EXPERIMENTAL CELL RESEARCH 313 (2007) 2236–2243

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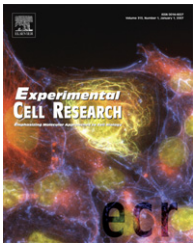

Review Article

The motility and dynamic properties of intermediate filaments and their constituent proteins

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ARTICLE INFORMATION

Article Chronology:
Received 23 January 2007
Revised version received
1 April 2007
Accepted 5 April 2007
Available online 12 April 2007

Keywords:
Vimentin
Keratin
Intermediate filaments
Molecular motors
Signal transduction

ABSTRACT

Intermediate filament (IF) proteins exist in multiple structural forms within cells including mature IF, short filaments or 'squiggles', and non-filamentous precursors called particles. These forms are interconvertible and their relative abundance is IF type, cell type- and cell cycle stage-dependent. These structures are often associated with molecular motors, such as kinesin and dynein, and are therefore capable of translocating through the cytoplasm along microtubules. The assembly of mature IF from their precursor particles is also coupled to translation. These dynamic properties of IF provide mechanisms for regulating their reorganization and assembly in response to the functional requirements of cells. The recent findings that IF and their precursors are frequently associated with signaling molecules have revealed new functions for IF beyond their more traditional roles as mechanical integrators of cells and tissues.

Contents

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Abbreviations: cdk, cell cycle-dependent kinase; DM-EBS, Dowling–Meara epidermolysis bullosa simplex; FISH, fluorescence in situ hybridization; FRAP, fluorescence recovery after photobleaching; GFP, green fluorescent protein; IF, intermediate filament; IFAP, IF associated protein; MAP kinase, mitogen activated protein kinase; mTOR, mammalian target of rapamycin; NF, neurofilament; siRNA, small interfering RNA

0014-4827/$ – see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.yexcr.2007.04.008

Introduction

Intermediate filaments (IF) are essential for maintaining the mechanical integrity of vertebrate cells and tissues. This has been convincingly demonstrated at the tissue level by discoveries that point mutations in epidermal keratins cause severe skin blistering diseases [1]. IF also play a role in maintaining cell morphology, as illustrated by changes in cell shape elicited by disrupting either vimentin IF networks by the microinjection of mimetic peptides [2] or peripherin networks by siRNA [3]. IF have also been shown to be involved in a variety of other processes including cell growth [4–6], stress responses [7–9], distribution of cellular organelles [10,11] and the transcellular migration of lymphocytes [12].

For many years, IF networks were considered to be static cytoskeletal structures. This was based on their well-established roles in maintaining the mechanical integrity and shape of cells, as well as their relative stability in cell free preparations. Over the past decade, however, many studies from different laboratories have shown that IF are, in fact, highly dynamic cytoskeletal structures with respect to the motions of entire IF networks, the rapid motility of their non-filamentous precursors and the turnover of subunits within individual IF. The first indication that polymerized IF were dynamic came from studies in which cultured cells were either microinjected with tissue-derived IF proteins [13,14] or transfected with IF cDNAs [15,16]. These studies showed that exogenous or newly synthesized IF proteins could rapidly become incorporated into endogenous IF networks. Subsequently, FRAP experiments demonstrated that photobleached vimentin IF could fully regain their fluorescence over relatively short time periods. In addition, recovery from photobleaching occurred simultaneously over the entire length of the bleached filament, indicating that subunit exchange occurs in a nonpolar fashion [17].

Our knowledge of the dynamic nature of IF took another leap forward following the introduction of GFP-tagged fusion proteins, which opened the way for detailed time lapse studies of different IF proteins in a variety of cell types. Studies making use of this technology clearly demonstrate that cytoplasmic IF exist in different structural forms and sizes, and most interestingly, that they are highly motile and undergo inter-conversion, depending on the functional state of the cell. IF are known to interact with a variety of signaling molecules and may also play a role in the distribution of these molecules as well as other cellular organelles [18]. The exciting finding that different structural forms of IF are interconvertible and motile has paved the way for looking into the functional linkage of these dynamics to other cellular activities. In this review, we describe the latest advances in our knowledge of the dynamic properties of IF and how their dynamic behaviors are connected to cellular events such as protein translation and the transport of signaling molecules.

The dynamic properties of IF in interphase cells

Time lapse image analyses of GFP-vimentin and GFP-keratin transfected cells have revealed several interesting features of the dynamic properties of IF in growing cultured cells. First of all, IF proteins are found in a range of morphologically distinct structures that have been subdivided into three groups: nonfilamentous particles, short filaments or “squiggles” and long “mature” IF (Fig. 1). These different forms of IF proteins are most readily observed during the attachment to and spreading of fibroblasts on solid substrates such as coverslips. During the spreading process there are extensive rearrangements and alterations in the organization of the IF cytoskeletal system. For example, during early spreading, although most vimentin is in a filamentous state concentrated in the perinuclear region, a significant fraction is found in the form of non-filamentous particles and squiggles [19]. The latter two structures are especially prominent near the edges of spreading cells. Interestingly, some vimentin particles are found to coalesce to form larger particles and other particles appear to form squiggles [19]. Squiggles, in turn, appear to anneal ‘end-to-end’ or to add on to existing long IF. Ultimately this process of constructing short and long IF results in the assembly of the extensive and complex IF cytoskeletal networks that occupy the region between the nuclear surface and the plasma membrane in fully spread cells (Fig. 2).

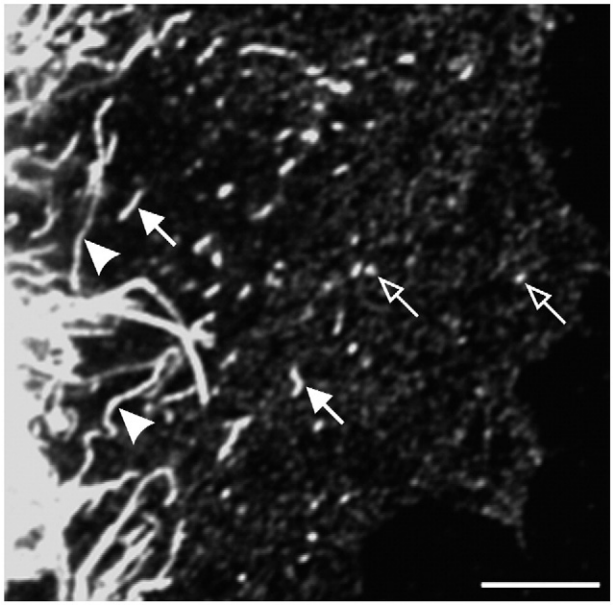

Fig. 1 – Immunofluorescence image of vimentin in a spreading mouse embryonic fibroblast. Note the existence of all three structural forms of vimentin: particles (open arrows), squiggles (filled arrows) and long filaments (arrowheads). Scale bar = 5 μm.

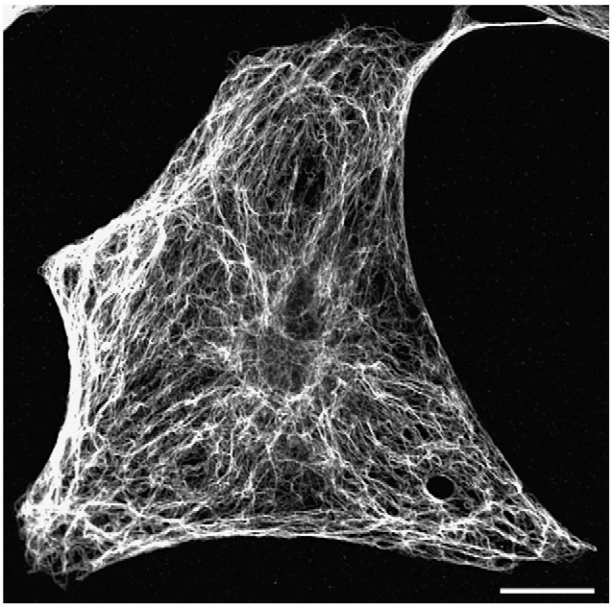

Fig. 2 – Immunofluorescence image of a mouse embryonic fibroblast with a fully spread vimentin network. Scale bar=20 μm.

The second and probably more interesting finding is the discovery that all three IF structures are motile. This is especially true of vimentin particles which were observed to undergo rapid movements at an average speed of 0.6 um/s [19] (see Fig. 3 and the supplementary video clip). Although the movement of vimentin particles is, in general, bidirectional in interphase cells, during cell spreading it is biased towards the cell periphery (anterograde movement). In addition, the motion of these particles is saltatory, as reflected by periods of rapid movement interspersed with pauses. Squiggles move at a relatively slower speed of about ~3 μm/min. Long vimentin IF are frequently seen to bend and undergo wave-like movements [20,21], and even when in very close proximity, individual IF appear to move past one another at different speeds and sometimes in different directions [22].

The motility of vimentin particles and squiggles [19], as well as the overall organization of IF networks [23], are sensitive to nocodazole treatment. This finding implicates microtubules and their associated molecular motors in IF motility. Direct evidence for vimentin–motor interactions was shown in experiments involving the microinjection of antibodies directed against conventional kinesin, a plus-end directed motor [19,24], and in studies in which over-expression of dynamitin was employed to disrupt the function of the minus-end directed motor dynein [25]. In the former case, the inhibition of anterograde motility resulted in the accumulation of IF in the perinuclear region, and as a result of dynein disruption, there was an accumulation of all forms of IF at the cell periphery. Furthermore, immunofluorescence, electron microscopic and co-sedimentation studies of cell-free preparations of IF networks have shown that vimentin particles, squiggles and long IF are associated with kinesin, dynein and microtubules, lending further support to their molecular interactions [19,25,26]. In a similar fashion, kinesin and dynein have also been implicated in the transport of neurofilaments [27–30].

The three structural forms, as well as their motile behaviors, are not unique to vimentin. Similar forms of keratin IF have also been reported in cultured epithelial cells [22,31–34]. However, the dynamic behavior of the keratins is different from vimentin in several respects. For example, keratin particles and squiggles appear to originate primarily in the actin-rich cell cortex and then elongate as they move towards the cell interior (retrograde movements; [22,34]). Time lapse observations of nascent keratin particles show that they frequently arise near focal adhesions and move along associated actin stress fibers [35]. The fact that these movements are sensitive to either treatment with cytochalasin D or a talin specific siRNA gives further support to the involvement of microfilaments in the distribution and organization of various keratin structures. Whether the keratin motility is mediated by a specific myosin motor is still unknown [35,36]. Other types of IF, including peripherin and NF triplet proteins, also form rapidly moving particles and squiggles. These have been observed in extending axons of PC12 cells [3,37] and in extruded squid axoplasm *in vitro* [38], indicating that fast transport of cytoskeletal IF proteins in axons, especially in the form of precursor particles, is possible. In cultured sympathetic rat neurons, long NFs have been reported to move bidirectionally and at speeds comparable with those of motile vimentin or keratin particles. However, the movements of these long IF are frequently interrupted by long pauses [39–41]. The interruptions are most likely related to the presence of the long C-terminal domains of two of the triplet proteins (NF-M and NF-H). In addition, myosin Va has been shown to be a NF associated protein [42]. This association may also contribute to the tug of war between microtubules and microfilaments for NF cargoes and play a role in the frequent pause during the NF transport. Taken together, these results provide a new insight into the mechanism underlying the phenomenon of slow axonal transport of cytoskeletal polymers. In most other cell types, mature long IF are also restricted in their movements in the cytoplasm. In homopolymer IF, such as those comprised of vimentin, extensive crosslinks by a family of proteins called plakins have been shown to form linkages between neighboring IF and between IF and microfilaments and/or microtubules [43].

IF organization within cells is altered in response to environmental stress

The organizational state of IF networks is profoundly altered by different forms of cellular stress including mechanical perturbation. For example, epithelial cells exposed to stretching forces exhibit accumulations of keratin-containing particles in keratinocytes expressing the DM-EBS mutant form of K14 (R125P), but not in cells expressing only wild-type proteins [44]. Prolonged exposure to relatively high fluid shear forces also causes the keratin IF network in lung epithelial cells to disassemble into small particles [45]. This disassembly of keratin IF has been shown to be mediated by PKC-δ-dependent phosphorylation of keratin K8 on serine residue 73. Endothelial cells, which contain extensive cytoskeletal networks of vimentin IF, are exquisitely sensitive to shear forces generated

by fluid flow on time scales ranging from a few millisecond to several days [46]. For example, rapid displacement of vimentin IF is seen upon exposing endothelial cells to moderate fluid shear forces within 3 min [47,48]. These displacements of IF are likely to play a key role in the activation of mechanical signaling pathways in endothelial cells. The potential roles of IF in mechanical signal transduction are further supported by the association of IF with components of cell/cell and cell/substrate adhesion complexes known to play a role in mechanical signaling. For example, keratin IF are associated with both integrin- and cadherin-associated cell surface junctions such as hemidesmosomes and desmosomes [49,50], while vimentin IF are linked to integrin- associated focal adhesions [51,52].

The dynamic properties of IF in dividing cells

The most dramatic changes in IF structure and organization are seen during mitosis, when cells undergo significant shape changes. The major restructuring event during this period is the dissolution of the nuclear envelope and the nuclear lamina. The lamina is mainly composed of nuclear lamins, the type V IF proteins found exclusively in the nucleus. The process of disassembly of the nuclear lamins/lamina during cell division is conserved among vertebrate cells, but the structural changes associated with cytoplasmic IF are not. These latter changes display both cell-type and IF-type specific features. For example, many cultured epithelial cells, such as PtK-2 and HeLa, express vimentin as well as certain keratins. In such cells, the two different IF types are organized into distinct filamentous networks. In PtK-2 cells, both vimentin and keratin IF networks remain intact throughout mitosis [53]. However, in HeLa cells the vimentin IF network retains its filamentous form while keratin IF networks are disassembled into non-filamentous particles [54,55]. In other cell types, such as BHK-21 and ST15A cells, the filamentous vimentin network disassembles into particles during late prophase [56,57]. This variability in the state of cytoplasmic IF in different cell types during mitosis suggests that there are cell type-specific biochemical mechanisms regulating the formation of non-filamentous particles. Furthermore, the presence of non-filamentous particles, in some, but not all cell types may reflect unique functions for these structures.

Phosphorylation is known to play an essential role in regulating the disassembly of IF in vitro [58,59]. For example, it has been shown that phosphorylation by cdk1 (also known as cdc2) kinase is essential for the formation of vimentin particles during mitosis of BHK-21 cells [60]. Since cdk1 is a highly conserved mitotic protein kinase present in all cell types, the retention of filamentous vimentin in most dividing cells suggests that additional factors unique to BHK-21 and ST15A cells may be required to facilitate particle formation. In this context, nestin, a type IV IF protein normally expressed in stem or progenitor cells, has been identified as one factor that operates in concert with vimentin phosphorylation to promote the disassembly of IF into vimentin/nestin containing non-filamentous particles in mitotic BHK-21 and ST15A cells. Other as yet unknown factors may also be required for the disassembly of keratin IF during mitosis. Members of the IFAP/plakin family of proteins, such as plectin, are potential candidates [61]. In nestin-negative cell types in which there is no disassembly of IF, the partitioning of IF into separating daughter cells is mediated by the localized phosphorylation and disassembly of IF in the cleavage furrow during late cytokinesis [62].

In those cells in which there is disassembly of IF into particles, the reformation of interphase IF networks in daughter cells following cell division appears to recapitulate the assembly sequences seen in spreading cells described above. For example, in BHK-21 cells, the vimentin particles are converted to short filaments during the anaphase/telophase transition. Immediately following cytokinesis, longer IF first appear in the centrosomal regions of each daughter cell and then gradually extend outward to the periphery to form a typical interphase IF network [56]. The reassembly of the keratin IF network from nonfilamentous keratin particles in newly divided daughter cells is quite different. It appears that keratin IF formation is initiated from the cell cortex. This cortical network then radiates inward over time to form an interphase network [33].

The functional significance of motile IF and their precursors

Little is known with regard to the functional significance of the formation of IF particles either in interphase or mitotic cells. However, particles are known to be IF precursors and their rapid motility clearly facilitates the targeted delivery of IF

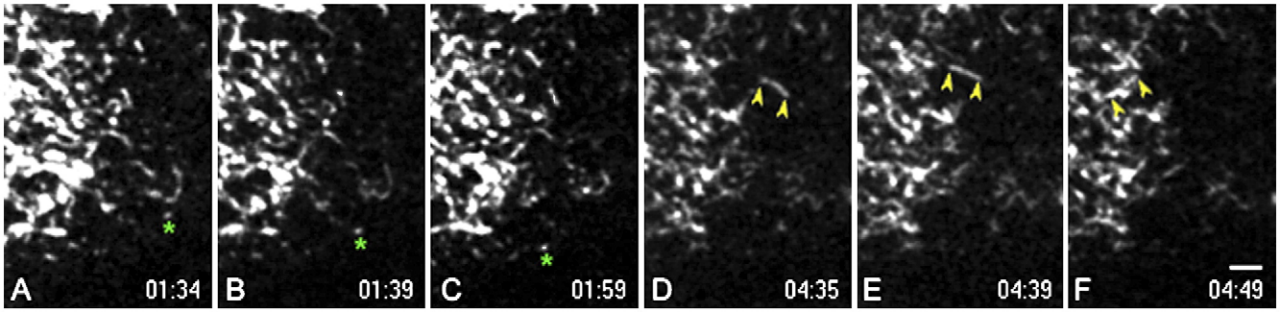

Fig. 3 – Selected image frames of Supplementary Video 1 showing a moving particle (panels A–C, highlighted with a green asterisk) and a moving squiggle (panels D–F, marked between two yellow arrows) in the same field of view but a few minutes apart. See Supplementary Video 1 for a larger field of view. Scale bar=2 μm.

proteins to sites where the formation of a new IF network is required, such as at the leading edge of a spreading cell. In addition, IF as well as nonfilamentous particles have been associated with signaling intermediates. These include associations with factors involved in cell growth such as MAP kinases [63], mTOR [4] and 14-3-3 proteins [64–66]; and cdk5 [9,67] and other apoptotic factors [7,8]. In some cases, the association of IF with these signaling molecules is regulated in a phosphorylation-dependent manner [64]. Taken together, motile IF structures could potentially play a role in facilitating the targeted distribution of signaling molecules to specific cellular compartments. An example is reported in a recent paper by Perlson and colleagues [63], in which the authors suggest that during axonal injury, enzymatically truncated vimentin is recruited as a transport scaffold that connects an activated MAP kinase to importin β and cytoplasmic dynein. The entire complex is then transported in a retrograde fashion along the axon towards the nucleus in the cell body where it initiates gene expression required for repairing the injury and regenerating a functional axon.

In a similar fashion, motile vimentin particles observed in certain cell types during mitosis could potentially facilitate the differential distribution of signaling molecules to one or both daughter cells. It is interesting to note here that some nestin-expressing progenitor cells undergo asymmetric division, resulting in the formation of two daughter cells, one containing nestin and one null for nestin. The nestin-retaining daughter cell remains in the proliferating cycle while the nestin-negative cell follows either an apoptotic or a differentiation specific pathway [68,69]. For cell types that do not disassemble their IF networks, the function of IF may be related to maintaining the structural connection of dividing cells with their neighbors. This is an important issue especially for epithelial tissues in which the mechanical integrity of an epithelial cell sheet depends mainly on IFs and their intercellular connections mediated by desmosomes [50]. Potentially, this interconnected IF structure may also provide spatial cues for the proper alignment of signaling molecules in dividing cells.

The assembly of IF proteins is coupled to protein synthesis

Not all IF particles are in a constant state of motion. For example, a fraction of them exhibit prolonged pauses during interphase [31]. This phenomenon is in part explained by a recent report [37] showing that ~30% of the peripherin particles in PC12 cells are non-motile. These stationary particles appear to be undergoing co-translational assembly. Evidence for this has been derived from RNA-based FISH studies and the simultaneous imaging of both peripherin mRNA and its protein product in live cells. Peripherin mRNA and protein particles appear to move independently along microtubules. However, only stationary mRNAs are associated with peripherin protein particles, suggesting that immobile mRNAs are translationally active. Once translation is complete, the ribosomes appear to dissociate, followed by the rapid movement of mRNAs away from the newly synthesized protein particle, possibly for degradation or translation elsewhere.

Although peripherin mRNAs have been observed to move throughout the cell, it is not yet obvious where they are going within the cytoplasm, and which signals, temporal and/or spatial, regulate their translational activity. Insights into the regulation of peripherin mRNA localization may be found in studies of another type III IF, vimentin. The 3′UTR of vimentin mRNA appears to be involved in its perinuclear localization in fibroblasts [70]. Replacement of this region with the 3′UTR of β-actin mRNA results in mislocalization of vimentin mRNA to peripheral regions of the cytoplasm and alters cell morphology [71]. Several RNA-binding proteins interact with the 3′UTR of vimentin mRNA, but the function of these interactions remains unclear [72]. Based on the well-established function of the 3′UTR and their various *trans*-acting factors in targeting and translational control of mRNAs [73], it is possible that one or more of these proteins is involved in the regulation of vimentin mRNA localization.

Despite rather extensive knowledge of the assembly properties of IF proteins *in vitro*, little is known regarding their assembly *in vivo*. The most obvious difference between the two situations is the precursor particle found in cells. The composition and structure of these particles remain unknown. Electron microscopic studies have suggested that they are heterogeneous in size (0.1–1 um in diameter) and contain aggregates of short protofilaments [19,54,56,74]. The relatively low level of soluble IF proteins (in tetrameric form) found in cells [75,76] and the rapid assembly of nascent protein into particles during translation [16,37], suggest that these insoluble structures are the main non-filamentous IF proteins in cells. In addition to giving rise to new filaments and their involvement in signal transduction, they may also move to sites using microtubule tracks in order to provide subunits such as tetramers or higher order structures for subunit exchange with existing IF polymers (also see above).

Future directions and conclusions

There are clearly many unanswered questions regarding the functional significance of and regulatory mechanisms responsible for the dynamic properties of IF and their precursors. Indeed, the structures of the various forms of IF proteins, including the mature 10 nm diameter filaments, remain unknown at high resolution. Hopefully, it will not be long before the molecular and biochemical properties between different IF structures are defined. Questions that arise in the immediate future include why, for instance, do the different IF structures exhibit different dynamic properties; how are the interconversions of the different morphological forms regulated; and how are these structures related to the *in vitro* IF assembly process which has been documented in considerable detail [77]? Beyond these most basic questions are more complex ones. For example, given that the expression of IF is developmentally regulated, are the dynamic properties of the different types of IF, and their different structural forms, related to specific functions at particular developmental stages? Answers to these questions should provide important insights into the links between the dynamic properties of IF and the transport of signaling molecules as described in this review. This may also lead to a better understanding of the non-mechanical functions of the large number of different types of IF in the specific signal transduction pathways that distinguish one differentiated cell type from another.

Acknowledgment

This work has been supported by a grant from the NIGMS.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.yexcr.2007.04.008.

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