keratin bundles, which, when disrupted by laser ablation, cause the cell to lose its shape and largely increase its area. (**E**). The vimentin network contributes to cortical tension during mitotic rounding. The loss of vimentin impairs rounding and induces abnormalities in chromosomal aggregation. (**F**). Both keratin and vimentin IFs slow down confined migration. Depletion of keratin or vimentin in different cell types increases their confined migration speed but promotes nuclear damage (red lightning sign).

The IF network can also reorganize in response to mechanical stresses [10][11][12][13][14]. Rearrangement of the network is nicely illustrated in epithelial domes. In this three-dimensional in vitro epithelial sheet model, cells undergo extreme deformations while the tension across the epithelial sheet is maintained constant [15]. In fully expanded domes, extremely stretched cells coexist with cells that barely change their shape. The stretched cells are characterized by the formation of unusually straight bundles of keratin IFs, which extend from the nucleus to the plasma membrane. Laser ablation of these IF bundles results in a rapid increase in the cell area [15] (**Figure 1** D). This suggests that keratin IFs in highly stretched cells are load-bearing elements that maintain the reversibility of cell shape after large deformations. At the cellular scale, cell stretching by hypo-osmotic stress partly depolymerizes vimentin and nestin filaments and redistributes them throughout the cells [13]. This rearrangement is essential to cell survival after hypo-osmotic shock, confirming the contribution of IFs to cell mechanical resistance.

Accumulating evidence shows that IF networks are rearranged when cells adapt to external mechanical challenges. Cells can generate contractile or pushing forces to reshape in order to accomplish specific functions such as cell division or migration. To divide, cells must actively generate forces to accommodate their shape changes and overcome mechanical constraints by the surrounding tissue. The IF network reorganizes extensively not only to adapt to but also to promote these changes [16][17][18][19]. As cells round up to facilitate the accurate positioning of the spindle and the correct segregation of chromosomes, cortical tension generated by the actin cortex increases. In HeLa Kyoto cells, vimentin IFs contribute to this increase in cortical tension by relocalizing to the cortex, where they interact with actin to control actin organization [20][21] (**Figure 1** E). In confined environments, the loss of vimentin becomes detrimental to the segregation of chromosomes, and chromosome lagging is often observed [20]. However, besides vimentin IFs, HeLa cells also express keratins, which also reorganize during mitosis and affect the organization of vimentin. Indeed, when cells express nestin, the reorganization of vimentin during cell division is different. In nestin-expressing ovary (CHO) cells, C6-2 glioma, BHK-21 fibroblast, and cerebellar ST15A cells, the vimentin network disassembles at the cleavage furrow and does not localize to the cortex [22][23]. It thus seems that the reorganization of IFs that accompanies the changes in cell mechanics depends on IF proteins that are expressed. The cell-type-specific composition of the IF network needs to be taken into account in future investigations.

Migration in confined environments requires resilient mechanical support to allow cell deformation but prevent damage as they pass through complex environments. IFs appear to provide the essential mechanical support. Keratin knock-out (KO) keratinocytes migrate faster when squeezing through small pores in a Boyden chamber assay. However, they also

frequently rupture and die [5] (**Figure 1** F). Similarly, vimentin depletion facilitates the migration of MEFs (mouse embryonic fibroblasts) in confined environments such as microchannels, collagen gels, and small pores [24][25] at the cost of nuclear alterations, nuclear envelop ruptures, and blebs [24] (**Figure 1** F). These observations were also confirmed in studies investigating the amoeboid migration of melanoma cancer cells [9]. Both keratin and vimentin networks appear to provide mechanical support to protect the nucleus against excessive deformations and maintain nuclear homeostasis during confined cell migration [24][25][26]. However, the switch from keratin to vimentin expression observed during the epithelial-to-mesenchymal transition (EMT) suggests that the two IF networks differentially contribute to the cell's mechanical properties [27][28][29][30]. The microinjection of purified vimentin into MCF-7 epithelial cells changes the cell shape to a mesenchymal cell morphology [28]. Whether this effect is solely due to the mechanical functions of IFs or also reflects their role in intracellular signaling and cell motility remains unclear. While IFs in general, and the organization of the cytoplasmic IF network in particular, are essential to provide cell mechanical resilience, it is tempting to speculate that the mechanical specificity of each type of IF participates in cell-type-specific mechanics. Therefore, the control of IF protein expression may be central to the acquisition of cell-type-specific mechanical behavior adapted to the properties of their microenvironment and the modifications of cell behavior observed in pathological situations.

3. Mechanical Properties of IF Networks and Single Filaments In Vitro

In contrast to actin microfilaments and microtubules, which interact with motors such as myosin, dynein, and kinesin, IFs do not bear molecular motors to provide mechanical forces. Instead, it is the fundamental structure of IFs that is at the heart of their mechanical properties (**Figure 2**). In vitro studies of reconstituted IF networks and single filaments have recently shed light on the structural bases of IF mechanical properties. In this chapter, we recapitulate the results obtained with oscillatory shear rheology experiments on in vitro assembled IF networks which demonstrate their high elastic properties. We discuss how the mechanical properties of IF networks rely on inter-filament interactions (**Figure 3**) and single filament mechanics (**Figure 4**) based on biophysical in vitro measurements.

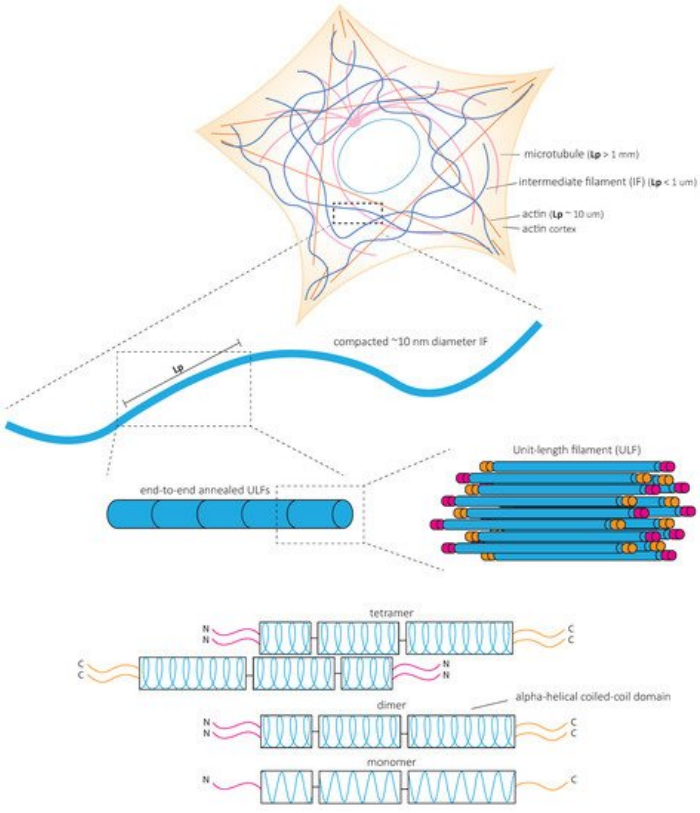

Figure 2. Intermediate filament structure. All intermediate filament (IF) proteins are formed by an α-helical 'rod' domain flanked by unstructured N- and C-termini that extend out of the assembled filament. The rod domain contains three coiled-coil domains responsible for the formation of IF dimers [31]. Dimers assemble in an anti-parallel fashion into apolar tetramers. Tetramers assemble via lateral associations into a unit length fragment (ULF) that anneal longitudinally and eventually form long 10 nm diameter filaments after lateral compaction. Soluble IF tetramers contribute to subunit exchange [32][33][34][35][36][37] and may influence the stability of the filaments and the mechanical properties of the network [37]. Lp = persistence length.

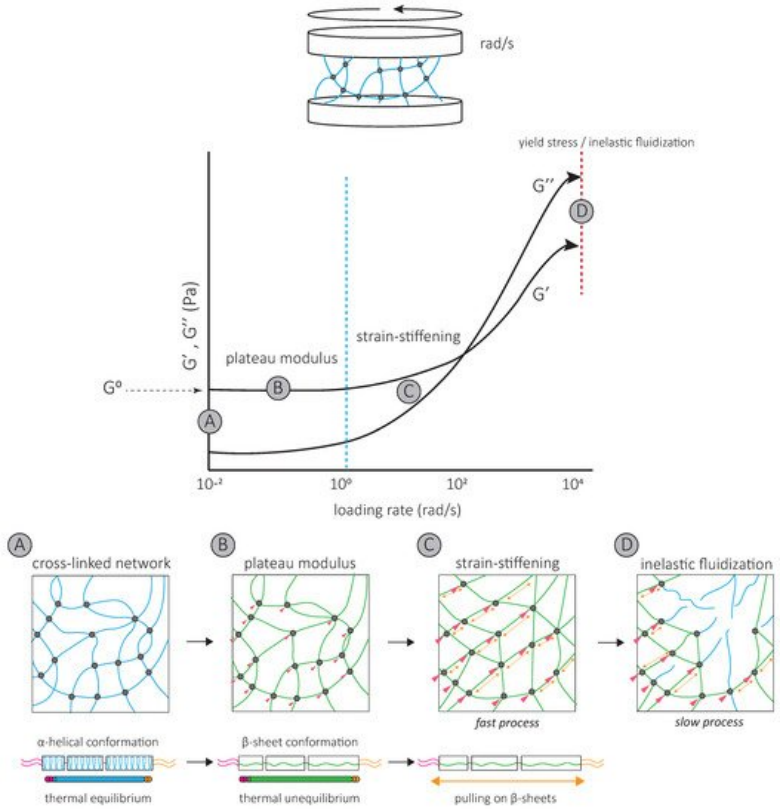

Figure 3. The mechanical properties of IF networks characterized by oscillatory shear rheology experiments of in vitro assembled networks (**A**). Deformation of the network using oscillatory shear rheology is achieved by network assembly between two plates and rotating one plate while the other is fixed, inducing a controlled deformation. Increasing the frequency of the rotations (rad/s) increases the stress and strain applied to the network. The response of the IF network to oscillatory shear is viscoelastic. Within the range of 0.01 and 10 rad/s, G' and G'' are independent of the oscillatory frequency, and a plateau is observed in both the G' and G'' curves. The corresponding G' is called the plateau modulus or G^0 (**B**). Further increasing the stress by increasing the oscillatory shear frequency results in strain-stiffening of the network ((**C**), start is indicated with blue dotted line). The network strain-stiffens up to a critical stress, the yield stress ((**D**), red dotted line), at which the network ruptures. This corresponds to the start of inelastic fluidization. The plateau modulus results from attractive forces between filaments (grey circles) to maintain the stretched β-sheet conformation of single filaments between crosslinks which are in thermal unequilibrium ((**B**), green filaments). The weaker inter-filament interactions between filament rod domains are thought to be responsible for the attractive interactions at low loading rates. Lower forces are required to resist the lower stresses (pink). Strain-stiffening results from the stretching of single filaments in β-sheet conformations ((**C**), yellow arrows) maintained by stronger inter-filament interactions. C-terminal tails of IFs are thought to be responsible for these inter-filament attractive interactions, which have to resist high stresses (pink). At the critical yield stress, the network ruptures as a result of the unbinding of multiple crosslinks (**D**). Strain-stiffening and inelastic fluidization compete at high stresses, resulting in a loading-rate-dependent rupture of the network [38]. Strain-stiffening is a fast process (**C**) while inelastic fluidization is slow (**D**). At fast loading rates, strain-stiffening dominates, and the network can resist higher stresses. At slow loading rates, the unbinding of crosslinks has time to occur within the same time frame as strain-stiffening, resulting in rupture of the network at lower stresses.

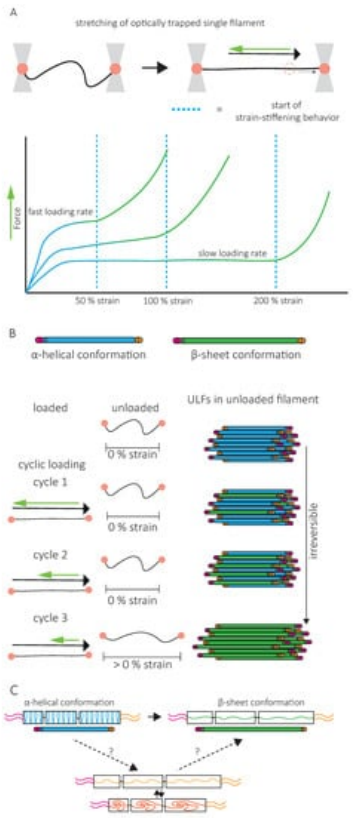

Figure 4. Single intermediate filament mechanics and underlying conformational changes. (**A**). Optical trap of a single intermediate filament and force–strain curves generated from stretching a single filament at different loading rates. The curve is characterized by a steep increase in force at low strains derived from the stretching of the alpha-helices (blue line and dimer). This is followed by a plateau in the curve at intermediate strains, attributed to the unfolding of α-helices to β-sheet conformations (green line and dimer). At higher strains, the filament strain-stiffens as a result of pulling on β-sheets. Both filaments stretched by atomic force microscopy and optical trapping demonstrate this mechanical response. For fast loading rates, strain-stiffening is already observed at 50% strain, while at slow loading rates, strain-stiffening is not observed before 200% strain. (**B**). For repeated loading and unloading cycles, the force needed to reach the same strain decreases with each cycle. This is attributed to part of the dimers being in a β-sheet conformation (green) while others remain in an α-helical conformation (blue). Lower forces are required to unfold the remaining α-helices. The filament length after the loading force is released is determined by the shortest elements, thus α-helices. After all α-helices have switched to a β-sheet conformation, elongation of the unloaded filament can be observed. Experiments have shown that the original mechanical properties are irreversible within physiologically relevant recovery times. The β-sheet conformation requires energy and is in thermal unequilibrium. Upon unloading, the filament is expected to return to its α-helical thermal equilibrium conformation. However, the irreversibility of its original mechanical properties does not fit this explanation. (**C**). To explain the irreversibility of the original mechanical properties of single filaments, a third disordered conformational state has been proposed (orange), which can elongate (yellow) but cannot return back to an α-helical con

The strain-stiffening behavior of the IF network, observed at high strains, results from further filament stretching between crosslinks (**Figure 3**) and is completely suppressed by the addition of a non-ionic surfactant [39]. Strain-stiffening is lost in networks formed by tailless filaments [39][40][41][42], showing that the filament C-terminal tails are responsible for the strong, attractive interactions necessary to withstand high stresses [39]. For instance, the characteristic side arms of neurofilament proteins are thought to be involved in the crosslinking of the network and provide resistance at large deformations [43].

The strain-stiffening of the network is limited by the rupture of the network [44][39][40][45] (**Figure 3**). The stress at which the network ruptures, called the yield stress, is much higher for IF than for actin networks. When the yield stress is reached, a softening of the network is observed, which depends on the nature and degree of inter-filament interactions. The softening of vimentin IF networks is transient, which also distinguishes them from actin networks [46][47], and indicates that filaments are not permanently fractured [48]. Strengthening attractive interactions by the addition of divalent cations [49][40][50][45][51] or permanently crosslinking the network [48] increases the yield stress, suggesting that the softening of IF networks is due to the loss of interactions between IF proteins [48] (**Figure 3**). The softening of vimentin IF networks is also loading-rate-dependent [48] (**Figure 3**). At slow loading rates, the disruption of transient C-terminal inter-filament interactions counteracts the strain-stiffening response [48]. Here, the mechanical response is dominated by the disruption of crosslinks which occurs before or at the same time as the stiffening of the network and results in a low yield stress (**Figure 3**) [48]. At fast loading rates, the strain-stiffening precedes the disruption of crosslinks, and the yield stress is much higher (

Figure 3) [48]. Similarly, transient interactions between neurofilament side arms are thought to be responsible for the reorganization of the network following disruption by large prolonged strains [43]. At fast deformations, these interactions provide the IF network with mechanical resilience [43].

A second specific characteristic of IFs is their high stretchability and strong resistance to breakage. In vitro, single IFs can be stretched about 250% before breakage and can even reach 350% for desmin filaments [52][53]. For low strains up to 100%, a steep increase in force is observed with increasing strain [53][54][55][56]. The force–strain curve plateaus for intermediate strains, and at higher strains, the force further increases, indicating strain-stiffening of the filament [56] (**Figure 4** A). X-ray experiments [57] and mathematical modeling approaches suggest that the steep linear increase in force at low strains results from the elastic stretching of the coiled-coil α-helical domains [56][58][31] (**Figure 4** A). Further stretching of the α-helical domains leads to their transition to β-sheets, reflected by the force plateau at intermediate strains. The stiffening of the filaments at higher strains corresponds to the necessity to exert higher forces to extend β-sheets further (**Figure 4** A). This conformational change and the stretching of single filaments in a β-sheet conformation between filaments in IF networks are essential to the high G 0 and strain-stiffening of the network, respectively (**Figure 3**).

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