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Intermediate filament dynamics: What we can see now and why it matters

Amélie Robert[±], **Caroline Hookway**[±], and **Vladimir I. Gelfand**[*]

Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago IL, USA

Abstract

The mechanical properties of vertebrate cells are largely defined by the system of intermediate filaments (IF). As part of a dense network, IF polymers are constantly rearranged and relocalized in the cell to fulfill their duty as cells change shape, migrate, or divide. With the development of new imaging technologies, such as photo-convertible proteins and super-resolution microscopy, a new appreciation for the complexity of IF dynamics has emerged. This review highlights new findings about the transport of IF, the remodeling of filaments by a process of severing and re-annealing, and the subunit exchange that occurs between filament precursors and a soluble pool of IF. We will also discuss the unique dynamic features of the keratin IF network. Finally, we will speculate about how the dynamic properties of IF are related to their functions.

Keywords

dynamics; keratin; neurofilament; severing and re-annealing; subunit exchange; transport; vimentin

Introduction

The cytoskeleton must be both rigid and flexible. It needs to be rigid to support cell shape, keep organelles in place, guide transport of cargo, and resist mechanical stress. At the same time, the cytoskeleton must be capable of the architectural adjustments required when cells change shape, migrate, or divide. Adjustments can be achieved by bending or moving the cytoskeletal structure or by local disassembly and reassembly of the cytoskeletal components. For two major components of the cytoskeleton in eukaryotic cells, filamentous actin (F-actin) and microtubules, dynamics have been investigated in great detail. But the metazoan cell cytoskeleton includes a third major component with a unique composition in each cell type, intermediate filaments (IF), for which the dynamic properties remain unclear.

Unlike F-actin and microtubules, which are made up of homogeneous globular proteins called actin and tubulin, respectively, IF are composed of one or more members of a large family of highly insoluble proteins encoded by multiple genes (more than 70 in human) [1].

[*]Corresponding author: Vladimir I. Gelfand, vgelfand@northwestern.edu.

[±]Co-first authors.

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These IF proteins are not globular and share similar central alpha-helical rod domains flanked by variable non-helical N- and C-termini. IF are divided into six types according to sequence homology in the rod domain (Table 1). Depending on their type, IF proteins form homopolymers and/or heteropolymers, and the expression of each type is regulated such that each cell type has its own IF signature. This extraordinary heterogeneity combined with additional post-translational modifications (PTM) of IF based on cellular context, allows cells to adjust their mechanical properties depending on the tissue and stage of development.

Another unique feature of IF is their mode of assembly, which is fundamentally different than that of the two other cytoskeletal components. F-actin and microtubules assemble into polar structures, and polymerization preferentially occurs at one extremity while depolymerization occurs at the other. In contrast, IF are non-polar filaments, and their turnover is not quite well understood. At least the assembly of homopolymeric IF has been well-characterized in vitro by studies conducted with vimentin and desmin type III IF (Fig. 1) (for a review see [2]). From this, assembly can be described by the following sequence: the rod domains of two IF polypeptides align in parallel to form a dimer. Two dimers associate laterally in an antiparallel fashion to form a non-polar tetramer, which is the smallest subunit of filaments observed in cells. Typically, eight tetramers assemble laterally into the unit-length filament (ULF), and ULF anneal end-to-end to form non-polar filaments. During the last phase of assembly, filaments mature to a width of approximately 10 nm as a result of radial compaction.

In vitro, IF proteins self-assemble into insoluble and quasi-unbreakable polymers without the help from co-factors or nucleoside triphosphates [3]. However, in cellulo, filaments coexist with particles (one or groups of ULF) and soluble (tetrameric) IF protein. Therefore, filaments undergo constant assembly and/or remodeling in the cell. These processes are likely regulated, raising the intriguing prospect that each form of IF might play different roles in the cell. In fact, compelling evidence demonstrates that IF function goes beyond a strictly mechanical role and includes participation in dynamic cell processes like adhesion, migration, and invasion [4, 5]. We propose that specific oligomeric forms of IF may be required for these functions, and the balance of the IF populations may influence these functions. Additionally, IF have been shown to play a role in cell signaling [6–13], in the filamentous [14] as well as in the soluble form [15, 16]. Therefore, regulating soluble IF may contribute to cell signaling.

The first studies to demonstrate that IF are dynamic showed fluorescence recovery after photobleaching (FRAP) of fluorescent-tagged versions of IF proteins, including both vimentin homopolymers [17, 18], as well as the heteropolymers, keratin [19], and neurofilaments (NF) [20]. However, the imaging used for these early studies did not provide sufficient resolution to image individual IF, and therefore could not discriminate between filament transport and filament remodeling. Now, with newly available technology, including the development of photoconvertible fluorescent proteins and super-resolution microscopy, we are just starting to understand the complexity of IF dynamics. Here, we will highlight new and exciting findings about IF transport, filament remodeling by a process of severing and re-annealing, and subunit exchange between ULF and the soluble pool of IF proteins. We will also discuss the unique dynamic features of the keratin IF network.

Finally, we will speculate about how the dynamic properties of IF ensure proper IF functions.

Intermediate filaments are a cargo for microtubule-based transport

One major mechanism by which IF are distributed throughout the cell is by their association with microtubules, which provide tracks for IF transport. Due to the complexity and density of IF networks and limitations in imaging, most studies of IF transport have focused on the movement of IF precursors at the cell edge. IF precursors consist of the smallest assembled pieces of filaments, particles (one or a few ULF), and short filament pieces called squiggles that are formed by the joining of several particles together or by the severing of longer filaments into short fragments. Movement of vimentin, neurofilament, and peripherin IF precursors is dependent on microtubules and microtubule-dependent motors [18, 21–26], while movement of keratin particles is dependent on F-actin [27]. Squiggles and particles of vimentin [23] and keratin [28] move at the cell periphery, join together, and create longer filaments that eventually incorporate into the network. Additionally, vimentin IF break down into squiggles and particles at the cell edge when lamellipodia formation is induced [29].

How the transport of IF precursors is regulated is not well known, but new clues are beginning to emerge. In particular, the polymerization-deficient mutant of vimentin (vimentin^{Y117L}) [30] has recently been used as a tool to study precursor transport [31] since its expression leads to the formation of ULF particles, but not filaments in cells. Therefore, a GFP-tagged version of vimentin^{Y117L} forms moving particles that are easy to visualize, facilitating the quantitative analysis of filament precursor motility in the absence of any background created otherwise by a dense and complicated filament network. Co-expression of GFP-vimentin^{Y117L} with a microtubule marker showed directly, for the first time, the transport of vimentin particles along microtubules tracks [31]. The same study also showed that F-actin is a major negative regulator of precursor transport. Tethering of vimentin precursors to actin-rich focal adhesions via plectin 1f or other cytoskeletal cross linkers could be part of this regulation ([32], see Box 1 for details). Furthermore, ULF transport is also directly modulated by signaling pathways independently of F-actin, as Rho-kinase activity inhibits ULF transport along microtubules while PAK2 stimulates it, even in the absence of F-actin [31]. Both enzymes have been shown to phosphorylate vimentin [33]. Rho-kinase and PAK are known to play a crucial role in actin organization during cell migration. Classically, Rho-kinase increases tension at the rear of the cell while PAK decreases it and stimulates actin reorganization at the front of the cell [34]. We can speculate that in addition to the role of each on actin, PAK promotes transport of ULF from the front of the cell to a new location, like the rear of the cell, while Rho-kinase slows down ULF transport to facilitate ULF incorporation into IF.

Box 1

Interplay between IF dynamics and cytoskeletal linker proteins

In order for the spatial regulation of IF functions, IF are targeted to different sub-cellular locations by cytoskeletal linker proteins. Known IF linker proteins are mostly members of the vast plakin family, which consist of large, modular proteins that cross-link

cytoskeletal networks to each other and to adhesion complexes. Other IF linkers are actin cross-linking proteins connecting IF to highly dynamic structures that trigger cell adhesion to the extracellular matrix. We speculate that some examples listed below affect IF dynamics by reducing IF transport or modulating filament remodeling.

	IF Interaction	Function
Plakins		
Desmoplakin	Keratin IF to desmosomes [35, 36]	KO of desmoplakin results in embryonic lethality due to the loss of integrity of the extra-embryonic ectoderm [37]. When desmoplakin expression is rescued in extra-embryonic tissues, the animal dies shortly after gastrulation from major defects in the heart muscle, neuroepithelium, and skin epithelium. This illustrates the importance of the linkage between keratin IF and desmosome plaques for epithelium integrity
BPAG1 BPAG1n	BPAG1 connects keratin IF to integrin α6β4 at hemidesmosome plaques [38, 39]. The neuronal form BPAG1n connects NF to actin-F [40, 41]	BPAG1 KO in mice results in compromised mechanical integrity of cells, abnormal migration of keratinocytes, and severe dystonia and sensory nerve degeneration in the animal [40, 42]
Epiplakin	Preferential binding to assembled keratin [43]. Some binding to vimentin and desmin IF [44]	Epiplakin is a good candidate as a negative regulator of IF transport or IF remodeling during stress conditions. Its binding to keratin filaments is induced in response to different stressors and promotes keratin bundling in proliferating keratinocytes during wound healing, suggesting a role for epiplakin to reinforce the keratin network during mechanical stress [45, 46]
Plectin	All major types of IF proteins bind to specific plectin isoforms [47] found at various cellular locations (for review see [48])	Plectin may affect IF dynamics: Plectin1f may be a negative regulator of vimentin transport, as it recruits vimentin at focal adhesions, promoting the end-to-end fusion of mobile vimentin particles to immobilized short vimentin filaments [32]. Furthermore, IF are less stable and more soluble in the absence of plectin in fibroblasts and keratinocytes [6, 14], suggesting plectin negatively regulates IF remodeling. We speculate that different forms of IF or those modified by particular PTMs could have different affinities for specific plectin isoforms
Other		
Fimbrin	Tetrameric vimentin in podosomes, filopodia and retraction fibers of adherent macrophages [49]	The vimentin-fimbrin complex may be involved in directing the assembly of the vimentin cytoskeleton at cell adhesion sites. Alternatively, binding of fimbrin to tetrameric vimentin could prevent filament assembly in these dynamic structures
Filamin-A	Vimentin to focal adhesion [50]	Association of filamin-A and vimentin during early stages of adhesion recruits vinculin and paxilin [50] to focal adhesions and regulates recycling of integrin β1 to the plasma membrane [51]. This regulation may rely on the PKC-dependent phosphorylation of vimentin that stimulates local vimentin disassembly to release the integrin and allow its integration into the plasma membrane [52]

Together, these results have suggested a model for organizing IF networks in cells: a dynamic population of short filament squiggles and particles move at the cell periphery in order to be joined together and incorporate into the otherwise static network, and the network is broken down into squiggles and particles, which can move to a new location when the network needs to be reorganized. However, this model overlooks the transport of

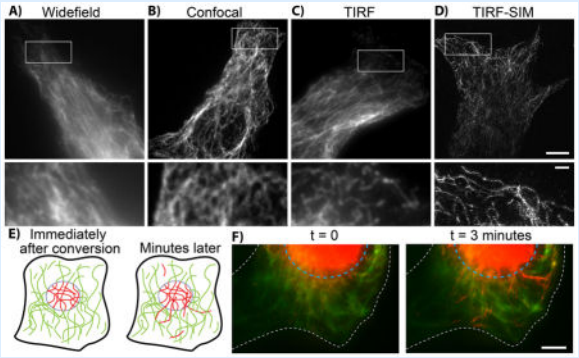
mature filaments within the network itself. There is now compelling evidence that several kinds of fully polymerized forms of IF are capable of transport like that of filament precursors, as will be discussed in the following paragraphs.

The first clue to suggest that fully polymerized IF are transported as cargo along microtubules by microtubule motors came from the dramatic reorganization of vimentin filaments after the injection of an inhibitory antibody against the heavy chain of kinesin [53]. More direct evidence has since come from recent live-cell imaging experiments. The technical challenges of imaging IF within dense networks has impeded the study of mature filament transport in most cell types. However, NF, the major IF type in neurons, can be studied in the axon where NF must get from their sites of synthesis and assembly to their final location. Imaging of GFP-tagged NF has demonstrated that NF are transported in axons, moving through NF-free gaps [54], or into photo-bleached segments [55], though NF also are transported in other forms (NF precursors) [56]. As in the case of vimentin squiggles and particles, transport of NF is dependent on microtubules and requires the microtubule-motors kinesin (in particular, Kif5A) and dynein [24, 57–64]. NF also associate with actin and myosin [65, 66]. NF transport can be characterized by periods of rapid movement that occurs when they are transported along microtubules, and long pauses that occur when they are off microtubule tracks [67, 68], transitioning between these states by myosin Va [66].

The development of photoactivatable and photoconvertible protein tags enabled the investigation of whether other mature forms of IF are also transported in cells, even as part of a dense network. The use of vimentin tagged with a photoconvertible protein combined with advanced forms of light microscopy revealed that vimentin IF move dramatically even within dense network regions (see Box 2) [69]. Not only was this the first study to show that mature filaments move throughout the cytoplasm, but it also presents a method for the quantification of filament transport. Such a method will facilitate future studies to determine the mechanisms and regulation of filament transport. For example, this method was used to demonstrate that transport of mature vimentin filaments depends on microtubules and microtubule motors, just as NF do, but not on the dynamics of microtubule polymerization. Additionally, super-resolution microscopy combined with two-color labeling of microtubules and vimentin directly shows the transport of IF along microtubules. Therefore, not only filament precursors, but at least two types of mature IF can actively be transported along microtubules.

Box 2

Imaging intermediate filament dynamics



Advances in microscopy methods and photoconvertible proteins have improved the study of intermediate filament dynamics.

First, new techniques in light microscopy, especially super-resolution microscopy, allow for greater detail in the merely ~10 nm-in-diameter intermediate filaments to be observed. Here, we compare four different modalities of light microscopy commonly used to image living cells and show how they perform for imaging vimentin intermediate filaments: (A) Widefield fluorescent microscopy excites fluorescent molecules both within and outside of the focal plane. As a consequence, the signal from filaments in focus can be obscured by the background created by those out of focus, especially in more central regions of the cell where the network is dense. Additionally, because cells are exposed to so much light, this technique can be phototoxic, especially when several frames must be collected, as in a time-lapse; (B) spinning disk confocal microscopy uses pinholes to narrow illumination to only a single plane of the sample, ~1 μm thick. This removes much of the out of focus light, greatly improving the signal to noise in images of vimentin filaments; (C) total-internal-reflection-fluorescence (TIRF) microscopy goes even further to improve signal to noise by narrowing the plane of illumination to only ~100 nm at the interface between the coverslip and the sample. This modality works very well to image vimentin filaments since there are several near the cell membrane, though filaments cannot be imaged deeper in the cytoplasm. Because the amount of light delivered to the sample is so restricted, this technique is also advantageous in minimizing phototoxicity; and (D) TIRF Structured Illumination Microscopy combines the advantages of TIRF microscopy with super-resolution. In this case, a set of images are collected using patterned illumination, and the interference pattern theses images create provides information in the sample beyond diffraction-limited resolution (hence super resolution, ~100 nm in this case). This allows fine detail within vimentin filaments to be observed. Scale bar, 5 μm, inset scale bar, 1 μm.

The second major advance is the development of photoconvertible proteins. In the case of vimentin, the photoconversion of a subset filaments within the dense network allows for the behavior of just a few filaments to be teased apart from the rest. Therefore, combining photoconversion and live-cell time-lapse imaging can be used to follow the

transport and remodeling of filaments within the network. (E) Depicts a schematic and (F) shows a real example of how photoconvertible-protein tagged vimentin can be used to study filament transport. Immediately after conversion (E and F left panel), photoconverted, red, filaments are restricted to the region of conversion, but after only a few minutes (E and F right panel), many photoconverted filaments are transported outside of this area. Scale bar, 5 μm.

The motors responsible for transporting filaments other than NF (including vimentin) on microtubules still remain to be identified. Since the motor known to transport NF, Kif5A, is enriched in neurons but only expressed at low levels in other cell types [70], the motors that transport NF and other IF may be different. It is also possible that IF are transported indirectly by their associations with other cargo. For example, vimentin might be dragged along with mitochondria via their interaction [71] as mitochondria are transported (or the other way around; some mitochondria tightly coupled with IF might be moved in the cell as IF are transported along microtubules). Additionally, since microtubules are capable of transporting each other in a process of microtubule-microtubule sliding [72], and IF associate with microtubules not only through motor proteins, but also through potential tethers like plectin [73], we speculate that IF may also be indirectly transported by piggy-backing off of sliding microtubules.

Future research will also need to address the regulatory mechanisms of mature filament transport as has been started with ULF (see above). Whether precursors and mature filaments are transported and regulated by the same mechanisms remains unknown. However, it is likely that if these populations are performing different functions in the cell, their transport will be regulated differentially.

Remodeling of mature filaments by a process of severing and re-annealing

For decades it has been known that the assembly state of filaments is linked to their phosphorylation state, with phosphorylation on serine (Ser) and threonine (Thr) residues generally promoting disassembly and dephosphorylation increasing stability [74, 75]. However, evidence that filaments reorganize by complete disassembly and reassembly is limited. One example is the disassembly of vimentin filaments via a phosphorylation-dependent mechanism that occurs during mitosis in BHK-21 cells, although these filaments remain intact during mitosis in most other cell types (see for example, [69, 76, 77]). Instead of complete disassembly and re-assembly, it has been proposed that filaments can exchange subunits along their length [19, 78, 79]. During this process, a filament looses subunits to a soluble form that can be incorporated somewhere else along the length of the filament (Fig. 2B).

More prominently than exchanging soluble subunits, vimentin IF and NF change their composition by a process of severing and re-annealing [69, 79–81]. This is based on the observation that when two differently labeled populations of filaments are mixed, after time, the labels are distributed in patched segments along the length of individual filaments, consistent with the formation of new filaments from the annealing together of the filament populations (Fig. 2A). In contrast, if filaments were to reorganize by disassembly and

reassembly or by exchange of subunits along their walls, uniform mixing of the labeled populations of IF (rather than the patches) would be expected along the length of a filament. In vivo, mixing of populations of differently colored IF to study dynamics of filament composition has been achieved through two main strategies, the fusion of cells expressing IF with different fluorescent protein tags [79], and photoactivation/photoconversion of a subpopulation of the IF within a single cell (Fig. 2D) [69, 81]. These experiments resulted in filaments with segments of each population along their length, and the decreasing length of segments with time suggests a constant process of severing and re-annealing [69, 79, 81].

The direct observation of subunit exchange within the mature filaments of living cells is quite challenging due to the limitations of current live-cell imaging technology. Initial in vitro experiments showed that even days after mixing two pre-assembled populations of differently labeled vimentin, filaments with patched segments are observed [80], arguing against the possibility of individual subunit exchange. However, recent in vitro experiments show that subunits within filaments can be exchanged, especially when filaments are polymorphic (having more or less than eight tetramers per cross-section). Nevertheless, the incorporation of subunits under physiological conditions is very slow, and severing and re-annealing is the major mechanism of filament turnover [82]. Interestingly, recent work has unveiled the surprising finding that microtubules are capable of incorporating tubulin subunits within their walls, occurring when microtubules are defective or damaged by external forces [83]. This raises the possibility such forces may also cause alterations in IF structure that may lead to the exchange of subunits within filaments. In this sense, IF in a cell type subjected to constant force might be more prone to subunit exchange.

The mechanisms responsible for severing and re-annealing are still unknown. Severing may occur by physically breaking/tearing apart filaments as can occur to microtubules during cell migration [84]. Alternatively, severing proteins, like those for microtubules [85] and actin microfilaments [86], may sever IF and could provide an additional level of targeting and regulation, however, such proteins have not been identified. How and where filaments may be targeted for severing will be an interesting topic for future research. It will also be interesting to determine how IF anneal. It is possible that a template enables the junction of IF so that the ends may find each other. The close association of IF with microtubules suggests microtubules may perform this function, although severing and re-annealing occurs even in the absence of microtubules [69]. Therefore, it is more likely that another structure facilitates IF annealing, which may be other neighboring IF.

Unlike mature IF, vimentin filament precursors actively exchange subunits

Since IF are highly insoluble, it is not surprising that the major mechanism of filament turnover occurs by severing and re-annealing rather than by exchange with soluble subunits [69, 81]. However, even if the amount of soluble vimentin subunits is barely detectable, a soluble pool of vimentin does exist in the cell in the form of tetramers [87, 88]. Interestingly, the size of the soluble pool of vimentin is modulated by signaling factors. For example, phosphorylation of vimentin by protein kinase A (PKA) in BHK-21 fibroblasts increases vimentin solubility [89]. A recent study also showed that vimentin solubility is influenced by changes in cellular tension and morphology [90].

However, since filaments are such stable structures, what is the source of this soluble pool? This raised the interesting question of whether ULF are as stable as mature filaments. A very different profile of soluble and insoluble pools of vimentin is found in vimentin^{Y117L} expressing cells (containing only ULF) compared to wild-type (mature-filament-containing) cells. In contrast to a barely detectable pool of soluble vimentin in wild-type cells, more than 50% of the vimentin present in cells expressing the vimentin^{Y117L} mutant is soluble (tetrameric), while the other 50% is insoluble and incorporated into ULF particles [88]. Unexpectedly, photo-conversion of a subset of particles formed in cells expressing vimentin^{Y117L} tagged with a photoconvertible probe has revealed that, in contrast to mature filaments, subunit exchange between ULF and the soluble pool of vimentin occurs within seconds (Fig. 2C and E) [88]. Surprisingly, subunit exchange in ULF is ATP-dependent, as ATP-depletion blocks exchange and reduces the soluble pool of vimentin. While it has been well-established that the assembly of actin microfilaments and microtubules requires nucleotide triphosphates, the requirement of ATP for ULF turnover is remarkable: Assembly of intermediate filaments in vitro occurs spontaneously, but this finding shows that an ATP-dependent mechanism regulates the initial steps of assembly in vivo.

To date, the mechanism responsible for the subunit exchange is unknown. The exchange is independent of the integrity of F-actin or microtubule networks and therefore does not involve active transport of subunits [88]. However, since the the exchange inhibition by the ATP depletion also reduces vimentin solubility, it is very likely that an ATP-dependent process regulates the dissociation of subunits from ULF. Attractive candidates for ATP-dependent mechanisms responsible for subunit exchange are the PTM that have been described for IF (Fig. 3A). These include phosphorylation, sumoylation, glycosylation, and ubiquitination [91]. Notably, the solubility of keratin and vimentin is regulated by sumoylation [92], and phosphorylation on Ser and Thr residues (p-Ser/Thr) typically promotes disassembly of filaments into ULF and increases IF protein solubility [93]. In particular, phosphorylation of the Ser38 residue of vimentin triggers filament disassembly during lamellipodia formation [29, 89]. However, ULF formed by the vimentin$^{S38A/Y117L}$ phosphomutant retain their capacity for subunit exchange, indicating that phosphorylation of Ser38 is not involved in the regulation of the subunit exchange [88]. However, since vimentin contains more than 35 phosphorylation sites in its head and tail domains targeted by multiple kinases and phosphatases, phosphorylation at another site could still be involved in the dissociation of vimentin tetramers from ULF.

Another possibility is that a transient ATP-dependent complex between vimentin tetramers and a chaperone protein prevents the oligomerization of tetramers (Fig. 3B). The association of IF with chaperones including small heat shock proteins (sHSP) was observed in a variety of cells expressing different types of IF [94–98]. This interaction may play a role during IF assembly since sHSP influence IF solubility [96]. Although sHSP are ATP-independent chaperones, they are regulated by ATP-dependent PTM-like phosphorylation [99], and sHSP cooperate with ATP-dependent chaperones [100].

What restricts subunit exchange to filament precursors and not mature filaments remains an open question. It may be that radial compaction and/or some PTM that occur during filament maturation prevent exchange after ULF have been incorporated into filaments. It would be

very interesting to test whether IF are capable of more readily exchanging subunits before compaction. Another possible explanation for the very low rates of subunit exchange in mature filaments could be that filament bundling blocks access for exchange. In this case, it is possible that subunit exchange rates vary with the extent of filament bundling, which differs by location within the cell, cell stress conditions and by the type of IF. Therefore, though exchange occurs rarely in mature vimentin IF and NF, it might occur more frequently in other types of IF.

The assembly and disassembly cycle of keratin filaments

Keratins are the most diverse cytoskeletal components in epithelial cells with more than 50 isotypes expressed. Keratin filaments are obligate heteropolymers formed by one keratin of Type I, and one keratin of Type II. Time-lapse imaging of fluorescently labeled keratins combined with FRAP [28] and photoactivation [101] has revealed very striking features of keratin dynamics. Observations made by the Leube group have demonstrated that keratin filaments undergo a constant cycle of assembly and disassembly in which the dynamics of keratin filaments are uniquely speciotemporally regulated (Fig. 4, see [102] for review). During the keratin cycle, the nucleation of filament precursors occurs in proximity to focal adhesions at the cell periphery [28, 103]. The newly formed filament precursors move toward and fuse to the peripheral keratin network via actin-dependent transport instead of the microtubule-dependent transport utilized by other IF [27, 101]. Keratin filaments continue to move centripetally in an actin-dependent manner and bundle [104]. These data have led to a model for the cycling of keratin in which the disassembly products of some keratin filaments diffuse to the cell periphery to be polymerized. Once polymerized, filaments reach the perinuclear region where some form a cage-like structure composed of highly bundled and stable filaments around the nucleus, and others are disassembled to begin the cycle again. The cycle was recently confirmed by the use of a new method for analyzing time-lapse image sequences to measure the local speed and direction of motion of keratin filaments and assess keratin assembly/disassembly in specific subcellular regions [105]. This method allows for the generation of assembly/disassembly maps for entire cells and has been used to monitor changes in keratin network dynamics in the same cell before and after treatment with specific drugs. Therefore, the method can be used as a powerful tool for analyzing mechanisms that locally control keratin dynamics.

According to the model of keratin cycling, soluble subunits are constantly released from the filament network suggesting that subunit exchange might be more prominent in keratin IF than for other IF. Furthermore, the complete inhibition of protein biosynthesis does not abrogate keratin filament formation and particle turnover [101, 106], consistent with the idea that keratin subunits are constantly recycled. We also observed that protein biosynthesis is not required for subunit exchange between ULF and the soluble vimentin pool (AR, unpublished data), which raises the possibility that keratin and vimentin IF might share a common turnover mechanism. Investigations might utilize the tagging of keratins with photoconvertible probes to test if remodeling by severing and re-annealing also contributes to keratin dynamics.

The biological role of intermediate filaments dynamics

The example of keratin emphasizes the idea that several mechanisms are involved in IF reorganization, and the dominant mechanism may depend on the type of IF as well as biological context. There are likely physiological reasons for which keratin remodeling differs from that of vimentin IF. Keratins are the predominant component of IF in epithelial cells, while vimentin is the major IF protein in mesenchymal cells. The perpetual renewal and repair of epithelium, crucial for the protective function of these cells to form selective barriers, involves constant and extensive rearrangement of the cytoskeleton. Keratins are necessary to maintain tissue integrity by interacting with desmosomes to provide intercellular adhesion. As a result, keratin-null mouse embryos die from severe growth retardation at embryonic day 9.5 [107] while mosaic KO mice survive 12 days but suffer from major epidermal damages [108]. It has been proposed that the cycle of assembly/disassembly of keratin allows rapid network remodeling without total network disruption, which would be disastrous for the integrity of the epithelium [102]. On the other hand, vimentin expressing mesenchymal cells can migrate more easily because they do not form stable cell-to-cell junctions. Vimentin promotes cell migration in several physiologically normal as well as pathological settings (see [4] for review). Cytoskeletal dynamics are essential to cell migration, and vimentin IF are no exception. In migrating cells, vimentin filaments extend in parallel to the lamella [109] but are excluded from the active lamellipodia, where only particles are present [29, 32]. Disassembly of vimentin IF might change cell stiffness [110], or increase flexibility at the leading edge of the cell to facilitate motility.

An increasing body of evidence suggests that, especially under cellular stress, functions of IF extend well beyond the mechanical and structural to direct participation in adhesion, migration, survival, and cell signaling [5]. Intriguingly, all of these processes are upregulated in tumor cells, which have greater vimentin solubility than in control cells [111]. A role for soluble vimentin was described in injured nerve, where it binds to phosphorylated Erk, preventing the deactivating dephosphorylation of Erk during its translocation [15, 16]. There are also examples of IF regulating gene expression. Following ectopic expression of oncogenes in pre-malignant breast epithelial cells, vimentin functionally contributes to EMT and is required for the regulation of the receptor tyrosine kinase gene AXL [8]. Additionally, a non-conventional role of keratin IF was recently described in skin tumors, where keratin 17 translocates to the nucleus and cooperates with the Aire protein to stimulate the transcription of pro-inflammatory genes [112].

We speculate that IF achieve their diversity of functions due to their presence in multiple forms. For example, protein complexes may select for a particular form of IF as a binding partner. It was shown that the cytolinker fimbrin binds only to vimentin tetramers [49], while epiplakin prefers keratin in its fully assembled form [46] (see Box 1 for details). It is also possible that different PTM serve as docking sites for protein complexes [113–115]. Although high-resolution data regarding the structure and organization of the different forms of IF are not yet available, it is very likely that sites for the binding of specific protein complexes and PTM are more or less accessible depending on the assembly state of the IF. In this sense, IF remodeling and subunit exchange in ULF become important players in the

regulation of IF function. Furthermore, spatial control of IF function could be achieved through filament or precursor transport combined with tethering to proper cytolinkers (see Box 1). For example, the binding of vimentin IF to plectin serves as a scaffold for the formation of invadopodias that facilitate cancer cell invasion [116].

Another function of subunit exchange in ULF could be to serve as a quality control mechanism to ensure proper ULF assembly has occurred prior to longitudinal annealing into long filaments. In this case, the binding of soluble vimentin tetramers to a chaperone in an ATP-dependent manner would make initial assembly reversible. Since there is a growing list of diseases associated with IF aggregation and signaling, better understanding how subunit exchange may contribute to these hypothesized roles could lead to important insights into disease comprehension and development of therapies.

Finally, it should be kept in mind that our knowledge of IF network dynamics is derived from data collected in cultured cells and might be different from that in cell sheets and tissues. The generation of a knock-in mouse that produces fluorescent-tagged keratin-8 recently allowed, for the first time, the monitoring of de novo IF network biogenesis [117]. Live-cell imaging of pre-implantation embryos showed that assembly of the first keratin-8 particles happens at the cell-cell junction after compaction of the 8-cell-stage embryo. FRAP analysis demonstrated the turnover of these particles, suggesting a role for subunit exchange during the first steps of IF network assembly. This study also showed that particles fuse together to elongate into filaments in the live animal like in cultured cells. However, whereas focal adhesions are the main site of particle biogenesis in cultured cells [28, 103], desmosomes perform this function in the embryonic context. Progress made in the field of genome editing, which has greatly facilitated the development of transgenic animals, will hopefully increase the amount of data collected on IF in vivo so that we may get a better comprehension of the physiological role of IF dynamics.

Conclusion

With the rapid advance of new imaging technologies, especially the development of super-resolution microscopy and new optical probes, we can begin to unlock the secrets of tightly packed IF networks. It is now possible to analyze and compare the dynamic properties, including transport and remodeling of many types of IF. We can also directly address the molecular mechanisms responsible for IF dynamics and how IF dynamics are regulated. For example, the motor proteins responsible for the bi-directional transport of many types of IF and their precursors have yet to be identified. Also, whether severing of filaments or dissociation of subunits requires helper proteins still needs to be determined. If helper proteins are involved, kinases and phosphatases are attractive candidates. As phosphorylation sites are in variable positions in the head or tail domains of each type of IF, each IF would likely have its own pattern of phosphorylation. Therefore, the regulation of IF dynamics could be cell-type specific, allowing cells to adjust IF dynamics depending on their physiological context. Once the molecular mechanisms that control remodeling of IF are elucidated, IF dynamics may be manipulated to distinguish the roles that various oligomeric forms of IF proteins play in different IF functions. There is a growing list of

pathologies associated with IF mutations. This and the fact that IF functions go beyond a strict role in cell mechanics, justify the efforts for deciphering IF dynamics.

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Abbreviations

BHK	baby hamster kidney
FRAP	fluorescence recovery after photo-bleaching
IF	intermediate filament
KO	knock-out
NF	neurofilament
PAK	p21-activated kinase
PKA	protein kinase A
PTM	post-translational modification
Ser	serine
sHSP	small heat shock protein
Thr	threonine
ULF	unit length filament

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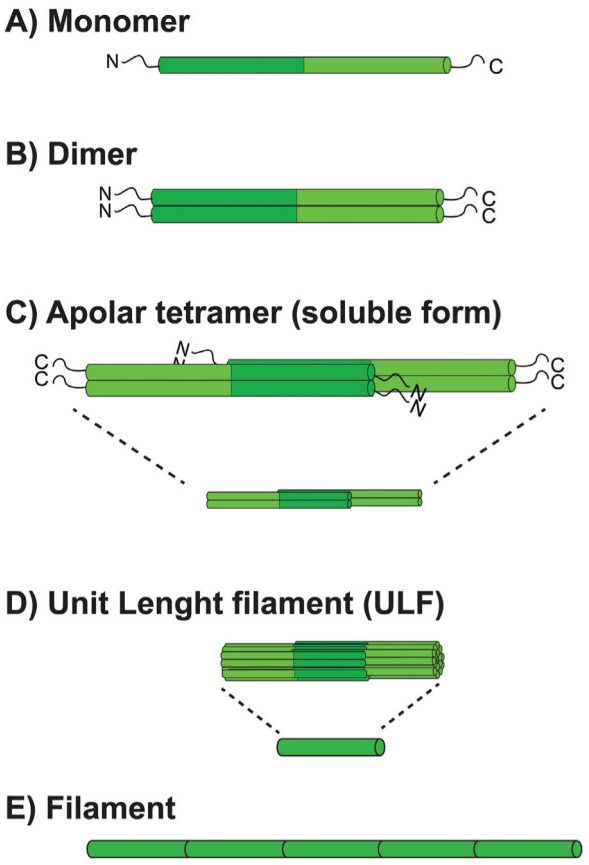

Figure 1.

Model of assembly of homopolymeric intermediate filaments. **A:** IF polypeptides comprise a highly conserved central alpha-helical rod domain (in green) and varying non-helical N- and C-termini. **B:** IF dimers are formed by the parallel alignment of the rod domains of two IF polypeptides. **C:** Two dimers associate laterally in an antiparallel fashion to form a non-polar tetramer, the smallest IF subunit observed in cells. **D:** Typically, eight tetramers assemble laterally into the unit-length filament (ULF), and **E:** ULF anneal end-to-end to form non-polar filaments. The last step of maturation is constituted of radial compaction into 10 nm wide filament (not illustrated).

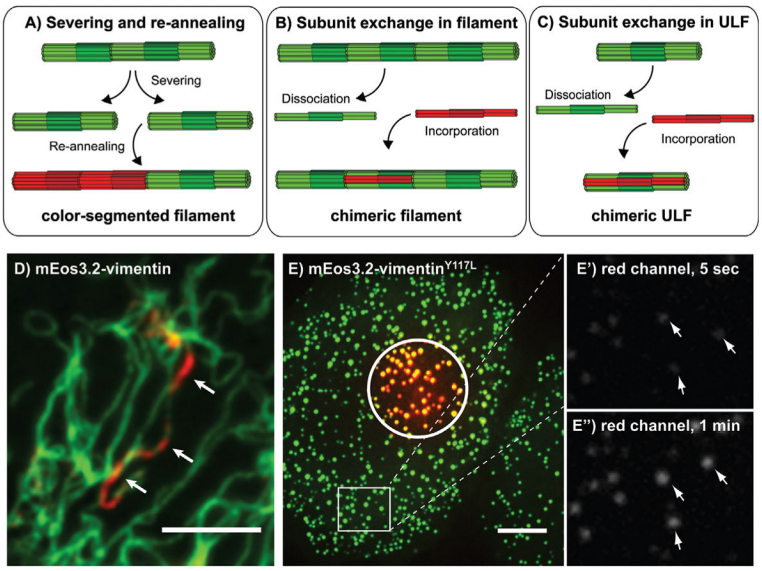

Figure 2.

Mechanisms of IF turnover. **A:** During severing and re-annealing, IF break into pieces of short filaments (in green) that can fuse end-to-end with another filament (in red). **B** and **C:** Subunit exchange consists of the dissociation of one tetramer (in green) from the wall of the filament (**B**) or from ULF (**C**) that is replaced by another tetramer (in red). **D:** TIRF microscopy of mEos3.2-vimentin 18 hours after photoconversion from green to red of a restricted area of the cell. The arrows point to a dual-color segmented filament, which is the result of severing and re-annealing. Scale bar, 5 μm. **E:** Live-cell spinning disk confocal microscopy shows the accumulation of red fluorescence in pre-existent ULF located outside of the initial photoconversion zone (circle), 5 seconds (**E′**) and 1 minute (**E″**) after photoconversion. Scale bar, 5 μm.

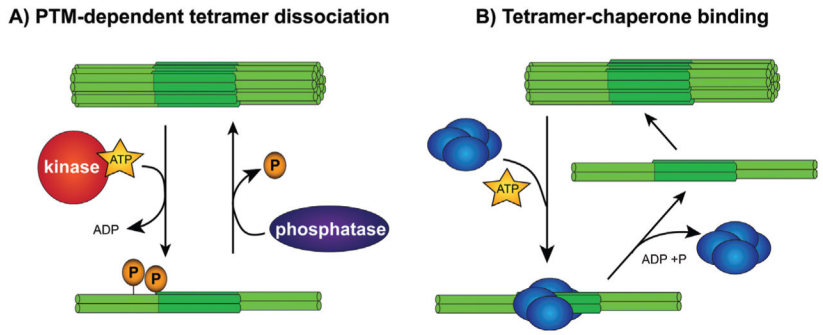

Figure 3.

Potential ATP-dependent mechanisms of subunit exchange. **A:** An ATP-dependent post-translational modification of an IF tetramer (example illustrated, phosphorylation) induces its dissociation from ULF. Removal of the modification (here by the action of a phosphatase) allows the lateral association of tetramers into ULF. **B:** ATP-dependent binding of an IF tetramer to a chaperone facilitates its dissociation from a ULF and maintains the tetramer's solubility. Release of the tetramer from the chaperone (for example via ATP hydrolysis) is necessary for the tetramer to laterally associate into ULF.

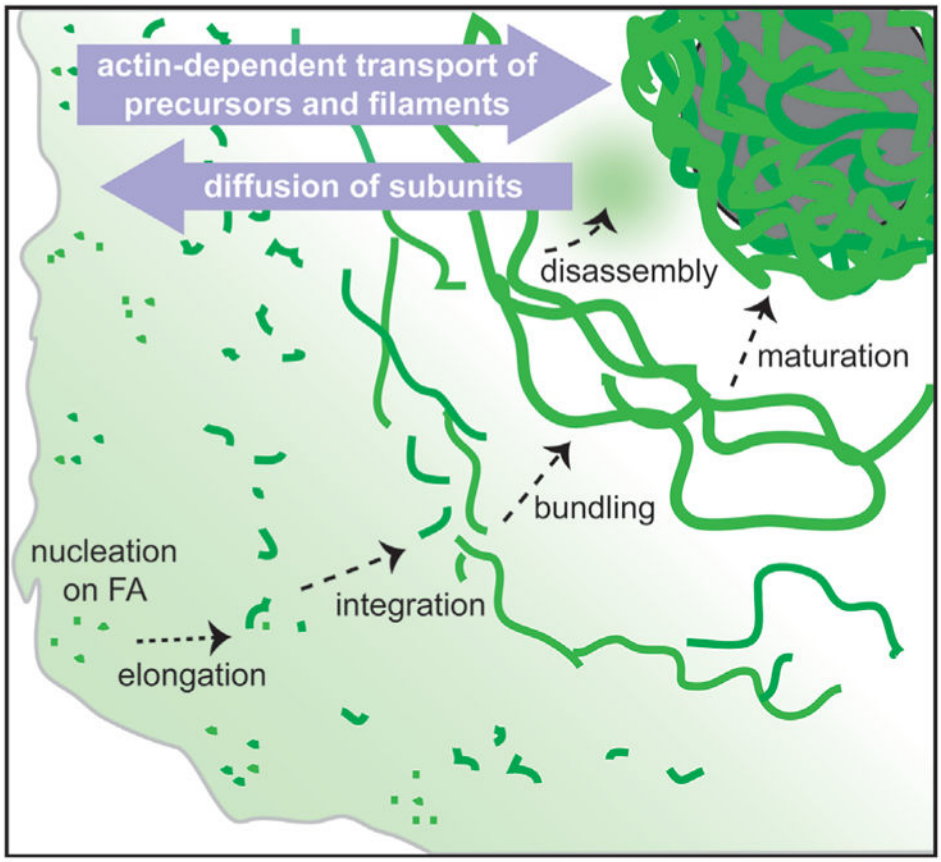

Figure 4.

The cycle of assembly/disassembly of keratin filaments. Soluble keratin oligomers assemble into particles (nucleation) at the cell periphery in proximity to focal adhesions (FA). These particles fuse into short filaments (elongation) and move toward the cell center driven by actin-dependent transport. As they move toward the cell center, short filaments are integrated into the peripheral keratin network (integration). Filaments bundle together as they continue their centripetal translocation toward the nucleus (bundling). Soluble oligomers dissociate (disassembly), diffuse throughout the cytoplasm, and are reused for another cycle of keratin filament assembly at the cell periphery. Alternatively, filament bundles are stabilized (maturation), forming a cage-like structure around the nucleus.

Table 1

Classification of IF proteins

Subtypes	Protein	Main tissue distribution
Type I	Keratin	Epithelia, hair, and nails
Type II	Keratin	
Type III	Vimentin	Mesenchyme
	Desmin	Cardiac, skeletal, and smooth muscles
	Syncoilin	Muscles
	GFAP	Glia, astrocytes
	Peripherin	Peripheral neurons
Type IV	Neurofilaments: NF-L, NF-M, NF-H	CNS and neurons
	α-Internexin	CNS and neurons
	Synemin	Neurons, astrocytes, and muscles
	Nestin	Neural stem cells
Type V	Lamins A, B, and C	Ubiquitous (nucleus)
Orphan	Filensin	Lens
	Phakinin	Lens