Intermediate filaments are dynamic and motile elements of cellular architecture

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Summary

Recent evidence showing that intermediate filaments (IFs) are dynamic, motile elements of the cytoskeletal repertoire of vertebrate cells has overturned the long-standing view that they simply form static 'space filling' cytoplasmic networks. In fact, many types of IF are now known to engage in a remarkable array of movements that are closely associated with their assembly, disassembly and subcellular organization. Some of these motile properties are intrinsic to IFs and others are attributable to molecular crosstalk with either microtubules or actin-containing microfilaments. This crosstalk is, to a large extent, mediated by molecular motors, including conventional kinesin and cytoplasmic dynein. These motors are responsible for the high-speed delivery of nonfilamentous IF precursors and short filaments to specific regions of the cytoplasm, where they assemble into long IFs. Interestingly, the patterns and speeds of IF movements vary in different cell types and even within different regions of the same cell. These differences in motility may be related to their interactions with different types of molecular motor and/or other factors, such as IF-associated proteins.

Key words: Vimentin, Neurofilaments, Keratin, Intermediate filaments

Introduction

Cell behavior and function is controlled to a large extent by a cytoskeleton that consists of three distinct, yet interconnected, filament systems: intermediate filaments (IFs), actin-containing microfilaments (MFs) and microtubules (MTs). There are many more IF proteins than the highly conserved proteins that make up MTs and filamentous actin. The IF proteins are encoded by one of the largest families of genes in the human genome (Hesse et al., 2001). This family consists of over 65 different genes, which encode five different categories of IF; four of these are localized in the cytoplasm (types I-IV – cytoskeletal IFs) and one resides in the nucleus (type V, the nuclear lamins – nucleoskeletal IFs). The expression patterns of IFs are cell- and tissue-type specific, providing each major cell type with a relatively specific 'fingerprint' of IF proteins. In contrast to the globular subunits of MFs and MTs, G-actin and tubulin, respectively, IF proteins are rod-shaped and can self-assemble in vitro into 10-nm filaments in a complex and hierarchical fashion in the absence of ATP and GTP (Strelkov et al., 2003). In addition, once IF proteins polymerize they remain insoluble under conditions that readily solubilize MTs and MFs (Zackroff and Goldman, 1979). Furthermore, in vitro preparations of fully polymerized IFs possess unique viscoelastic properties that render them more resistant to deformation and breakage due to mechanical strain (Janmey et al., 1991; Janmey et al., 1998). On the basis of these biochemical and physical properties, the conventional textbook view of IF has been one of a static cytoskeletal system that provides the cell with a mechanism for resisting mechanical stress and deformation (e.g. Coulombe et al., 2000). However, recent studies of a variety of different types of live cells have revealed that IFs and their precursors are remarkably dynamic and exhibit a complex array of motile activities related to their subcellular assembly and organization.

The assembly and motility of vimentin in fibroblasts

Various techniques have provided insights into the mechanisms underlying the dynamic properties of the type III IF vimentin. When soluble vimentin is microinjected into cells, it rapidly forms discrete particles, which are subsequently incorporated into the endogenous vimentin IF network (Vikstrom et al., 1989; Vikstrom et al., 1992). This shows that endogenous type III IFs can incorporate exogenous vimentin, suggesting that their structure is regulated by an equilibrium between subunits and polymers. The existence of this equilibrium state has been directly demonstrated by fluorescence recovery after photobleaching (FRAP), following the microinjection and incorporation of fluorophore-tagged vimentin. FRAP analyses have shown that IF subunit exchange is nonpolar and occurs along the entire length of polymerized IFs (Vikstrom et al., 1992). However, these initial FRAP studies were limited in both their spatial and temporal resolution owing to the rapid photobleaching of the fluorophore-conjugated IF proteins. Over the past few years, the use of green fluorescent protein (GFP)-tagged IF proteins has permitted detailed time-lapse studies, allowing for the first time detailed analyses of the entire photobleach recovery process. These studies have shown explicitly that IFs display a broad range of dynamic and motile activities.

The assembly properties of vimentin have been most extensively characterized in BHK-21 cells (Ho et al., 1998; Prahlad et al., 1998; Yoon et al., 1998). Studies of fixed cells

Table 1. Different types of intermediate filaments and their movements

Cytoplasmic intermediate filament type	Protein studied	Cell type	Structural form observed	Published results	*n*=	Time moving (%)	Time pausing (%)	Antero movements (%)	Retro movements (%)	Average antero motility (mm/sec)	Range antero (μm/sec)	Average retro motility (μm/sec)	Range retro (μm/sec)
Type I	Keratin 13	AK13-1	Particles (dots)	Windoffer and Leube, 1999					Mostly Retro		0.002-0.008		0.002-0.008
	Keratin 18	PtK2	Squiggles	Yoon et al., 2001	37	18*	82	16	84	0.004±0.03	0.002-0.01	0.004±0.03	0.002-0.01
Type II	Keratin 5	NEB-1	Particles	Liovic et al., 2003	16						>0.1		>0.1
	Keratin 8	PtK2	Filaments	Yoon et al., 2001	14					0.001±3.33E-05		0.001±3.33E-05	
Type III	Vimentin	BHK-21	Particles	Helfand et al., 2003	53	45	55	66	34	0.39±0.24	0.08-1.65	0.43±0.26	0.08-1.40
			Squiggles	Yoon et al., 1998	32			97	3	0.06±0.03	0.02-0.19	0.06±0.03	0.02-0.19
			Filaments	Yoon et al., 1998	23					0.004±0.002		0.004±0.002	
	Vimentin	PtK2	Squiggles	Yoon et al., 2001	32			70	30	0.06±0.04	0.006-0.15	0.06±0.04	0.006-0.15
			Filaments	Yoon et al., 2001	29					0.15±0.11		0.15±0.11	
	Vimentin	3T3	Squiggles	Martys et al., 1999						0.25		0.25	
	Peripherin	PC12	Particles	Helfand et al., 2003	77	75	25	65	35	0.33±0.24	0.08-1.45	0.30±0.20	0.08-1.54
			Squiggles	Helfand et al., 2003	50	70	30	62	38	0.31±0.29	0.08-1.21	0.30±0.28	0.08-1.00
Type IV	NF-M	Rat sympathetic neurons	Short filaments	Wang et al., 2000	69	27	73	83	17	0.38	0.02-1.21	0.49	0.15-1.82
	NF-M	Rat sympathetic neurons	Short filaments	Wang et al., 2001	69	33	67	69	31	0.56	0.15-1.26	0.62	0.17-1.35
	NF-M	Rat sympathetic neurons	Particles	Wang et al., 2001	17	56	44	22	88	0.08	0.06-0.10	0.32	0.13-0.60
	NF-M	NB2a/d1	Particles	Yabe et al., 1999				>80%	<20%	0.025±0.005			
	NF-H	Rat sympathetic neurons	Short filaments	Roy et al., 2000	73	20	80	71	29	0.67	0.14-1.7	0.57	0.11-1.4
	NF-L, NF-M, NF-H	Purified bovine spinal cordrrr	Short filaments	Shah et al., 2000						0.27	~0.1-0.85	0.29	~0.1-1
	NF-L, NF-M, NF-H	Axoplasm from *Loligo pealei*	Particles	Prahlad et al., 2000	10						0.5-1.0		0.5-1.0

processed for immunofluorescence at time intervals during the spreading process, following trypsinization and replating, have revealed that the formation of typical IF networks is a complex, multistep process (Prahlad et al., 1998). Shortly after replating, vimentin IFs form a juxtanuclear cap and numerous nonfilamentous, non-membrane-bound protein aggregates (‘particles’) dispersed throughout the cytoplasm. Within 1-2 hours the number of vimentin particles decreases and there is an increase in the number of short vimentin filaments, termed ‘squiggles’. In extensively spread cells (i.e. 4-6 hours after replating), particles and squiggles are apparent only in the thinnest peripheral regions, probably owing to the abundance of long fibrils that typify interphase IF networks throughout the cytoplasm. Particles and squiggles might therefore be precursors in the assembly of long IFs (Prahlad et al., 1998). Indeed, observations of live cells expressing GFP-vimentin show that individual particles are frequently converted into squiggles. When considered together, the results of these experiments suggest that at least three morphologically distinct structural forms of IF (particles, squiggles and long IFs) are assembled in a highly regulated process to form the extensive cytoskeletal networks that typify spread cells.

Time-lapse studies of cells expressing GFP-vimentin clearly show that many vimentin fibrils constantly change shape and appear to assemble, disassemble, shorten and elongate throughout the cytoplasm (Martys et al., 1999; Yoon et al., 1998). IF motility has also been visualized as movements of photobleached bars made across the long axis of fibrils that contain GFP-vimentin. Specifically, bleach zones made on closely spaced parallel fibrils can translocate either anterogradely (towards the cell surface) or retrogradely (towards the nucleus) at average rates of ~0.2-0.3 μm/min (Table 1) (Yoon et al., 1998). These experiments show that long IFs can move bidirectionally in all regions of the cytoplasm and that these movements require energy.

The most remarkable movements of IF proteins have been revealed through time-lapse observations of spreading cells expressing GFP-vimentin. These studies have shown that IF proteins can move at speeds up to 1-2 μm/s (Table 1) (Prahlad et al., 1998). Similar movements of vimentin particles have been described in well-spread fibroblasts (Table 1) (Helfand et al., 2002). The movements of these particles are

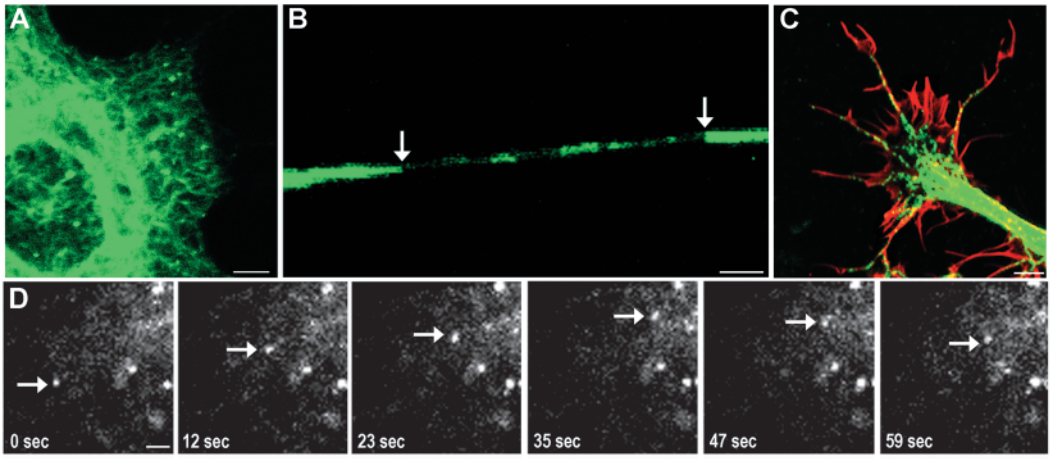

Fig. 1. Neural intermediate filaments move bidirectionally throughout all regions of nerve cells. (A) Differentiated PC12 cells expressing GFP-peripherin show that particles and squiggles, the precursors to long IF, are present in the peripheral regions of cell bodies. (B) These structures can be visualized in the neurites of live cells expressing GFP-peripherin after photobleaching (arrows denote the bleached region). (C) Peripherin particles can also be observed throughout the central and peripheral domains of the growth cones of nontransfected PC12 cells following fixation and staining for peripherin (green) and actin (red). (D) A series of images taken from a live PC12 cell expressing GFP-peripherin. The peripherin particle (see arrows) moved at rates of up to 0.58 μm/s and reversed directions in a region of the cell body during the observation period. Bars, 5 μm (A-C) and 2 μm (D).

saltatory and bidirectional (see Fig. 1D for peripherin, another type III protein), and most take place along MT tracks (Prahlad et al., 1998). However, the majority (~65-70%) are directed anterograde (i.e. towards the cell surface).

The mechanisms responsible for the bidirectional MT-dependent movements of vimentin particles are related to their association with conventional kinesin and cytoplasmic dynein. Indeed, immunofluorescence studies have shown that conventional kinesin is associated with vimentin particles, squiggles and even long IFs (Helfand et al., 2002; Prahlad et al., 1998). The first suggestion that conventional kinesin mediates the movements of IFs relative to MTs came from studies in which kinesin antibodies were microinjected into fibroblasts (Gyoeva and Gelfand, 1991; Prahlad et al., 1998). The results showed that cytoskeletal IFs are reorganized into perinuclear aggregates owing to the inhibition of anterograde movements towards the plus-ends of MT. The results of in vitro studies confirm these in vivo observations; the tail portion of kinesin heavy chain and a specific 62 kDa kinesin light chain appear to be required for the interactions between IFs and kinesin (Avsyuk et al., 1995; Liao and Gundersen, 1998). A significant fraction of cellular kinesin co-isolates with IF-enriched cytoskeletal preparations (Avsyuk et al., 1995; Prahlad et al., 1998). Kinesin thus appears to be responsible for the MT plus-end-directed movements of vimentin particles, squiggles and long IFs, and these interactions seem to be required for the proper formation and maintenance of IF networks. Kinesin has also been implicated in the anterograde movements of another type III IF protein, peripherin, and the type IV neural IF proteins (Helfand et al., 2003; Prahlad et al., 2000; Xia et al., 2003; Yabe et al., 1999).

More recently, the retrograde movements of vimentin particles, squiggles and filaments have been shown to depend on the minus-end-directed motor complex that consists of cytoplasmic dynein and dynactin (Helfand et al., 2002). Immunofluorescence and electron microscopic observations reveal that many of the components of dynein, including the heavy chain, intermediate chain and light intermediate chains, as well as the dynactin-associated proteins, p150^{glued}, actin-related protein-1 (Arp-1) and dynamitin, are present at the intersections between the various structural forms of vimentin and MTs. In addition, these are also present in IF-enriched cytoskeletal preparations (Helfand et al., 2002; Shah et al., 2000). A more direct approach to ascertain whether dynein contributes to IF motility has used dynamitin overexpression to inhibit dynein function (Burkhardt et al., 1997; Echeverri et al., 1996). Under this condition, the different structural forms of type III vimentin and type III peripherin reorganize towards the peripheral regions of BHK-21 and PC12 cells, respectively (Helfand et al., 2002). The inhibition of dynein and dynactin also alters the motile properties of vimentin particles, causing ~92% of these structures to move in the anterograde direction, probably owing to the residual activity of kinesin (Helfand et al., 2002). Dynein and dynactin are therefore essential for the maintenance and organization of type III IF networks.

Even though kinesin and cytoplasmic dynein/dynactin are associated with rapidly moving IF precursors such as vimentin particles and squiggles (Helfand et al., 2002; Prahlad et al., 1998), long IFs also move, but these movements are much slower (for a review, see Chou and Goldman, 2000). Because MT-based motors can move cargoes at rates that are relatively independent of their size in vitro (Hunt et al., 1994), other factors are probably responsible for the slower motility of long IFs in vivo. One plausible explanation for these differences lies in the number of interactions between longer IFs and IF-associated proteins (IFAPs). For example, IFAPs such as plectin might form crossbridges between IFs and MTs (Svitkina et al., 1996), thereby acting as brakes for the rapid transport of long IFs (Chou and Goldman, 2000). Such crossbridges may be required for the stabilization of fully polymerized long IFs in specific regions of the cytoplasm experiencing mechanical stress and deformation.

The assembly and motile properties of keratins

Keratin IFs are obligate co-polymers of type I and type II IF proteins. Their dynamic properties were initially studied by monitoring the incorporation of microinjected soluble keratin into cells expressing keratin IF networks (Miller et al., 1993; Miller et al., 1991). As in the case of vimentin (see above),

keratin particles form immediately after the microinjection of type I keratin protein. Soon thereafter, the keratin particles are incorporated into the endogenous bundles of keratin filaments (tonofibrils) in a concentration-dependent manner (Miller et al., 1993). Injection of excess type I keratin, however, induces the disassembly of the endogenous co-polymer, which suggests a requirement for an equal balance between type I and type II in the maintenance of a stable keratin network (Miller et al., 1993; Miller et al., 1991).

Several groups have studied the motile properties of keratin IFs in living epithelial cells transfected with GFP-linked keratins such as K5, K8, K13 and K18 (Table 1) (Liovic et al., 2003; Windoffer and Leube, 1999; Yoon et al., 2001). Windoffer and Leube have observed undulations of tonofibrils in A-431 epithelial cells that express GFP-K13 (Windoffer and Leube, 1999). Studies of PtK2 cells expressing GFP-K8 and GFP-K18 have also revealed that tonofibrils bend frequently to generate waveforms that are propagated along their longitudinal axes (Yoon et al., 2001). In cells that express both keratin and vimentin, such as PtK2 cells, the overall rates of keratin IF translocation are approximately three times slower than those of long vimentin filaments (~0.06 μm/min vs ~0.15 μm/min) (Yoon et al., 2001). Similarly, keratin squiggles move 15 times more slowly than vimentin squiggles (Yoon et al., 2001). Interestingly, the majority (~84%) of keratin squiggles move in a retrograde fashion, whereas ~70% of vimentin squiggles move towards the cell surface. The results of dual FRAP experiments in PtK2 cells doubly transfected with yellow fluorescent protein-K8 and cyan fluorescent protein-vimentin show that there is an ~18-fold slower recovery of keratin fibrils (t=~106 minutes), compared with vimentin fibrils (t=~6 minutes), in the same area of the cytoplasm (Yoon et al., 2001). These findings suggest that the factors regulating the subunit exchange processes for different types of IF vary significantly even within a single cell.

A possible explanation for the different motile properties of keratin and vimentin IFs may lie in differences in their capacity to associate with MTs and motor proteins. For example, we have known for many years that the overall organization of keratin IF networks remains relatively unaltered after treatment with MT inhibitors (Osborn et al., 1980; Yoon et al., 2001), whereas the vimentin IF network is dramatically reorganized, primarily into a perinuclear cap (Goldman, 1971). The lack of an obvious association between the vast majority of keratin IFs and MTs is also supported by the finding that disruption of dynein function in epithelial cells, by either microinjection of a dynein antibody or dynamitin overexpression**,** does not obviously alter the organization of keratin IFs (Helfand et al., 2002; Yoon et al., 2001). From these results, it is obvious that the vast majority of keratin IF movements in epithelial cells are independent of MTs and their associated motors. There is evidence, however, that a small number of MT-associated, rapidly moving keratin particles, similar to the vimentin particles, exists in epithelial cells (Liovic et al., 2003).

Approximately 30% of the motile keratin squiggles continue to translocate in the absence of MTs, whereas ~99% of vimentin squiggles stop moving (Yoon et al., 2001). This suggests that movements of keratin IFs are mainly attributable to other cytoskeletal networks, such as MFs. Indeed, keratin tonofibrils are closely associated with actin-containing stress fibers (Green et al., 1986), and treatment of epithelial cells with actin-depolymerizing drugs such as cytochalasin B disrupt the overall organization of the keratin IF network (Green et al., 1987). Recent studies have also shown that actin influences the organization, assembly and movements of keratin IF networks in extracts of *Xenopus laevis* eggs (Weber and Bement, 2002). It is likely, therefore, that different types of IF interact with different MT-based and actin-based motors, such as myosin (see below), and that these interactions are responsible to a great extent for the cytoskeletal crosstalk that distinguishes one cell type from another.

Differences in the association between individual types of IF and the various MT- and actin-based motors probably reflect important physiological distinctions among different cell types. As mentioned above, every major cell type appears to possess a unique IF expression profile. For example, the major IF protein found in fibroblasts is vimentin, and in epithelial cells it is keratin. Epithelial cells move at much slower rates (7 μm/h) than fibroblasts (82 μm/h) (Middleton, 1973). The associations between vimentin and MTs and their molecular motors might permit the rapid turnover and reorganization of the vimentin network that accompanies the changes in cell shape observed during fibroblast locomotion. Evidence supporting this hypothesis stems from the finding that fibroblasts from vimentin-null mice exhibit impaired locomotor behavior (Eckes et al., 2000; Eckes et al., 1998). The slower movements and dynamics of keratin IFs in epithelial cells may be related to their interactions with actin-based motors such as myosin. Myosins move more slowly than the major MT-associated motor proteins, and this slower motility may be related to the increased stability (measured by FRAP recovery) of keratin IFs compared with vimentin IFs.

The motile properties of neural intermediate filaments: fast vs slow transport

Neurons are ideal for studies of IF motility because they have remarkably long cytoplasmic processes, dendrites and axons, which can extend beyond one meter in situ. Because the bulk of protein synthesis takes place within the cell body of neurons, the majority of newly formed proteins, such as those that make up IFs and their associated proteins, must be transported in an anterograde fashion to reach the distal regions of axons and dendrites. There is also retrograde transport of proteins and organelles, including cytoskeletal proteins such as IFs, towards the cell body (Glass and Griffin, 1994). This bidirectional transport system is commonly referred to as axonal transport. On the basis of experiments employing pulse labeling with radioactively labeled amino acids, axonal transport has been subdivided into fast and slow components (Hammerschlag, 1994; Hoffman and Lasek, 1975; Hoffman and Lasek, 1980). Membranous organelles, for example, undergo fast transport (~50-400 mm/day) and cytoskeletal proteins undergo slow transport (~0.3-8 mm/day) (Brown, 2000). If the slow transport system was used exclusively for the turnover of cytoskeletal structures, such as the different types of neural IF, this would take years in the most distal regions of axons. Moreover, such ‘slow’ IF transport is inconsistent with the known motile and dynamic properties of IF proteins in other cell types, such as fibroblasts and epithelial cells.

Recently, direct observations of living sympathetic neurons expressing the type IV IF composed of the neurofilament (NF)

triplet proteins NF-L, NF-M and NF-H have shed new light on cytoskeletal protein transport along axonal processes (Roy et al., 2000; Wang and Brown, 2001; Wang et al., 2000). These studies used GFP-NF-M and GFP-NF-H to reveal that NFs, ranging in length from 1.0 μm to ~16 μm, and NF particles (morphologically similar to vimentin and keratin squiggles and particles (see above)) move at rates as high as ~1.8 μm/s (Roy et al., 2000; Wang and Brown, 2001; Wang et al., 2000). However, the movements of these different structural forms of NF are often interrupted by long pauses; they move only ~27% of the time (Wang et al., 2000). In fact, in mature sensory axons it has been estimated that NFs spend as much as 99% of their time pausing during their journey from the cell body towards the tip of an axon (Brown, 2000). These results have altered the traditional view of IF motility to one of rapid movements over very short distances. Because there are frequent pauses, their net movement is in the slow axonal transport category (Roy et al., 2000; Wang and Brown, 2001; Wang et al., 2000). A small proportion of NF protein, perhaps not readily visualized by light microscopy, might nevertheless move in the fast axonal transport system. In support of this, there is evidence from in vivo radiolabeling experiments that a small amount of NF protein can move at fast transport rates of 72-144 mm/day (Lasek et al., 1993).

Recently, studies of the type III neural IF protein peripherin have shed additional light on the rapid transport of cytoskeletal proteins. Peripherin is abundantly expressed in developing motor neurons, in PC12 cells and in the mature neurons of the peripheral and enteric nervous systems (Leonard et al., 1988; Parysek and Goldman, 1987; Portier et al., 1983a; Portier et al., 1983b). Detailed analyses of a large number of nonfilamentous peripherin particles and squiggles in PC12 cells have revealed movements at >1 μm/s (see Table 1 and Fig. 1). The rates of the movement of both forms of peripherin vary according to their intraneuronal location (Helfand et al., 2003). For example, the motility of individual peripherin particles and squiggles contained within cell bodies and growth cones is distinguished by their frequent (~40-50% of the time) and rapid reversals of direction (Fig. 1). By contrast, such reversals are very rare (~6-8% of the time) for these same structures in neurites (Helfand et al., 2003). These observations suggest that peripherin particles and squiggles contained within neurites can travel longer distances in shorter time periods relative to the distances traveled within either cell bodies or growth cones. In fact, these peripherin structures engage in rapid movements more than 70% of the time, and >20% move at rates equivalent to 50-133mm/day (Helfand et al., 2003). Therefore, a subpopulation of peripherin structures can move at rates similar to those recorded for membranous organelles in fast axonal transport (Brown, 2000). These fast-moving IF protein complexes could provide sufficient numbers of subunits for the normal turnover of IF, regardless of their distance from the cell body.

The maximum motility rates recorded for peripherin and type IV NFs are very similar (Table 1) (Helfand et al., 2003; Roy et al., 2000; Wang et al., 2000). In addition, both in vitro and in vivo studies have shown that NF and peripherin are associated with MTs, kinesin and cytoplasmic dynein (Helfand et al., 2003; Prahlad et al., 2000; Shah et al., 2000; Yabe et al., 2000; Yabe et al., 1999). Therefore, the mechanisms governing the intracellular transport of NFs and peripherin are not fundamentally different from each other or the IF-transport systems present in other cell types (see above). The overall distances traveled by the microscopically resolvable populations of type III IF proteins and the type IV IF (NF) proteins are significantly different (Table 1). These differences are attributable to their dramatically different pause times. A comparison of the short NFs with the similar peripherin squiggles shows that NF structures pause two to three times more frequently (Table 1) (Helfand et al., 2003; Wang et al., 2000).

These dramatic differences in pause times may be related to differences in IF structure. Specifically, the triplet proteins of NF are significantly more complex in structure than the homopolymeric peripherin. Furthermore, both NF-M and NF-H have unusually long, highly charged C-terminal ‘tails’ that project from the core IF structure (Hirokawa et al., 1997; Hisanaga and Hirokawa, 1988). These tail domains could promote filament stability by interacting with other NFs, as well as with other cytoskeletal systems, such as MTs (Chen et al., 2000; Hisanaga and Hirokawa, 1988; Jung et al., 2000; Nakagawa et al., 1995). Phosphorylation of these non-α-helical tail domains could also play a role in NF transport. For example, phosphorylation of the conserved KSP repeats in the tail domains of NF-M and NF-H may control the overall three-dimensional conformation of NFs, thereby regulating their association with the molecular motors required for transport (Yabe et al., 2000) (for a review, see Shea, 2000). Evidence for this comes from the finding that hypophosphorylated NFs are associated to a lesser degree with conventional kinesin than are extensively phosphorylated NFs (Yabe et al., 2000). In a fashion similar to that proposed for IFAPs such as plectin (see above) (Chou and Goldman, 2000), the tail domains of NFs may act, in a phosphorylation-dependent manner, as a brake in the regulation of NF transport. Support for this idea comes from studies of transgenic and knockout mice. NF-H-null mice show accelerated transport of NF-M and NF-L, whereas transgenic mice overexpressing NF-H show significant decreases in NF-M and NF-L transport rates (Collard and Julien, 1995; Marszalek et al., 1996; Zhu et al., 1998). Furthermore, phosphorylation of the NF-H tail domain increases pause times, thereby regulating the rate of NF transport (Ackerley et al., 2003). However, transgenic mice engineered to express NF-H lacking the entire C-terminal tail domain show no gross alterations in the rate of NF transport, which suggests that the tail domain of NF-H is not the crucial determinant of NF transport (Rao et al., 2002a). However, this latter study did not rule out the possibility that compensatory changes in NF-M, which also possesses a long tail domain, can maintain transport rates. The tail domains of NF-M and NF-H are thus likely to be involved in the regulation of NF transport. Therefore, the relatively sustained rapid movements observed for peripherin particles and squiggles may reflect the absence of the long phosphorylated tail domains that are characteristic features of NFs.

Another possible explanation for the slower rates of NF motility may be related to their associations with other non-MT-based cytoskeletal components. Recent immuno-electron microscopic, co-immunoprecipitation and blot-overlay analyses have shown that myosin Va associates with NF proteins, particularly NF-L. Since myosin Va is a processive, actin-based motor, NFs could presumably move in association

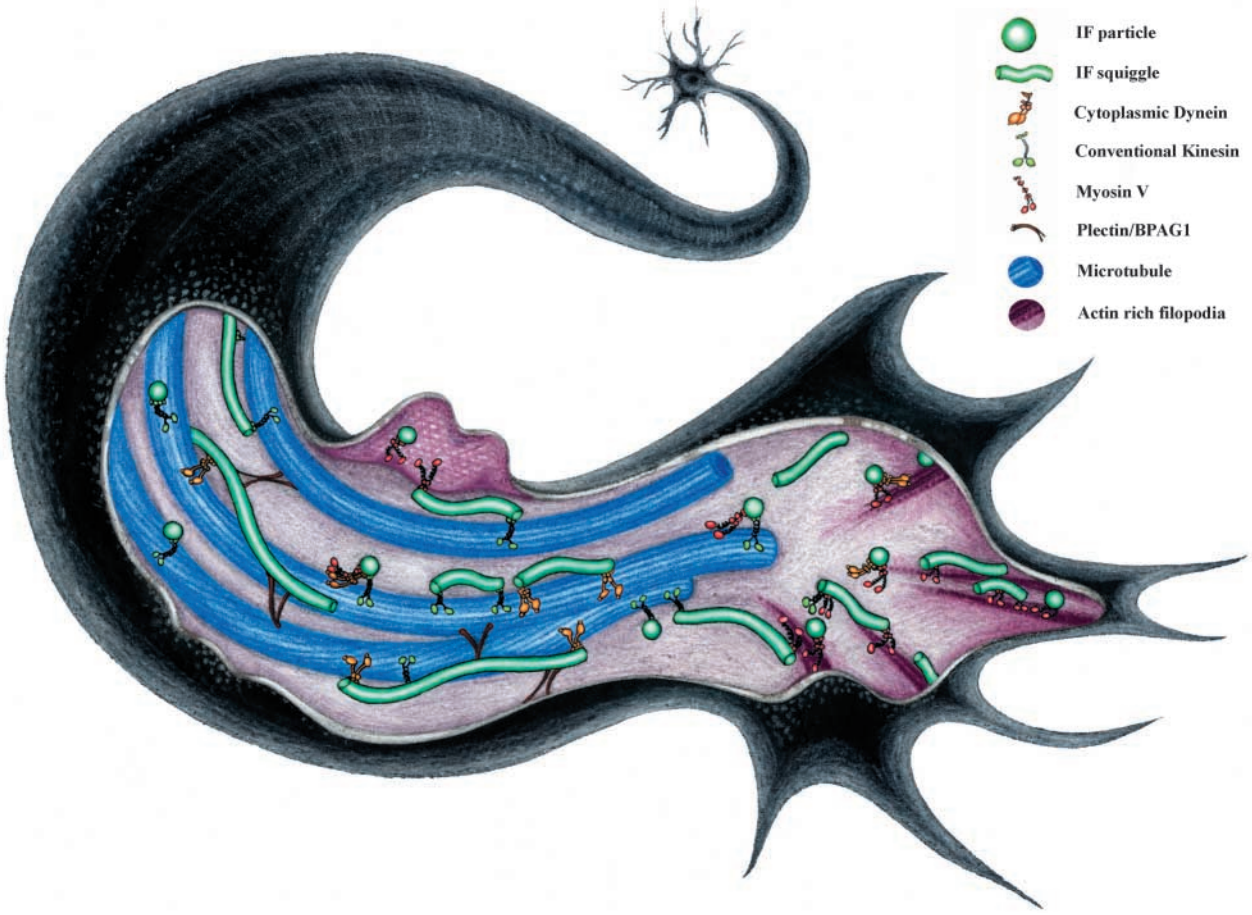

Fig. 2. A model for neural intermediate filament transport. Neural IF proteins in the form of nonfilamentous particles, squiggles and longer IF (green) move rapidly along microtubles (blue) in association with kinesin (yellow) and cytoplasmic dynein and dynactin (orange). These motors are responsible for the timely delivery of neural IF particles and squiggles, the precursors to the long IF, to all regions of the neuron including the growth cone (on the right side of cell). Long neural IFs also move along neurites, albeit more slowly than the precursors. Their slower motility might be due to associations with IF-associated proteins such as plectin and bullous pemphigoid antigen (BPAG) (red). In addition, a population of neural IF proteins may move along actin structures (purple) in association with myosin Va (pink) in the cortical and peripheral domains of the growth cone.

with actin (Rao et al., 2002b) (Fig. 2). Because there is a continuous tug-of-war between actin- and MT-based motors (Gross et al., 2002), myosin Va could act as a brake for NF transport that antagonizes the overall rate of NF transport. By contrast, peripherin might not associate with myosin Va to the same degree, and therefore, the MT-based movements of peripherin might not be as extensively inhibited.

The dramatic differences between the rates of NF motility along axons may reflect important differences in the specific functions of different types of IF. After axotomy, for example, regenerating dorsal root ganglion neurons (Oblinger et al., 1989) and motor neurons (Troy et al., 1990) show significant increases in the levels of both peripherin mRNA and protein. This increased expression coincides with significant decreases in the levels of NF proteins and their respective mRNAs. Regenerating axons are significantly thinner than normal axons. Their normal diameter is re-established only after the regeneration process is complete, as NF levels return to normal (Hoffman et al., 1985). These changes in IF expression suggest that peripherin is involved in neurite outgrowth during early development and regeneration, whereas NFs are involved mainly in regulating axonal caliber in terminally differentiated mature neurons. This possibility is also consistent with the differences observed in the motile properties of NFs and peripherin. From a functional perspective, the fast axonal transport of peripherin might be required to deliver IF precursors to neurites and thus provide sufficient initial cytoplasmic stability to sustain the rapid axonal outgrowth that typifies developing and regenerating neurons.

Intermediate filament dynamics and neurodegenerative diseases

The recent revelations of the mechanisms responsible for neural IF transport are beginning to shed light on the pathological processes responsible for several types of human neurodegenerative disease. Neural IF accumulations are the pathological hallmark of neuropathies including amyotrophic lateral sclerosis (ALS), Charcot-Marie tooth disease type 2 (CMT2), Parkinson's disease, progressive supranuclear palsy and giant axonal neuropathy (Al-Chalabi and Miller, 2003; Miller et al., 2002). As discussed above, there is a direct

relationship between the motility, assembly and cytoplasmic organization of IFs. Therefore, alterations in neural IF assembly or motility could lead to pathological accumulations. Mutations in the NF proteins are associated with CMT2, Parkinson's disease and ALS (Al-Chalabi et al., 1999; Figlewicz et al., 1994; Jordanova et al., 2003; Lavedan et al., 2002; Lee et al., 1994; Mersiyanova et al., 2000; Rooke et al., 1996). Specifically, mutations in the N-terminal domain and the 2B region of the rod domain of the NF-L subunit that occur in CMT2 have been shown to disrupt the distribution and axonal transport of neurofilaments in dorsal root ganglion neurons (Brownlees et al., 2002). Similar mutations in the 2B domain of NF-M are associated with Parkinson's disease (Lavedan et al., 2002). Mutations and deletions in the tail domain of NF-H have also been reported in sporadic forms of ALS (Al-Chalabi et al., 1999; Figlewicz et al., 1994; Rooke et al., 1996; Tomkins et al., 1998). Interestingly, many of the motor neurons from ALS patients exhibit gross alterations in axonal transport, such as an augmented mean anterograde speed and a diminished amount and speed of retrograde traffic (Breuer and Atkinson, 1988; Breuer et al., 1987). These point mutations could cause the accumulation of NF proteins by altering their normal assembly and/or their associations with molecular motors or IFAPs. Alternatively, mutations of domains of NF proteins that have been implicated in the regulation of motility, such as in the tail domains of NF-H or NF-M (see above), could dramatically alter the transport of NF into and within axons.

Because IFs are now known to be important protein cargoes for MT-associated motors such as cytoplasmic dynein and conventional kinesin (Helfand et al., 2003; Helfand et al., 2002; Prahlad et al., 2000; Prahlad et al., 1998), a disruption in any one of the numerous components involved in the motility of neural IFs, including MTs and their associated proteins (MAPs), kinesin and any one of the many different subunits that comprise cytoplasmic dynein and dynactin (Allan, 2000; Goldstein and Yang, 2000) could alter the normal organization of neural IF networks. An increasing amount of evidence supports this hypothesis because mutations in motor proteins involved in IF transport have been associated with many NF-accumulating human neurodegenerative diseases. For example, mutations in kinesin have been associated with the NF accumulations in CMT2 (Zhao et al., 2001), and mutations in the gene encoding the p150^{glued} subunit of dynactin have been associated with a slowly progressive, autosomal dominant form of lower motor neuron disease (Zhao et al., 2001). Similarly, MAPs, such as tau, appear to be involved in the regulation of anterograde motors such as conventional kinesin (Stamer et al., 2002; Trinczek et al., 1999). Disruption of normal tau expression by transient overexpression mimics many of the disruptions in IF organization and motility observed in numerous disease states (Ebneth et al., 1998; Stamer et al., 2002; Trinczek et al., 1999). Recently, mutations in the mouse cytoplasmic dynein heavy chain have been associated with Lewy-body-like inclusions containing abnormal aggregates of NF proteins, and mice that have these mutations exhibit many of the features of human neurodegenerative diseases (Hafezparast et al., 2003). Similarly, functional disruption of dynein by the overexpression of the p50 (dynamitin) subunit of dynactin is associated with a late-onset, progressive motor neuron disease in transgenic mice. The motor neurons of these mice also exhibit large accumulations of NF proteins, which are similar to those observed in the motor neurons of ALS patients (LaMonte et al., 2002).

Microtubule motors provide the means for delivering neural IF cytoskeletal components from their major site of synthesis (in the cell body) to the most distal regions of axonal processes in a highly regulated and timely fashion. It is therefore not surprising that a failure in any one of the components of this delivery system could be directly related to the pathogenesis of numerous types of neurodegenerative disease. A complete understanding of both the components and regulatory factors involved in this transport system will undoubtedly provide additional insights into the mechanisms responsible for these devastating neurological diseases, and will ultimately provide leads for new therapeutic interventions.

Concluding remarks

Obviously, not all IFs move in the same way. The different types of cytoplasmic IFs have significantly different N- and C-terminal amino acid sequences. These domains are involved in both their assembly properties and their interactions with other proteins, such as those associated with molecular motors. In turn, these associations with motors are responsible for many of the motile properties of IFs and also appear to be involved in regulating the subunit exchange that is involved in maintaining IF structure. These motile and dynamic properties of IF can also influence different types of cell behavior, such as cell migration and neurite outgrowth. As for future studies of IF, many important problems remain to be addressed. For example, it will be important to isolate and compare the molecular constituents of particles obtained from different types of IF. It will also be interesting to determine how IF structures such as particles, squiggles and long IFs associate with the different motor proteins. Studies such as these will undoubtedly yield additional insights into the mechanisms responsible for organizing and maintaining IF networks within cells. Such knowledge will certainly shed new light on many human disease processes in which IF motility is disrupted.

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