THE DYNAMIC AND MOTILE PROPERTIES OF INTERMEDIATE FILAMENTS

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■ **Abstract**   For many years, cytoplasmic intermediate filaments (IFs) were considered to be stable cytoskeletal elements contributing primarily to the maintenance of the structural and mechanical integrity of cells. However, recent studies of living cells have revealed that IFs and their precursors possess a remarkably wide array of dynamic and motile properties. These properties are in large part due to interactions with molecular motors such as conventional kinesin, cytoplasmic dynein, and myosin. The association between IFs and motors appears to account for much of the well-documented molecular cross talk between IFs and the other major cytoskeletal elements, microtubules, and actin-containing microfilaments. Furthermore, the associations with molecular motors are also responsible for the high-speed, targeted delivery of nonfilamentous IF protein cargo to specific regions of the cytoplasm where they polymerize into IFs. This review considers the functional implications of the motile properties of IFs and discusses the potential relationships between malfunctions in these motile activities and human diseases.

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INTRODUCTION

Analysis of the human genome reveals that there are more than 65 different
genes encoding intermediate filament (IF) proteins (Hesse et al. 2001). These have
been subdivided into five different types, four of which are located in the cytoplasm
(i.e., types I–IV) and one type, the lamins, which reside in the nucleus (Hesse et al.
2001). Therefore, the structural building blocks of IFs are not highly conserved,
and different cell types express different types of IFs. Intermediate filaments are
readily distinguished from other major cytoskeletal elements with respect to their
biochemical properties because they remain insoluble under conditions that readily
solubilize microtubules (MT) and actin-containing microfilaments (MF) (Zackroff
& Goldman 1979). This has led many investigators to assume that they form sta-
ble structures in vivo. Furthermore, measurements of their viscoelastic properties
reveal that they are much more resilient to applied forces and mechanical de-
formation than either MT or actin filaments (Janmey et al. 1991, Janmey et al.
1998). These properties are supported by the findings in vivo that mutations in IF
genes decrease cell and tissue resistance to mechanical stress, giving rise to a va-
riety of diseases including fragile skin syndromes and myopathies (for review, see
Carlsson & Thornell 2001, Galou et al. 1997, Irvine & McLean 1999). Taken
at face value, these findings suggest that IFs are relatively static polymers and
that their functions are restricted to establishing and maintaining the mechanical
integrity of cells. However, results from recent studies employing live imaging
of green fluorescent protein (GFP)-tagged IF proteins demonstrate that IFs form
dynamic, motile networks. These newly elucidated properties of IFs have impor-
tant functional implications for their involvement in protein trafficking, cellular
motility, intracellular signaling, the regional control of cytoplasmic architecture,
and the pathological processes of many human diseases.

THE DYNAMIC PROPERTIES OF INTERMEDIATE FILAMENTS: SUBUNIT EXCHANGE AND ORGANIZATIONAL ALTERATIONS

One of the earliest insights into the dynamic properties of IFs came from morphological studies documenting their organization throughout the cell cycle. In interphase cells, cytoplasmic IF proteins typically form a network that extends from the nuclear surface to the cell periphery. In some cell types, during the transition from late prophase to metaphase, the IF network disassembles and is reorganized into protofilamentous aggregates (for example see Rosevear et al. 1990). These aggregates, or particles, become concentrated near the mitotic spindle poles in late telophase where they appear to assemble into a juxtanuclear cap of IFs. This cap appears to be a focal point for the formation of IF networks in daughter cells as they respread following cytokinesis (Chou et al. 1990, Jones et al. 1985, Rosevear et al. 1990, Windoffer & Leube 2001).

Little is known about the mechanisms regulating the disassembly and reassembly of IFs during mitosis. In BHK-21 cells, the disassembly of vimentin (a type III protein) IFs into nonfilamentous particles corresponds to an increase in its phosphorylation by MPF (Chou et al. 1990, 1996; Tsujimura et al. 1994). However, it appears that other factors contribute to the formation of IF particles during mitosis. One of these is nestin, a type IV IF protein (Chou et al. 2003). Nestin cannot form IFs on its own, but it can co-assemble with vimentin forming heteropolymeric IFs (Steinert et al. 1999). Furthermore, nestin can inhibit vimentin assembly into IFs in vitro in a concentration-dependent manner (Steinert et al. 1999). In addition, it appears that nestin facilitates the disassembly of IFs when vimentin is phosphorylated at a specific site in the N-terminal domain during mitosis (Chou et al. 2003). This type of regulation of IF assembly states may not be restricted to nestin because other type IV IF proteins such as synemin (Bellin et al. 1999, Schweitzer et al. 2001), paranemin (Hemken et al. 1997, Schweitzer et al. 2001), and syncoilin (Newey et al. 2001) have been identified that can assemble only in the presence of vimentin or other types of IF proteins.

Some of the initial clues that IF networks are dynamic during interphase came from observations of cells responding to drugs or environmental stress. For example, it was shown that IF networks are rapidly reorganized into thick bundles or perinuclear aggregates of IFs in response to different stress conditions such as heat shock and serum deprivation (Collier et al. 1993, Djabali et al. 1997, Perng et al. 1999). It has also been shown that the induction of mechanical stress rapidly deforms IF networks in cells (Helmke et al. 2000, 2001). Similar observations of IF network rearrangements have been reported in cells after exposure to toxic compounds such as acrylamide and 2,5 hexanedione (Durham et al. 1983) or in response to different kinases such as protein kinase C, cAMP-dependent kinase, calcium/calmodulin-dependent kinase, and p21-activated kinase (Ando et al. 1991, Geisler et al. 1989, Goto et al. 2002, Inagaki et al. 1987, Tokui et al. 1990, Yano et al. 1991). The rapid changes in IF networks in response to these wide-ranging

chemical and physical conditions suggest that they are not static cytoskeletal systems. To the contrary, IFs exhibit a wide range of flexible properties that permits them to respond to both intracellular and extracellular stimuli through processes involving changes in their cytoplasmic organization and their state of assembly.

More direct analyses of the dynamic properties of IFs have been undertaken. For example, when either biotinylated or fluorophore-tagged IF proteins are microinjected into the cytoplasm of cells (Miller et al. 1991, 1993; Mittal et al. 1989; Vikstrom et al. 1989, 1992; Wiegers et al. 1991), they form discrete particles immediately, which are subsequently incorporated into the endogenous IF network within 2 h (Miller et al. 1991). In addition, these experiments show that the incorporation is dose dependent as high concentrations of microinjected soluble protein significantly alter the organization and assembly state of the endogenous IF network (Miller et al. 1993). Because the microinjected proteins are successfully incorporated into existing filaments in a dose-dependent manner, this suggests that a dynamic equilibrium exists between a soluble and insoluble pool of IFs. This equilibrium state has been confirmed by fluorescence-recovery-after-photobleaching (FRAP) studies following the microinjection of fluorophore-tagged vimentin and more recently in cells expressing GFP-tagged vimentin. The results of these studies demonstrate that IF subunit exchange is nonpolar and occurs along the entire length of polymerized IFs (Vikstrom et al. 1992, Yoon et al. 1998). Furthermore, these studies show that fluorescence recovery takes place with a $t_{1/2}$ (half time for full recovery) of ∼5 min (Yoon et al. 1998). Other studies showing similar properties of IFs have involved the transfection of cells with cDNA encoding for either wild-type or mutant IF proteins. For example, following transient transfection of epithelial cells with keratin cDNA, the newly expressed protein is incorporated into endogenous IFs without any visible alterations in the endogenous network (Albers & Fuchs 1989, Ngai et al. 1990). However, when mutant keratins are expressed, there are gross alterations in the assembly state of keratin networks (Albers & Fuchs 1989). All these early attempts to carry out detailed analyses of IF dynamics were limited owing to either the processes involved in visualizing tagged proteins in fixed cells or the rapid photobleaching of microinjected fluorochrome-tagged IF proteins. However, over the past few years, the use of GFP-tagged IF proteins has provided the opportunity to carry out microscopic observations with increased temporal and spatial resolution. These studies conclusively demonstrate that IFs engage in a broad range of motile and dynamic activities.

INTERMEDIATE FILAMENTS EXHIBIT A WIDE RANGE OF MOTILE ACTIVITIES

Slow Movements

Observations of fibroblasts expressing GFP-vimentin fusion proteins demonstrate that many fibrils of the vimentin IF network move constantly in a wave-like fashion (Ho et al. 1998, Yoon et al. 1998). Individual fibrils frequently change their shapes and move slowly as demonstrated by the translocation of bleach zones during

fluorescence recovery. Furthermore, vimentin fibrils exhibit independent motile properties as demonstrated by the finding that closely spaced fibrils can move either retrograde or anterograde with respect to the nucleus. These fibrils move at average rates of ~0.2–0.3 μm/min (Yoon et al. 1998). Interestingly, the rate of translocation is similar to that reported for MT and actin-containing microfilaments (Gorbsky & Borisy 1989, McKenna & Wang 1986, Waterman-Storer & Salmon 1998). In addition, it has been shown that both the movements of IFs and their photobleach recovery require energy (Yoon et al. 1998).

In epithelial cells, GFP-tagged keratin IF bundles (tonofibrils) also exhibit bidirectional bending movements (Windoffer & Leube 1999, Yoon et al. 2001). In many cases, waveforms appear to be propagated along the length of tonofibrils (Yoon et al. 2001). When small photobleached bars are made perpendicular to the long axes of tonofibrils, they move slowly at an average rate of ~0.06 μm/min, which is more than 3 times slower than vimentin fibrils (Yoon et al. 2001). Furthermore, closely spaced tonofibrils also behave independently with respect to their translocation, bending, and waveform movements. Fluorescence-recovery-after-photobleaching analyses demonstrate a steady-state exchange between keratin subunits and keratin IFs with a $t_{1/2}$ of 110 min. This rate of keratin fluorescence recovery, and therefore subunit exchange, is approximately 20 times slower than that recorded for vimentin IFs (Yoon et al. 2001).

Fast Movements

The most striking motile activity described for IF proteins has come from studies of IF network assembly during the spreading of fibroblasts (Prahlad et al. 1998). At early time points after trypsinization and replating, vimentin is concentrated both as filaments in a juxtanuclear cap and as numerous nonfilamentous, nonmembrane-bound particles in the region between the cap and the cell surface. These particles are morphologically similar to those observed during mitosis (Franke et al. 1982, Rosevear et al. 1990). Time-lapse observations made of spreading cells reveal that the vimentin particles move rapidly at speeds up to 1–2 μm/s (Figure 1A) (Prahlad et al. 1998). These movements are saltatory, bidirectional, and they follow MT tracks. Similar rapid movements of particles along MT have also been described for vimentin in spread fibroblasts, for type IV IFs in cultured neurons and in vitro utilizing nerve cell extracts (Helfand et al. 2002, Prahlad et al. 2000, Roy et al. 2000, Wang et al. 2000, Yabe et al. 2001). For example, vimentin particles have been observed to move at rates up to ~1.7 μm/s in the peripheral regions of spread fibroblasts. Although these movements are bidirectional, ~65% are anterograde (Helfand et al. 2002).

During the later stages of the fibroblast spreading process, there is a decrease in the relative number of vimentin particles and an increase in the number of squiggles, defined as short IFs with two visible ends. Based on these observations of fixed cells processed for immunofluorescence, it has been hypothesized that particles and squiggles are precursors in the assembly of long IFs (Prahlad et al. 1998). This is supported by direct observations of live cells in which GFP-tagged vimentin

particles have been observed to form squiggles (Prahlad et al. 1998). Subsequent imaging studies have demonstrated that IF squiggles also move along MT at speeds greater than 1 μm/s (see Figure 1*B*) (B.T. Helfand & R.D. Goldman, unpublished observations), and they appear to interact in tandem to form longer IFs (B.T. Helfand & R.D. Goldman, unpublished observations). These observations suggest that the formation of IF networks is a highly regulated process in which particles can be moved rapidly to specific locations where they transform into squiggles. As indicated above, similar motile structures (particles and squiggles) have been observed in a wide variety of cell types from different species both in vivo and in vitro (Helfand et al. 2002, Prahlad et al. 2000, Shah et al. 2000, Wang et al. 2000, Yabe et al. 2001). In addition, slower moving precursors, mainly in the form of squiggles, have been reported for keratin in epithelial cells (Windoffer & Leube 1999, 2001; Yoon et al. 2001). Taken together, these studies demonstrate that the various structural forms of IFs appear to be ubiquitous structural precursors to long IFs.

THE MOTILE PROPERTIES OF NEURAL INTERMEDIATE FILAMENTS

Neurons are one of the more interesting cell types that have been used for studying IF transport. These cells possess unusually long asymmetric cytoplasmic processes. The longest of these is the axon, which in humans can reach lengths of a meter or more. Because most of the protein synthetic machinery is located in the cell body, it has been assumed that newly synthesized proteins are transported to distal regions of nerve cells by a complex process known as axonal transport. Based on radioisotopic pulse labeling experiments, axonal transport has been divided into both fast- and slow-moving components (Hammerschlag 1994; Hoffman & Lasek 1975, 1980). Under this classification system, mitochondria, Golgi vesicles, lysosomes, and other membrane-bound organelles move as components of fast transport [50–400 mm/day (Brown 2000)]. In contrast, it has been thought for many years that cytoskeletal elements, such as neural IFs and their associated proteins, are components of a slow axonal transport system [∼0.3–8 mm/day (Brown 2000)]. At these slow rates, it could take months or even years for the turnover of neural IF subunits [e.g., neurofilaments (NF) containing three type IV IF proteins: NF-L, NF-M, and NF-H] in the most distal regions of the longest axons. These findings are inconsistent with the most recent revelations regarding the rapid movements of some of the structural forms of IF proteins such as vimentin particles and squiggles (see above).

Recently, direct observations of live sympathetic neurons expressing GFP-tagged NF-M and NF-H have demonstrated that NF squiggles and particles, very similar to the structures found in fibroblasts (see above), are transported bidirectionally at rates up to ∼1.8 μm/s (Roy et al. 2000, Wang & Brown 2001, Wang et al. 2000). However, the movements of these structures are frequently interrupted by long pauses, and therefore move only ∼27% of the time (Wang et al. 2000). These results alter the traditional view of slow NF transport to one characterized by rapid movements over short distances. The net movement, therefore, remains in the slow

component category (Roy et al. 2000, Wang & Brown 2001, Wang et al. 2000). However, it is still possible that there are rapidly moving forms of NF protein, some of which may lie beyond the resolution of the light microscope. In support of this, there is evidence from radioisotope labeling experiments showing that some NF protein can move at rates between 72 and 144 mm/day (Lasek et al. 1993), which is consistent with fast transport. This fast-moving, albeit small, amount of NF protein could be responsible for providing sufficient quantities of subunits necessary for the normal turnover of NF regardless of their distance from the cell body.

The Role of Microtubules in the Organization, Dynamic Properties, and Motility of Intermediate Filaments

From a historical perspective, relationships between IFs and MT have been known for many years. The first clues came from electron microscopic studies that demonstrated their close association in a number of cell types. For example, IFs and MT form closely associated parallel arrays throughout the cytoplasm in fibroblasts (Goldman 1971). More recently, it has been shown that a specific subset of detyrosinated MT (Glu-MT) are involved in these associations (Gurland & Gundersen 1995). Other results supporting an interaction between IFs and MT come from studies using inhibitors such as colchicine and nocodazole. When cells are treated with either of these inhibitors or injected with tubulin antibody, vimentin IFs are reorganized into perinuclear aggregates coincident with the depolymerization of MT (Goldman 1971, Gordon et al. 1978, Kreitzer et al. 1999).

It has also been shown that IF subunit exchange and turnover is to a great extent dependent upon the presence of MT (Yoon et al. 1998, 2001). For example, the rates of fluorescence recovery of GFP-vimentin IFs decrease by ∼60% in the absence of MT (Yoon et al. 1998). In addition, nocodazole inhibits the vast majority of vimentin particle, squiggle, and long IF movements, thereby preventing normal IF network formation (Ho et al. 1998, Prahlad et al. 1998, Yoon et al. 1998). Similarly, nocodazole treatment slows down the movements of keratin squiggles and increases the time required for fluorescence recovery after photobleaching (Windoffer & Leube 1999, Yoon et al. 2001). In the case of nerve cell extracts, NF particles have been observed to be associated with MT (Figure 2*A*). In preparations of squid axoplasm, many of these particles move rapidly along MT tracks (Prahlad et al. 1998). Short NF (squiggles) obtained from bovine spinal cord extracts have also been observed to move along MT (Shah et al. 2000). This transport appears to be mediated by both plus- and minus-end-directed MT-associated motor proteins (for review see Chou et al. 2001).

Interactions Between the Different Structural Forms of Intermediate Filaments, Microtubules, Kinesin, and Dynein

To date, all of the analyses on MT-based IF motility demonstrate that IF movements are bidirectional, but the majority (∼65%–70%) of these movements are directed anterograde or toward the cell surface (Helfand et al. 2002, Prahlad et al. 1998,

Roy et al. 2000, Wang et al. 2000). Therefore, a likely candidate for providing the motive force for these movements is the plus-end directed motor, conventional kinesin. Support for this is derived from the observation that the vimentin IF network is reorganized into a juxtanuclear aggregate in fibroblasts following the microinjection of kinesin antibody (Gyoeva & Gelfand 1991, Prahlad et al. 1998). A similar result has been obtained in CV-1 cells expressing a dominant-negative conventional kinesin heavy chain (Navone et al. 1992). The most likely conclusion drawn from these findings is that kinesin is required to maintain an extended IF network in the direction of the plus-ends of microtubules.

Immunofluorescence studies also demonstrate that kinesin is associated with IF particles, squiggles, and even long IFs (see Figure 2*B*) (Helfand et al. 2002; Prahlad et al. 1998, 2000; Yabe et al. 1999, 2000). The association between the extensive arrays of long IFs that typify interphase cells and kinesin is obscured by the overall immunofluorescence pattern generated by kinesin antibody, which is punctate throughout the cytoplasm. However, when cells are chilled to 4°C, the patterns of kinesin resemble the staining of long IFs, presumably owing to the stabilization of the association between kinesin and IF cargo (Prahlad et al. 1998). Under these conditions, the amount of kinesin present in IF-enriched cytoskeletal preparations is increased (Prahlad et al. 1998). Other biochemical analyses suggest that the tail region of kinesin heavy chain and a specific 62-kDa kinesin light chain are required for the interactions with IFs (Avsyuk 1995, Liao & Gundersen 1998). On the basis of all the available information, it appears that the association of kinesin with IFs is required for the normal anterograde movements of IF precursors and for the proper assembly and maintenance of extended IF networks (Figure 3).

Although the majority of vimentin IF movements are anterograde, ~30–35% are retrograde [i.e., directed toward the nucleus (Helfand et al. 2002, Roy et al. 2000, Wang et al. 2000)], suggesting that the minus-end-directed MT motor, cytoplasmic dynein, is also involved in regulating their motility. Cytoplasmic dynein is a large complex consisting of heavy chains, intermediate chains, light intermediate chains, and light chains (for review see King 2000). Furthermore, to function efficiently, it associates with dynactin, another large complex that contains ~11 different subunits, including dynamitin, actin-related protein-1 (Arp-1), and p150^{Glued} (for review see Allan 2000). Recently, both in vivo and in vitro experiments have demonstrated that cytoplasmic dynein and dynactin are essential for the normal retrograde motility of different forms of IF protein (Helfand et al. 2002, LaMonte et al. 2002, Shah et al. 2000) (see Figure 3). Immunofluorescence and electron microscopic analyses of IF networks have shown that many particles, squiggles, and long IFs are associated with both dynein and dynactin (Figure 2*C*) (see Helfand et al. 2002, Shah et al. 2000). However, a more direct approach to determine the role of dynein in IF motility has involved the overexpression of the dynamitin subunit of dynactin. This dissociates the dynactin complex, thereby inhibiting dynein function in vivo (Burkhardt et al. 1997, Echeverri et al. 1996). Dynamitin overexpression induces a displacement of IF networks toward the cell surface in fibroblasts and in motor neurons (Helfand et al. 2002, LaMonte et al.

2002). Under these conditions, ~92% of vimentin IF particle motility is directed toward the cell surface, supporting the continued function of kinesin (Helfand et al. 2002). Both dynein and dynactin are therefore essential for the maintenance and organization of IF networks (see Figure 3).

Observations of bovine spinal cord extracts have revealed that isolated short NF (squiggles) can move rapidly toward the minus ends of MTs at rates expected for dynein function (Shah et al. 2000). When these preparations are exposed to antibodies directed against the dynein intermediate chain, NF motility becomes biased toward MT plus ends (Shah et al. 2000). Furthermore, IF-enriched cytoskeletal preparations contain dynein and dynactin subunits (Helfand et al. 2002, Shah et al. 2000). When similar cytoskeletal preparations from cells overexpressing dynamitin are analyzed, a decrease in the concentration of most of the components of dynein and dynactin is found. Interestingly, the concentration of dynamitin present in these preparations appears unaltered (Helfand et al. 2002). This is not surprising, as dynamitin has been shown to link dynactin to other organelles. For example, dynamitin interacts with ZW10 and links dynactin to the kinetchore (Starr et al. 1998), and similarly, dynamitin links dynactin to Golgi membranes through interactions with Bicaudal-D2 protein (Hoogenraad et al. 2001).

Motor Coordination and Intermediate Filament Motility

As mentioned above, the net movement of most IF structures is biased toward the cell surface, suggesting that kinesin function is normally dominant over dynein function. However, analysis of single IF particles or squiggles demonstrates that the movements are discontinuous and frequently reverse their direction (Helfand et al. 2002, Prahlad et al. 1998, Roy et al. 2000, Wang et al. 2000). This particle behavior can be explained by the finding that the majority of IF protein structures are associated with both kinesin and dynein (Helfand et al. 2002, Prahlad et al. 1998), although it is not known how motors of opposite polarity bind to and move a single type of cargo such as an IF particle. Two models have been proposed to explain such dual motor regulation (Gross et al. 2002b). In one model, the two motors compete for directionality of movement by engaging in a molecular tug-of-war. The other, a coordinated model, proposes that the oppositely polarized motors work in concert to move structures in an efficient fashion.

The answer to which hypothesis is more likely to be correct for IF motility comes from experiments in which IF particle movements were monitored following the inhibition of dynein by dynamitin overexpression (Helfand et al. 2002). If IF-associated motors engage in a tug of war, then the disruption of dynein function should enhance the overall function of kinesin. In this scenario, IF particles would be expected to move more rapidly toward the cell surface. In contrast, if the motors associated with IFs work in a coordinated fashion, then impairment of the retrograde motor may be expected to decrease the efficiency of the anterograde motor. Under these conditions, it has been shown that IF particles move toward the cell surface at rates that are indistinguishable from controls (Helfand et al. 2002).

However, we have also observed by tracking individual particles that the number of stops or pauses increases significantly in dynamitin overexpressing cells (B.T. Helfand & R.D. Goldman, unpublished results). These results demonstrate that the molecular motors responsible for the motility of IF particles are most likely coordinated. This type of coordination is not surprising, as similar results have been reported for lipid droplet movements in *Drosophila* embryos (Gross et al. 2002b) and pigment granule movements in *Xenopus laevis* melanophores (Gross et al. 2002a).

Different Movements for Different Structural Forms of Intermediate Filaments

As described above, kinesin and dynein are associated with IF particles, squiggles, and long IFs (Helfand et al. 2002; Prahlad et al. 2000, 1998; Yabe et al. 1999). However, long IFs move at much slower rates ($\sim$0.3 μm/min) compared with rapidly moving particles and squiggles ($\sim$0.6 μm/s). Because it has been demonstrated that motors, such as kinesin, are capable of moving cargoes relatively independent of their size or length (Hunt et al. 1994), then it is likely that other factors must be responsible for the decreased rate of motility of long IFs in vivo. An explanation for these differences may lie in the association of long IFs with other proteins such as MTs and MFs that mediate and stabilize their interactions with different cell structures. Possible candidates for such interactions include IF-associated proteins (IFAPs). For example, IFAPs such as plectin and bullous pemphigoid antigen 1 (BPAG1), and some of their various splice variants, can form cross bridges between IFs, MT, and actin filaments (for review see Leung et al. 2002). As is the case for molecular motors, disruption of these cytoskeletal cross-linkers also alters the organization of IF networks. For example, dystonia musculorum (dt/dt) mutant mice lacking the BPAG1 gene exhibit severe degeneration of primary sensory neurons (Brown et al. 1995) and abnormal accumulations of IFs within axons (Brown et al. 1995, Guo et al. 1995, Yang et al. 1999). These accumulations are most likely the result of a failure to properly stabilize the interactions between IFs and other cytoskeletal components such as MT, leading to the deregulation of IF motility and organization (see Figure 3).

Microtubule-associated proteins (MAPs), such as tau, MAP4, or MAP2, also appear to be involved in the regulation of the attachment and detachment of motors from microtubules, potentially altering the transport of major cargoes such as IFs (Bulinski et al. 1997, Ebneth et al. 1998, Stamer et al. 2002, Trinczek et al. 1999). In support of this finding, it has been shown that overexpression of tau protein in CHO cells induces an accumulation of IF protein within the perinuclear area by inhibiting kinesin binding to MT (Ebneth et al. 1998, Stamer et al. 2002, Trinczek et al. 1999). Therefore, the overexpression of tau could cause, for example, vimentin particles and squiggles to accumulate and assemble long IFs in the juxtanuclear region, the phenotype also seen following the microinjection of kinesin antibodies (Gyoeva & Gelfand 1991, Prahlad et al. 1998). Another MAP, MAP2, has been shown to bind to IFs (Bloom & Vallee 1983), and there is evidence that this MAP reduces

the attachment of kinesin motors to MT (Seitz et al. 2002). Similarly, MAP4 overexpression induces a reduction in MT-based motility (Bulinski et al. 1997). It is likely that there are other MAPs, yet to be discovered, that specifically limit minus-end directed dynein-based motility.

THE INTRINSIC STRUCTURAL PROPERTIES OF INTERMEDIATE FILAMENTS MAY ALSO REGULATE THEIR MOTILITY

In addition to IFAPs and MAPs, it is also possible that IF proteins themselves modulate their motility. This is supported by considering the differences between the motile properties of homopolymeric vimentin IFs and the heteropolymeric neural IFs comprised of the NF triplet proteins. As described above, in vivo and in vitro studies of fibroblasts and neurons show that the different structural forms of IFs are associated with MT, kinesin, and cytoplasmic dynein (Helfand et al. 2002, Prahlad et al. 2000, Prahlad et al. 1998, Shah et al. 2000, Yabe et al. 1999). Therefore, the basic mechanisms governing IF transport in these two cell types are not fundamentally different. However, whereas NF movements are often interrupted by long pauses (Wang 2000; see above), vimentin particles move most (>60%) of the time (B.T. Helfand & R.D. Goldman, unpublished observations). One explanation for these differences may be related to the structure of the triplet proteins composing NF. Both NF-M and NF-H have unusually long, highly charged C-terminal tails that project from the core IF structures (Hisanaga & Hirokawa 1988). It has been suggested that these domains promote filament stability and slow NF transport (Chen et al. 2000, Hisanaga & Hirokawa 1988). This increased stability could be due to cross bridges between NF and MT formed by the C-terminal domains of NF-M and NF-H (Miyasaka et al. 1993). These bridges could, therefore, be major factors in determining the number of pause intervals regulating NF transport in axons.

Other factors involved in regulating NF motility may be related to their state of phosphorylation (for review see Pant et al. 2000). For example, phosphorylation of numerous KSP (Lys-Ser-Pro) repeats is thought to regulate the configuration of the tail domains of human NF-H and NF-M. In turn the conformation of these domains is thought to regulate intra-NF and NF-MT bridges (Eyer & Leterrier 1988, Gotow & Tanaka 1994, Gotow et al. 1994, Leterrier et al. 1996, Miyasaka et al. 1993, Sanchez et al. 2000). It has also been suggested that phosphorylation of these same sites determines their association with the molecular motors involved in transport (Yabe et al. 2000). For example, hypophosphorylated NF appear to associate to a lesser degree with kinesin than extensively phosphorylated NF (Yabe et al. 2000).

Actin-Based Intermediate Filament Motility

There is evidence supporting a role for MF in some types of IF movements. In this regard it is of interest to compare differences in the motile properties of

vimentin and keratin because they are frequently expressed as separate IF networks in cultured epithelial cells. For example, it has been shown that in a given cytoplasmic region of the same PtK2 cell, keratin squiggles move >15 times slower than vimentin squiggles (Yoon et al. 2001). In addition, keratin squiggles move mainly retrograde, whereas vimentin squiggles move mainly anterograde within similar regions (Prahlad et al. 1998, Windoffer & Leube 2001, Yoon et al. 2001). Keratin squiggles also continue to move in the presence of nocodazole, whereas vimentin squiggles do not (Yoon et al. 2001). These results are most likely related to the fact that keratin IFs do not appear to associate with MT-based motors to the same degree as vimentin IFs. In support of this, the overall organization of keratin IF networks remains relatively unaltered after treatment with MT inhibitors such as colchicine or nocadazole, whereas vimentin IFs in the same cell form a juxtanuclear cap (Osborn et al. 1980, Yoon et al. 2001). In addition, 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).

In light of the findings that most keratin movements are independent of MT, it is important to note that close associations between keratin tonofibrils and actin/MF bundles (stress fibers) have been reported in epithelial cells (Green et al. 1986). In addition, treatment of epithelial cells with drugs such as cytochalasin D, known to inhibit actin function, disrupt the organization of keratin IF networks (Green et al. 1987). In vitro it has been shown that actin influences the organization, assembly, and movements of keratin IF networks in extracts of *Xenopus* eggs (Weber & Bement 2002). Therefore, it appears that different mechanisms and cytoskeletal interactions account for the different motile properties of vimentin and keratin IFs.

Recent evidence has shown that myosin motors can also mediate interactions between IFs and actin-containing MF. This is based on the finding that more than half of the total myosin Va in neurons associates with NF. The deletion of this myosin results in the altered distribution of NF within axons (Rao et al. 2002). Although the link between some forms of IF motility and myosin has been shown only in this one study of neurons, undoubtedly there are similar myosin-mediated interactions between IFs and MF in a wide variety of other cell types (see Figure 3).

DISRUPTIONS OF INTERMEDIATE FILAMENT TRANSPORT COULD BE CRITICAL FACTORS IN A VARIETY OF DISEASES

In light of the findings that IFs and their constituent proteins are major cargoes for MT-associated motors such as cytoplasmic dynein and kinesin (Helfand et al. 2002, Prahlad et al. 1998), it is likely that subtle changes in IF transport could lead to significant alterations in the distribution, organization, and function of IF networks. For example, if the mechanisms regulating the motile properties of the

different forms of IFs are disrupted, they would most likely accumulate in different subdomains of cells. These alterations in IF network organization could cause dysfunctions with respect to the mechanical integrity of different subcellular domains (Goldman et al. 1996), intracellular signaling (Tzivion et al. 2000), and cell motility (Eckes et al. 1998, Singh & Gupta 1994). In support of this, accumulations of IFs are frequently noted as the pathological hallmark in a wide range of human diseases including Mallory bodies in alcoholic cirrhosis (Jensen & Gluud 1994) and vimentin aggregates in skin fibroblasts of patients with giant axonal neuropathy (Bousquet et al. 1996, Pena 1982). In the case of neurons, cytoplasmic aggregates of neural IFs have been described in many neurodegenerative diseases, including the spheroids in amyotrophic lateral sclerosis (ALS) (Toyoshima et al. 1989), Lewy bodies in Parkinson's disease (Galloway et al. 1992), Rosenthal fibers in Alexander's Disease and glioblastoma multiforme (Brenner et al. 2001, Hwang & Borit 1982), and NF aggregates in Charcot-Marie Tooth type II Disease and fetal alcohol syndrome (Perez-Olle et al. 2002, Saez et al. 1991). These aggregates are considered to be major factors in the pathogenesis of these diseases (for review see Julien 2001). In some cases, it has been proposed that aggregates of neural IFs clog axons, thereby preventing the axonal transport of organelles and nutrients (Williamson & Cleveland 1999). In this scenario, neural IF aggregates may strangle neurons, ultimately resulting in their untimely demise.

At the present time, little is known about the precise mechanisms responsible for the accumulation of neural IFs in these different diseases. Some clues have been derived from studies of ALS suggesting that transport defects may be correlated with the aberrant accumulation of IFs (for review see Julien 2001). ALS is the most common motor neuron disease in adults. It involves the selective death of upper and lower motor neurons, leading to skeletal muscle atrophy and ultimately death due to respiratory failure. Approximately 5 to 10% of all ALS cases are familial, with a small proportion of these linked to mutations in Cu/Zn superoxide dismutase 1 (SOD1) (Cudkowicz et al. 1997, Rosen et al. 1993). The vast majority of cases are sporadic, suggesting that the causes may be multifactorial (Julien 2001). However, in all cases the accumulation of IFs remains the pathological hallmark of ALS. It is possible, therefore, that this accumulation is related either directly or indirectly to altered IF transport. In support of this, time-lapse observations of live motor neurons obtained from the median nerves of patients with sporadic ALS demonstrate that transport of cytoplasmic organelles is abnormal (Breuer & Atkinson 1988, Breuer et al. 1987).

Other aspects of the properties of motor neurons may also be relevant to IF accumulations in motor neuron disease. These neurons are typically large with respect to the cross-sectional diameter of their axons, the maintenance of which requires a constant supply of neural IFs or their constituent proteins, known to play important roles in determining axon caliber (Hoffman et al. 1984, 1987; Marszalek et al. 1996). Furthermore, NF proteins are the most abundant proteins in the axons of large motor neurons (Hoffman et al. 1984), so it is likely that their MT-based axonal transport system is extremely important in the regulation of

their proper distribution and turnover. It follows that a disruption in any one of the numerous components involved in regulating the motile activities of IFs, including MT, kinesin and its various light chains, and over 16 different subunits making up cytoplasmic dynein and dynactin (see above), could cause neural IF aggregation and subsequently lead to neuronal death. This hypothesis is supported by recent findings in both humans and mice that exhibit disruptions in kinesin, dynein, or dynactin (LaMonte et al. 2002, Hafezparast et al. 2003, Puls et al. 2003, Xia et al. 2003). For example, transgenic mice that overexpress dynamitin in their motor neurons have late-onset progressive neurological disease characterized by motor neuron degeneration, loss of innervation, and muscle wasting. The motor neurons of these mice exhibit an inhibition of retrograde axonal transport and subsequent accumulations of neural IFs that are morphologically similar to those observed in human cases of ALS (LaMonte et al. 2002). In addition, mice lacking the neuronal-specific conventional kinesin heavy chain show alterations in neural IF transport that are associated with a reduction in axon caliber, neuronal degeneration, and hind limb paralysis (Xia et al. 2003). In addition, there is evidence demonstrating a direct relationship between mutations in NF proteins and impaired axonal transport in neurodegenerative disease (Brownlees et al. 2002, Perez-Olle et al. 2002). Other studies have identified mutations in the KSP repeats of the NF-H subunit in ∼1% of sporadic ALS cases. As indicated above, the subdomains containing these repeats are also thought to be involved in the regulation of NF transport (see above) (Cleveland 1999). These mutations could also affect the association of NF with motor proteins, or they may perturb intra-NF or NF and MT interactions resulting in the aggregation of IFs.

Novel Expression Patterns of Intermediate Filaments Frequently Accompany Changes in Cell Motility

It is becoming more and more apparent that in some pathological situations, cells express novel IF proteins. For example, atypical vimentin expression is observed in many epithelial cell–derived human tumors, including metastatic breast carcinoma, uveal melanomas, tumors of the oral mucosa, and prostate cancer (Heikinheimo et al. 1991; Hendrix et al. 1992, 1996, 1998; Lang et al. 2002). In fact, vimentin expression has frequently been used by pathologists to grade and diagnose different types of tumors (Huszar et al. 1983, Shuster et al. 1985, Sommers et al. 1992, Thomas et al. 1999). Based on recent insights into the dynamic and motile properties of different types of IFs, it is possible that the induction of vimentin expression may not simply represent an epiphenomenon, but rather it may be directly related to altering the molecular architecture of epithelial cells such that they become more mesenchymal-like (termed the epithelial-mesenchymal transition, or EMT) with respect to their shape and motile behavior. For example, MCF-7 cells are nonmetastatic human breast ductal epithelial cells that express only keratin IFs. However, following transfection with vimentin cDNA and the formation of a type III IF network, these cells exhibit increased motile activity, invasiveness,

and tumorgenicity (Hendrix et al. 1992, Thompson et al. 1992). Other experiments have demonstrated that there is a 70% reduction in the migration of highly invasive breast cancer MDA-MB-231 cells after decreasing the amount of vimentin by an antisense approach (Hendrix et al. 1996, 1997). It has also been shown that fibroblasts from vimentin-null mice move much more slowly than normal fibroblasts (Eckes et al. 1998, 2000), and vimentin expression is turned on in migrating epithelial cells during wound healing in monolayer cultures (Gilles et al. 1999). Taken together, these results demonstrate that the expression of vimentin is highly correlated with increased cell motility. Although the mechanisms responsible for the EMT remain unknown, it is important to consider the potential significance of the findings that vimentin IF networks, and not keratin IF networks, depend extensively on MT and their associated motors (see above). It is therefore possible that the aberrant expression of vimentin in epithelial cells introduces a novel type of cytoskeletal cross talk with MT. This cross talk could be more conducive to the rapid reorganization of IFs and other cytoskeletal constituents required for the changes in cell shape, motility, and mechanical properties that accompany the EMT observed in many metastatic tumors.

CONCLUSIONS

Recent findings have uncovered a remarkable array of mechanisms regulating the dynamic and motile properties of IFs. These mechanisms require molecular motors such as conventional kinesin, cytoplasmic dynein, and in the case of nerve cells, myosin Va. These motor proteins are necessary to move IFs and their precursors, particles, and squiggles along cytoskeletal tracks of either microtubules or microfilaments. This rapid transport system is required for the maintenance and proper organization of IF networks, as well as for the targeted and timely delivery of IF precursors to specific areas of cells, where the formation and/or active remodeling of IF networks may be required for a variety of cell functions. For example, the rapid transport of IF particles to the cell surface and their subsequent assembly may play an important role in signal transduction. In support of this, there is evidence that IFs are associated with numerous factors, such as 14-3-3 protein, that are known components of the signal transduction machinery (Tzivion et al. 2000).

The evidence supporting a wide array of motile activities and the finding that IFs can polymerize locally in cells from nonfilamentous precursors open up many avenues of research. The results obtained from ongoing studies of IF assembly in numerous laboratories will ultimately lead to a greater understanding of the unique properties and functions of IFs, which are one of the major protein structures found in vertebrate cells. It will be especially important to isolate and identify the molecular constituents of IF particles in order to determine how these nonmembrane-bound precursors interact with motor proteins and how these interactions are related to the assembly of IFs. Once in hand, this information should contribute significantly to the overall understanding of the physiological functions of IFs in normal and diseased cells.

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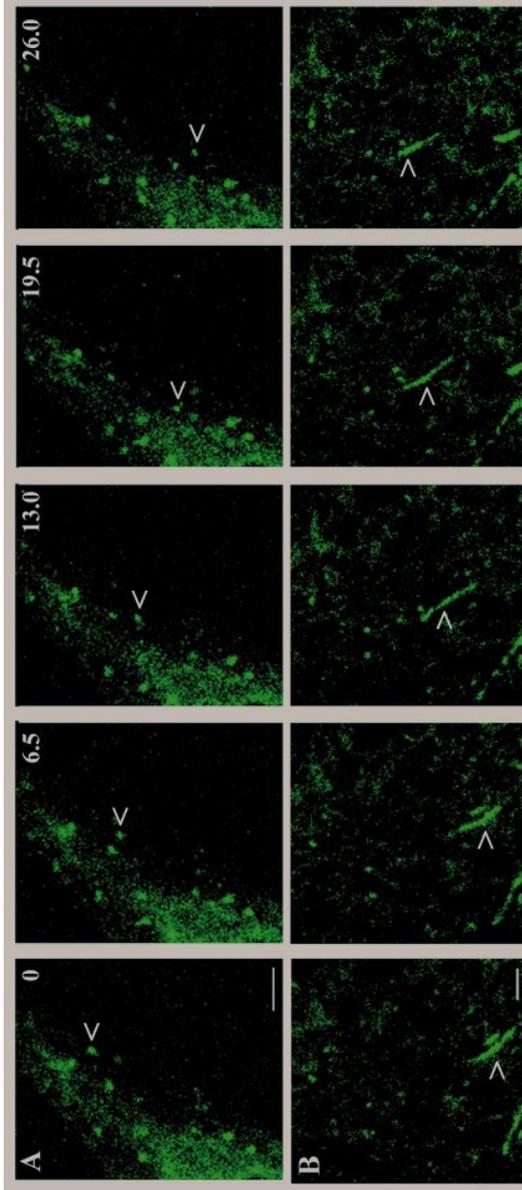
Figure 1 Rapid movements of IF precursors. Particles and squiggles, the precursors to long IFs, have been observed to move rapidly in fibroblasts and neurons. For example, live images of GFP-vimentin particles at the edge of a well-spread BHK-21 fibroblast are observed to move at rates up to 1–2 μm/s (*row A*). The particle marked by the arrowhead in row A moved at an average speed of 0.43 μm/s with a peak of 0.92 μm/s. Peripherin, which is the major type III IF protein present in neurons of the peripheral nervous system, also moves at similar rates as shown in row *B*. The GFP-peripherin squiggle indicated (see *arrowheads*) moved toward the cell surface of a PC-12 cell at a maximum rate of 0.87 μm/s. Images were captured for both *A* and *B* at the time intervals (s) indicated at the upper right corner. Size bars = 5 μm.
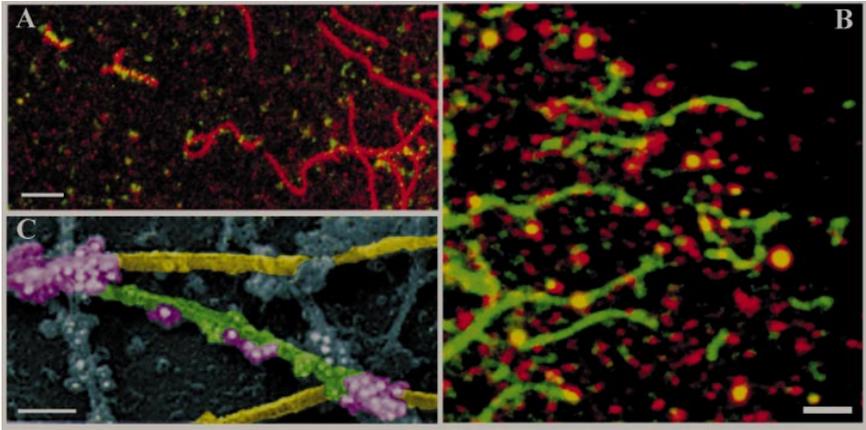
Figure 2   The different structural forms of IFs associate with MT, conventional kinesin, and cytoplasmic dynein. The different structural forms of IF proteins appear to move along MT in association with kinesin, dynein, and dynactin. (*A*) For example, double label indirect immunofluorescence reveals that neurofilament particles (*green*) associate with MT (*red*) in extruded axoplasm from the giant axon of *Loligo pealei* (Prahlad et al. 2000). (*B*) Visualization of vimentin (*green*) and conventional kinesin (*red*) in spreading BHK-21 cells prepared for double label immunofluorescence demonstrates that many of the vimentin particles and squiggles associate with the anterograde motor (seen as *yellow*) (Prahlad et al. 1998). (*C*) Spread BHK-21 cells were processed for double-label immuno-gold platinum replica electron microscopy (see Helfand et al. 2002 for details). Antibodies directed against vimentin are labeled with 10-nm gold particles and dynein heavy chain with 18-nm gold particles. Some of the vimentin IFs (*pseudocolored green*), and some of the dynein heavy chain antibody locations (*pseudocolored pink*) are indicated. Two MT are highlighted in yellow. Size bar *A*,*B* = 10 µm; *C* = 100 nm.

See legend on next page
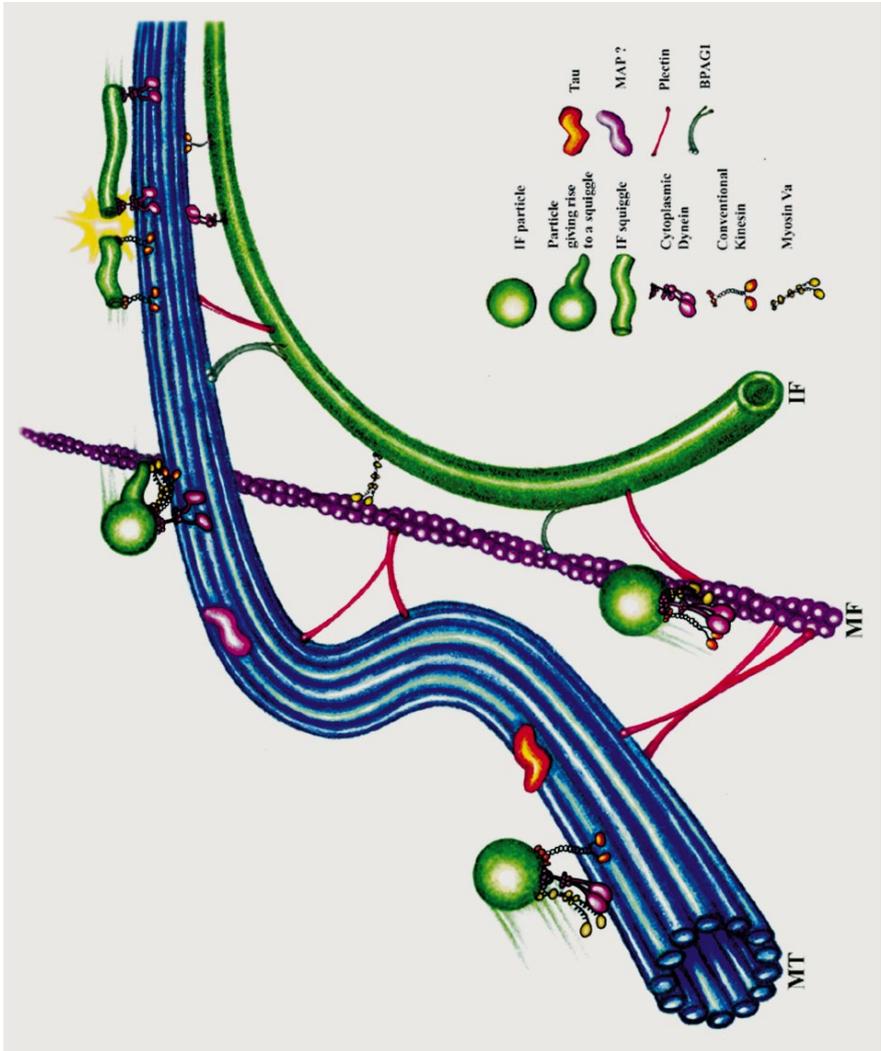
Figure 3   A model for IF transport. Non-membrane bound IF precursor particles associate with conventional kinesin and cytoplasmic dynein. These motors provide the motive force for the delivery of IF particles along microtubule tracks to specific regions of the cytoplasm. The anterograde transport is influenced, at least in part, by MAPs, such as tau, that appear to regulate the attachment of kinesin to MT. Other unidentified MAPs probably regulate retrograde transport mediated by dynein in a similar fashion. Upon reaching their cytoplasmic destination, particles are converted into squiggles. Squiggles continue moving along MT driven by the same motor proteins until they are linked together (depicted as the *yellow* region between two squiggles) to form longer IFs. Longer IFs also appear to be moved along MT by kinesin and dynein; however, their movements are slower owing to associations with IFAPs, such as BPAG1 and plectin. These latter cross-bridging elements could act to stabilize long IFs relative to their interactions with other cytoskeletal networks. In addition, it is possible that IF particles and other IF structures also move, albeit more slowly, along MF in association with myosin Va.

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