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Intermediate Filaments: Versatile Building Blocks of Cell Structure

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Summary

Cytoskeletal intermediate filaments (IF) are organized into a dynamic nano-fibrillar complex that extends throughout mammalian cells. This organization is ideally suited to their roles as response elements in the subcellular transduction of mechanical perturbations initiated at cell surfaces. IF also provide a scaffold for other types of signal transduction which together with molecular motors ferries signaling molecules from the cell periphery to the nucleus. Recent insights into their assembly highlight the importance of co-translation of their precursors, the hierarchical organization of their subunits in the formation of unit length filaments (ULF), and the linkage of ULF into mature apolar IF. Analyses by atomic force microscopy reveal that mature IF are flexible and can be stretched to over 300% of their length without breaking, suggesting that intrafilament subunits can slide past one another when exposed to mechanical stress and strain. IF also play a role in the organization of organelles by modulating their motility and providing anchorage sites within the cytoplasm.

Introduction

Intermediate filaments (IF) assemble into extensive cytoskeletal networks that appear to connect the cell surface with the nucleus and provide cells with important mechanical properties (Figure 1 and Figure 2 [1,2]). At the cell surface, IF interact with desmosomes, hemidesmosomes, focal adhesions and the extracellular matrix via a variety of linker proteins [3–5]. There is also evidence that IF associate with factors on the outer nuclear membrane which, in turn, connect to components of the nuclear lamina [4,6]. These observations suggest that IF form a continuous network of nanofibrils along which signals from the cell's exterior can be transmitted to the nuclear surface and the nucleoplasm. The extensive distribution of IF provides an enormous surface area which can act as a scaffold for the binding of numerous types of regulatory and signaling molecules [8,9]. The IF system has also been shown to associate with membranous organelles such as mitochondria, the Golgi apparatus and vesicles, as well as with other cytoskeletal components such as actin filaments, microtubules, and their associated molecular motors [4,6,10]. Thus the IF system is positioned to perform significant roles in the internal organization and positioning of organelles and other cytoplasmic components. In humans, over 70 genes encode the protein subunits that assemble into the 10–12 nm filaments located in the cytoplasm [11]. The

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expression of these subunits is developmentally regulated and subunits are frequently mixed and matched. IF protein subunits are therefore the building blocks of a potentially enormous variety of protein polymers that serve as customized scaffolding materials in different cell types.

Recent Insights into Intermediate Filament Structure and Assembly

All IF proteins have similar structural features; a conserved central α -helical rod domain flanked by non- α -helical N- (head) and C-terminal (tail) domains. The central rod is composed mainly of seven residue repeats (heptad repeats) that enables the formation of a coiled-coil structure [12]. The non- α -helical head and tail domains are variable in size and sequence, contributing to the diversity of the IF superfamily.

Intermediate filament proteins interact to form coiled-coil dimers in different ways. For example, Type III IF proteins such as vimentin interact to form homodimers, whereas heterodimers of Types I and II are required for keratin IF assembly [12]. In all IF systems, the two chains associate in parallel and in register to form dimers, the basic building blocks required for IF assembly. Qualitative analyses in low ionic strength solutions at neutral pH suggest that IF dimers associate rapidly to form anti-parallel, half-staggered tetramers that associate laterally to form unit-length filaments (ULFs). A recent study using small angle X-ray scattering in combination with analytical ultracentrifugation has revealed that tetramers interact laterally to form octamers and that four of these comprise the ULF [13] (•). Proof that IF grow by the addition of ULFs, and not dimers or tetramers, has come from an important quantitative analysis of the assembly of vimentin IF using electron and scanning force microscopy (SFM). The results show that the most likely mechanism of assembly in vitro involves rapid formation of ULFs, followed by their annealing to elongate IF [14] (••) (Fig. 3). In simple buffer conditions IF elongate at 3 μ m per hour at 37°C at a protein concentration of 0.2 mg/ml. This corresponds to an effective elongation of one ULF /45sec. At higher protein concentrations under more physiological conditions, the rate of elongation is faster (H. Herrmann, personal communication). Assembly in vivo is most likely to be much faster, since IF protein concentration is higher (~2–3% of total fibroblast protein, unpublished observations) and physiological conditions are optimal (e.g., the extent of phosphorylation [15,16]). Once longer IF are formed, there is a “radial compaction” phase in which molecular re-arrangements result in a decrease in filament diameter from ~17 nm to the mature dimension of 10 nm [17]. The regulation of this compaction mechanism remains unknown. Furthermore, growing filaments of different lengths can also anneal at their ends to form even longer filaments in vitro [14] (••).

It is of interest to compare the steps in IF assembly in vitro with those that take place in vivo. Vimentin and other Type III IF proteins such as peripherin exist in several structural forms including non-filamentous particles, short filaments (or squiggles) and long filaments [18,19]. During the early stages of fibroblast and nerve cell spreading, much of the IF protein is in the form of particles that are subsequently converted into short filaments. The short filaments, in turn, appear to link in tandem to form longer IF [18]. While the exact state of IF protein in the particles is unknown, it is likely that they contain high concentrations of filament precursors such as ULFs. If this is the case, the formation of short filaments likely involves end-to-end interactions among the ULF comprising the particles. Overall these steps in vivo are similar to the end to end assembly of short IF seen in vitro (see above and [14] ••). Live cell imaging studies monitoring the translation of peripherin, a Type III IF protein, suggest that 30% of the non-filamentous particles are assembled in an mRNP complex by a process termed dynamic co-translation. Intriguingly, over 50% of these complexes contain more than 1 and up to 30 IF specific mRNAs. Therefore these appear to be large IF translation factories capable of optimizing synthesis of IF protein chains in a

small cytoplasmic domain. It is interesting to speculate that these domains could provide a high local concentration of newly synthesized subunits which could be co-translated into dimers and the higher order structures required for ULF assembly [20] (••).

Within epithelial cells, the steps in keratin IF assembly resemble those reported for vimentin. Short keratin IF assemble from particulate precursors and these appear to fuse and become integrated into IF networks [21–24]. Interestingly, the assembly of at least some keratin IF from their precursors appears to be associated with focal adhesions located in lamellipodia of epithelial cells [25] (••). There is also evidence that vimentin IF associate with focal adhesions and can modulate those associated with $\alpha\text{v}\beta 3$ integrin in endothelial cells [26]. Mechanical stress enhances the association of bundles of vimentin with focal contacts, altering their dynamic properties [27], and silencing vimentin expression results in smaller focal contacts and decreased adhesion [27].

It should be noted that in vivo the various vimentin and keratin precursors are motile due to their associations with microtubule [28] and actin filament based motors [25] ••. Importantly, their motile properties are required for their proper assembly into the extensive IF networks that typify interphase cells [19,24,25]. In this fashion IF have also been implicated as important players in the cross talk among the different cytoskeletal systems [9].

Intermediate Filaments: Structure and Nanomechanics

In vitro studies have revealed that the properties of IF are uniquely adapted for dealing with mechanical stress. Intermediate filaments are both strong and flexible polymers. Previous studies have shown that vimentin IF networks reconstituted in vitro can withstand strains of over 100% without losing their elasticity. In fact, the more the vimentin networks are strained, the more resistant they became to further deformation. This behavior is referred to as 'strain stiffening' [29] •. Reconstituted keratin IF also exhibit this property and keratin mutations known to cause epidermolysis bullosa simplex (EBS), a skin blistering disease, altered these properties to reduce their strain stiffening capacity [30]. This may help explain the mechanisms underlying the mechanical changes in epidermal cells responsible for EBS.

Using atomic force microscopy (AFM), the mechanical properties of individual IF have been determined. Kreplach and colleagues [31] •• have imaged single IF in physiological buffers using a low applied force, and then increased the force along one scan line to pull the same filament. These analyses have been carried out for desmin (Type III), keratin (Types I and II) and neurofilaments (Type IV). In all 3 cases the length of IF increased by 250–350% before they ruptured. This was accompanied by a significant decrease in the width of the filaments, suggesting that the subunits in stretched filaments can slide past one another. Filament stretching may also be accompanied by an α -helix to β -sheet transition in the central rod domain [31]••. This experimental approach is limited because the force applied to an IF could not be accurately determined under conditions where the IF was attached to a substrate. To overcome this problem, another method was developed in which a single filament was placed over a hole in the substrate and imaged with the AFM tip using increasing forces under physiological conditions. Successive images were analyzed to determine the deflection of single vimentin IF under a known applied force, revealing a bending modulus of 300–400 megapascals (MPa) [32]. By comparison, F-actin is much stiffer, with a bending modulus of ~2,000 MPa. These findings demonstrate the extensive flexibility of IF polymers. In summary, these AFM studies suggest that the flexibility and extensibility of IF are most likely related to the hierarchical interactions of their subunits, especially the anti-parallel/ staggered arrangement of the dimers within the tetramers/ octomers that form the core structures of ULF (Fig 3).

The unique structural organization of IF is in contrast to microtubules and microfilaments which are basically linear polymers of globular subunits. The molecular organization and physical properties of IF help to account for the fact that they are flexible, tough filaments. Intriguingly, measurements of IF persistence lengths (a basic mechanical property quantifying the stiffness of a polymer) appear to vary over a wide range from several hundred nanometers to several μm [33,34]. This variability may be related to the different methods used to observe IF, or it may also reflect the variability in their properties when placed in different solutions or environments (see discussion in [34]). Although persistence lengths have not been measured accurately in live cells, it has been shown that IF frequently exhibit bending movements, sometimes appearing as the propagation of wave forms, and these changes in shape appear to involve configurations well within the range of values seen for the different measurements of persistence length in vitro [19,24].

Intermediate Filaments: Roles in Mechano-transduction and Signaling

The mechanical properties of IF in vitro and the fact that in most cells they form interacting networks between the cell surface and the nucleus (Figure 1 and Figure 2) leads to the hypothesis that this system provides tracks or scaffolds for the transduction of mechanical perturbations in a cell's environment throughout all compartments of the cell. There is some evidence in support of this hypothesis in vascular endothelial cells subjected to shear stress across their surfaces. These cells exhibit extensive three dimensional networks of vimentin IF between the nucleus and the cell membrane, which respond rapidly to a shear stress of 12 dynes/cm² exerted at their apical surfaces. The response involves a rapid displacement of IF (~1 μm within 3 min.) While these movements of IF occurred in 3-D throughout the cytoplasm, there was relatively more translocation at the apical surface compared to the basal surface of the cell [35]. Keratin filaments (tonofibrils) also respond rapidly to shear stresses exerted at the surface of epithelial cells. For example, tonofibrils in live PtK2 cells move throughout the cytoplasm in the direction of flow, within 2–4 minutes of the initiation of a shear force of 15 dyn/cm². After 6 min of flow, the system stabilizes and no further displacement is seen [24].

Another intriguing signaling function for IF proteins has been reported in neurons. Immediately following sciatic nerve crush injury, the MAP kinases, Erk1 and Erk2, are phosphorylated and activated. At the same time vimentin is synthesized from pre-stored mRNA. Following synthesis it appears that vimentin is cleaved by calpain into a 'soluble', but truncated form [36]. This fragment binds active Erks, importin β , and dynein to form a retrograde transport complex. Binding to the vimentin fragment prevents the activated kinases from being dephosphorylated during their journey towards the nucleus along microtubules [37] ••. Once this complex reaches the cell body, activated ERKs are released for transport into the nucleus. Thus a non-polymerized form of vimentin plays an essential role in signal transduction.

Polymerized IF networks also play roles in numerous other signal transduction pathways by providing a scaffold or platform that interacts with signaling molecules including MAP kinases, mTOR, various 14-3-3 protein isoforms, Cdk5, and apoptotic factors. This has been the subject of extensive recent reviews and therefore is not covered here [8,9,38,39].

IF and the Cytoplasmic Organization of Organelles: Possible Roles in Tethering and Positioning

Although there is a significant literature on the interactions between IF and membranous organelles, little attention has been paid to this important area of research until quite recently. It has been known for many years that IF, as well as microtubules, interact with

membranous organelles such as mitochondria, the Golgi Complex and more recently, lysosomes. Mitochondrial associations with vimentin IF were first described by electron microscopy (e.g. [40,41]). In nerve cells, the subcellular organization and movement of mitochondria are associated with the IF comprised of the neurofilament triplet proteins (neurofilaments) [42,43]. In vitro the interactions between mitochondria and neurofilaments are dependent upon the membrane potential of mitochondria and the state of phosphorylation of the C-terminal domains of neurofilament heavy (NF-H) and medium (NF-M) chains. Furthermore it has been shown that anti-NFH disrupts binding between mitochondria and neurofilaments [44]. In cardiomyocytes, desmin IF have been shown to interact with mitochondria and in a desmin knockout mouse, the morphology, distribution, number, and function of mitochondria are abnormal [5]. These mice die of dilated cardiomyopathy and heart failure.

Vimentin IF are also associated with the Golgi complex. For example, when neurofilaments are induced to aggregate by injecting excess NF-H into chicken and mouse embryo dorsal root ganglion cells, the golgi complex is fragmented and dispersed throughout their cell bodies [43]. In addition, it has been shown that vimentin IF bind to formiminotransferase cyclodeaminase (FTCD), a peripheral component of the Golgi complex, both in vitro and in cells. Although this enzyme is known to be a bifunctional enzyme that catalyzes two sequential reactions in the histidine degradation pathway, it also links the Golgi complex to the IF cytoskeleton [45].

A recent study of the bidirectional motility of melanosomes in *Xenopus* melanophores demonstrates that vimentin IF networks may play a role in the movements of these organelles. It was shown that the expression of a dominant negative vimentin mutant destroyed the organization of IF which permitted the organelles to engage in uninterrupted run lengths that were 50% longer than in the presence of vimentin IF. This observation suggests that IF, rather than just obstructing motility, may act to tether organelles and regulate their cytoplasmic transport and organization [46].

Vimentin IF also interact with lipid droplets, the repositories of cellular fatty acids. Specifically, IF form a “cage” around these organelles [47,48]. Although the specific function of the IF cage is unknown, there is evidence that it is involved in formation and stabilization of nascent droplets, as altering the organization of the vimentin IF network causes an inhibition of lipid droplet formation [49]. Interestingly, the IF associated with droplets appear to be resistant to the effects of drug-induced depolymerization of microtubules, a treatment that usually causes the retraction of vimentin IF into a perinuclear cap [41,47]. In addition to stabilizing lipid droplets, vimentin may participate in linking them to other organelles. Finally, vimentin IF may play a role in fatty acid (FA) metabolism, as it has been suggested that they are required for the release of fatty acids from adipocytes through an interaction with the $\beta 3$ adrenergic receptor [50].

Conclusions

Intermediate filaments play a key role in establishing and maintaining the mechanical integrity of cells. Recent studies in vitro have provided insights into the molecular mechanisms responsible for IF assembly, revealing their unique structure that resembles cables or ropes assembled from numerous repeating rod-like elements. Studies of single IF reveal that this organization results in very flexible filaments that can be stretched several fold before they break. When analyzed in vitro, reconstituted IF networks exhibit strain hardening properties that distinguish them from other cytoskeletal networks. Progress is also being made in understanding the assembly of IF in vivo and this appears to involve co-translational assembly of subunits. However, the precise understanding of IF assembly

awaits the determination of their structure at the atomic level, which is a technically challenging crystallographic problem. IF also play key roles in signal transduction, cytoskeletal cross talk and in organizing the cytoplasm by their attachments to and tethering of organelles in the cytoplasm. It is becoming increasingly clear that IF act as a nano-fibrillar scaffold involved in nuclear/cytoplasmic/cell surface communication and, as such, are major determinants of cellular architecture and subcellular organization.

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Mathematical modeling considered eight different potential pathways for vimentin filament elongation, and concluded that assembly involves rapid formation of ULFs followed by ULF and filament annealing. Importantly, growth by tetramer addition to filament ends did not contribute significantly to filament elongation.]

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Figure 1. Vimentin intermediate filament network

A Chinese hamster ovary (CHO) cell fixed and processed for immunofluorescence using anti-vimentin. The IF surround the nucleus and extend to the cell periphery.

Figure 2. Keratin filament network

Rat kangaroo kidney epithelial cells (PtK2) were fixed and processed for immunofluorescence with anti-keratin. The extensive network of tonofibrils (bundles of IF) ranges throughout the cytoplasm and appears to connect the cell surface with the nucleus where there is a juxtannuclear cage.

Figure 3. IF Assembly

Based on a variety of biochemical, physical chemical, electron microscopic and X-ray diffraction studies, the assembly pathway for intermediate filaments is now understood in reasonable detail. Individual protein chains self-assemble to form a parallel, non-staggered coiled-coil dimer (A), and two of these dimers come together in an antiparallel, manner to form a tetramer (B). In turn, 2 tetramers associate to form octamers, and finally 4 octamers join to form the ULF (unit length filament) (C). Individual ULFs join end-to-end to form short filaments (D), and these then grow into longer filaments by the continued addition of ULFs as well as the end-to-end fusion of existing filaments. (The A_{12} mode of antiparallel overlap of IF dimers is illustrated; see reference [12].)