



Inroads into the structure and function of intermediate filament networks

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ABSTRACT

Although intermediate filaments are one of three major cytoskeletal systems of vertebrate cells, they remain the least understood with respect to their structure and function. This is due in part to the fact that they are encoded by a large gene family which is developmentally regulated in a cell and tissue type specific fashion. This article is in honor of Ueli Aebi. It highlights the studies on IF that have been carried out by our laboratory for more than 40 years. Many of our advances in understanding IF are based on conversations with Ueli which have taken place during adventurous and sometimes dangerous hiking and biking trips throughout the world.

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1. Introduction

This paper is written as a tribute to Ueli Aebi's remarkable career in the fields of structural and cell biology. In honor of his formal retirement from the University of Basel we have chosen to relate our own history of discovery in the area of intermediate filament structure and function, a field of research in which Ueli has made major contributions.

2. Historical overview

Intermediate filaments (IF) remain the least studied and understood of the three major cytoskeletal systems that are expressed in virtually all vertebrate and many invertebrate cell types. From a historical perspective, this is probably related to the fact that even though their constituent subunits typically form 10 nm filaments, the protein composition of these subunits is not conserved as they are encoded by ~75 different genes. Contributing to the complexity is the fact that the expression of these genes is developmentally regulated in a cell and tissue type specific fashion. As a result of the enormous diversity in their subunit composition, it took many years for cell biologists and biochemists to realize that all of the 10 nm IF seen in different types of cells were derived from the same large protein family. It is likely that the first descriptions of IF were made over a hundred years ago by the pioneering studies of cytologists and histologists who developed various methods to generate contrast in the bright field microscope (for review see

(Wilson, 1928)). These methods employed various fixatives and stains which revealed extensive arrays of filamentous structures in nerve cells and in epithelial cells (Wilson, 1928). In the former, the fibrillar patterns are highly reminiscent of the organization and distribution of neurofilaments composed of the Type IV IF proteins (e.g., neurofilament triplet proteins) and in epithelial cells, the "fibrillae" or "tonofibrils", are now known to be bundles of closely packed Types I and II keratin IF (Fig. 1A).

Following the introduction of techniques developed for studying cell fine structure using electron microscopy, 10 nm IF were described in numerous cell types (Goldman and Follett, 1969) (see Fig. 2). However, the nomenclature used in the 1960s and 1970s to describe them caused significant confusion. During this period IF were called 10 nm and 100 Å filaments, decafilaments, 9 nm filaments, 110 nm filaments, 80–100 Å filaments, 'intermediate filaments' in muscle cells (the latter name is based on the cross sectional diameter of IF which lies between actin and myosin filaments), neurofilaments in neurons, tonofilaments for the keratin IF in epithelial cells, glial fibrillary acidic filaments in glial cells and sarcoplasmic filaments for skeletal muscle cell IF. In addition, the names of their purported protein subunits varied widely and included muscle skeleton or desmin; glial fibrillary acidic protein (GFAP); fibroblast 10 nm filament protein, fibroblast intermediate filament protein, vimentin or decamin; peripheral and brain neurofilament protein; keratin and tonofilament protein, etc. (for review see (Eriksson and Thornell, 1979)). Eventually, the name of intermediate filaments became widely accepted, based mainly on the fact that the diameter of IF was between that of actin/microfilaments and microtubules.

It was not until the late 1970s and early to mid-1980s that cell biologists began to realize that, although there were differences,

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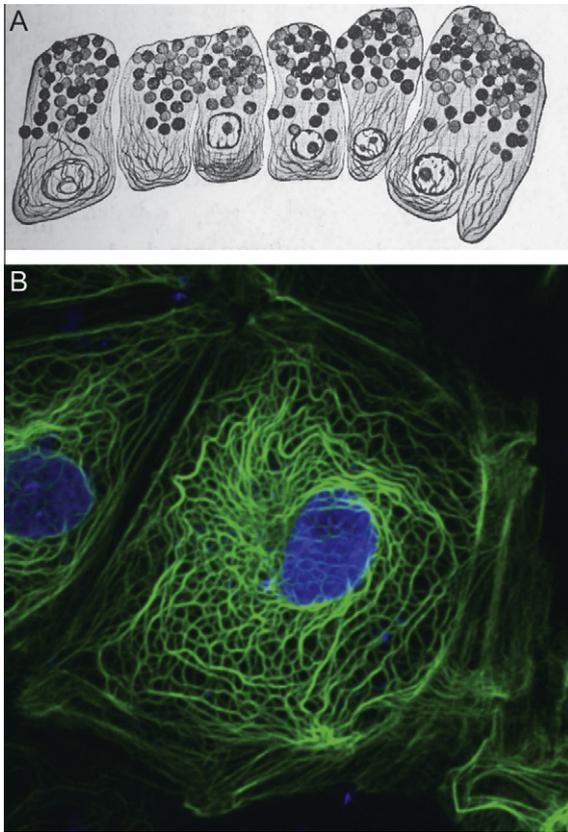


Fig. 1. (A) Early observations of secretory epithelial cells in the mudpuppy pancreas. Note the “fibrillae” which most likely represent keratin IF bundles or tonofibrils. Work of Mathews in the laboratory of E.B. Wilson. From reference [Wilson, 1928](#). (B) A PtK2 epithelial cell fixed and processed for immunostaining with anti-keratin. Note the similarities in the tonofibrils seen in the preparations spanning 80 years of research.

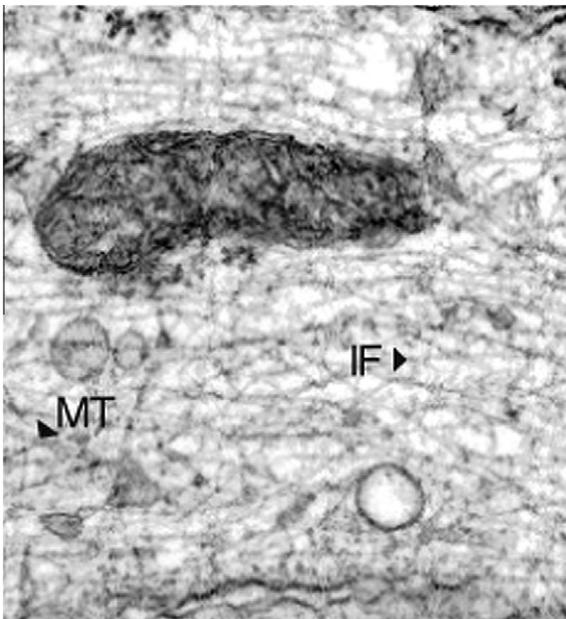


Fig. 2. Electron micrograph of a human fibroblast showing the cytoplasm containing numerous 10 nm vimentin IF (IF) and a few microtubules (MT).

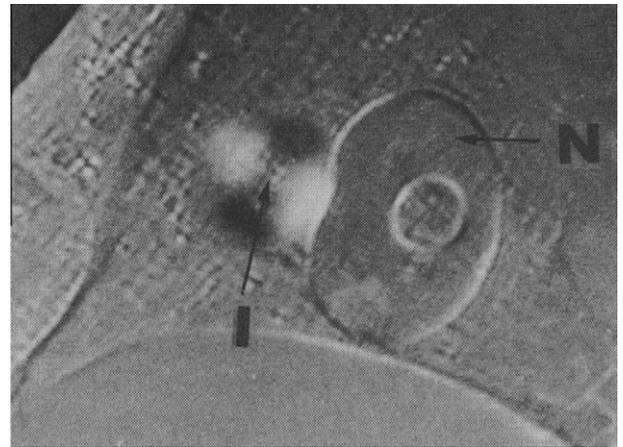


Fig. 3. A living BHK-21 cell observed during the early stages of cell spreading by polarized light microscopy. The birefringent sphere comprised of dark and bright sectors surrounds an isotropic region (I). This structure is closely associated with the nucleus (N). From reference [Goldman and Follett, 1970](#).

there were also very significant similarities in the proteins comprising the different IF systems. These similarities began emerging when comparative structural and biochemical methodologies, along with the introduction of cloning and sequencing of cDNAs, made it possible to determine the specific properties of IF derived from a wide range of cell types. Now we know that IF assemble from a coiled-coiled dimer. The region responsible for dimer formation is the highly conserved, α -helix rich, central rod domain which is a common feature of all cytoskeletal IF proteins. These dimers associate in a hierarchical fashion to form IF which differ from each other primarily in the size and the amino acid composition of their N- and C-terminal non- α -helical domains. These discoveries are based primarily on work from the laboratories of Peter Steinert, David Parry, Ueli Aebi and Harald Herrmann ([Downing et al., 1983](#); [Herrmann et al., 1996, 1999](#); [Parry et al., 1985, 1986, 2007](#); [Steinert et al., 1976](#)). During this same period, the development of antibodies directed against IF proteins and advancements in fluorescence microscopy revealed the presence of extensive arrays of complex cytoskeletal networks of IF, typically extending from the perinuclear region to the cell surface ([Starger et al., 1978](#); [Sun and Green, 1978](#)) ([Fig. 1B](#)). We now know that these complex networks engage in many functions ranging from cell shape determination and mechanics to signal transduction and cell motility.

3. Early observations leading to the isolation and initial biochemical characterizations of IF

Our first IF isolation procedures were based upon observations of live cells using polarized light microscopy. We discovered that during the early stages of the spreading of fibroblasts such as BHK-21 cells, there was a prominent juxtannuclear “birefringent sphere” (or juxtannuclear cap) which became elongated into birefringent fibers as cells flattened and took on the shape of fibroblasts. The middle of this spherical region was isotropic while the surrounding anisotropic region exhibited positive birefringence with respect to its circumferential axis ([Fig. 3](#)). This latter finding indicated that filamentous components were oriented with their long axes wound around the center of the isotropic core. Electron microscopy revealed that the birefringent sphere consisted exclusively of closely associated masses of IF organized in the fashion predicted by the polarized light findings. No microtubules or microfilaments were detected within these juxtannuclear arrays of

IF in the early stages of cell spreading. An association with microtubules only became apparent as the cells flattened into the typical shapes of fibroblasts. We also speculated that the organizational changes in IF were in some fashion responsible for aspects of the motile properties of cells (Goldman and Follett, 1970). Other factors involved in the formation of the juxtannuclear caps of IF were revealed in experiments in which we used colchicine to disassemble microtubules in BHK-21 cells. The results demonstrated that IF retracted in a retrograde fashion and became concentrated next to the nucleus to form a juxtannuclear cap, as the microtubules disassembled. Support for interactions between IFs and microtubules also came from our earlier electron microscope studies showing a close relationship between these two cytoskeletal elements (Goldman, 1971; Goldman and Follett, 1970) (see Fig. 2). These results showed that the normal organization of IF in fibroblasts depends on microtubules (Goldman, 1971).

Based upon the observations of the formation of juxtannuclear IF caps, we developed a procedure for their preparation exploiting their close association with nuclei. Briefly, we lysed BHK-21 cells either during the early stages of cell spreading or following colchicine treatment and subsequently fractionated the nuclei in these lysates with their IF caps attached. The nuclei were then lysed in high salt and subsequently digested with DNAase I to remove DNA/chromatin. This latter step also removed actin from these preparations (Hitchcock et al., 1976). This method rapidly produced relatively pure fractions of intact IF caps which retained their birefringent properties. Electron microscopy revealed that pellets of these IF caps contained large numbers of intact IF

(Fig. 4). When intact IF obtained from these preparations were analyzed by SDS-PAGE, the major proteins migrated as bands of ~54 and 55 kD (arrowheads Fig. 4) which later we realized consisted of two Type III IF protein subunits, vimentin and desmin (Starger and Goldman, 1977; Starger et al., 1978). We also observed a high molecular weight protein (arrow in figure) which was subsequently identified as the IF associated protein, plectin (Clubb et al., 2000). It is also interesting to note that the ~60–70 kD bands migrating above the cytoskeletal IF proteins were later identified as the Type V nuclear lamins (Aebi et al., 1986; Goldman et al., 1986; Zackroff et al., 1984) (Fig. 4).

4. Early insights into the common structural features of IF

We first began to realize that there may be similarities in IF from different cell types when we met Peter Steinert at the 1976 Cell Motility Meeting held at the Cold Spring Harbor laboratory (Goldman et al., 1976). Peter was one of the world's leading authorities on the keratins. This stemmed from his Australian wool chemistry roots and his training under a distinguished hair and wool keratin protein chemist, George Rogers in the Department of Biochemistry at the University of Adelaide. It was at this meeting that Peter and I realized that the 10 nm filaments my lab was studying and isolating from mesenchymal cells (e.g., vimentin IF) might be similar to the keratins that he was isolating from epithelial cells. Following this meeting Peter and I immediately initiated a collaboration which resulted in numerous publications until his untimely and tragic death in 2003. Our first paper demonstrated

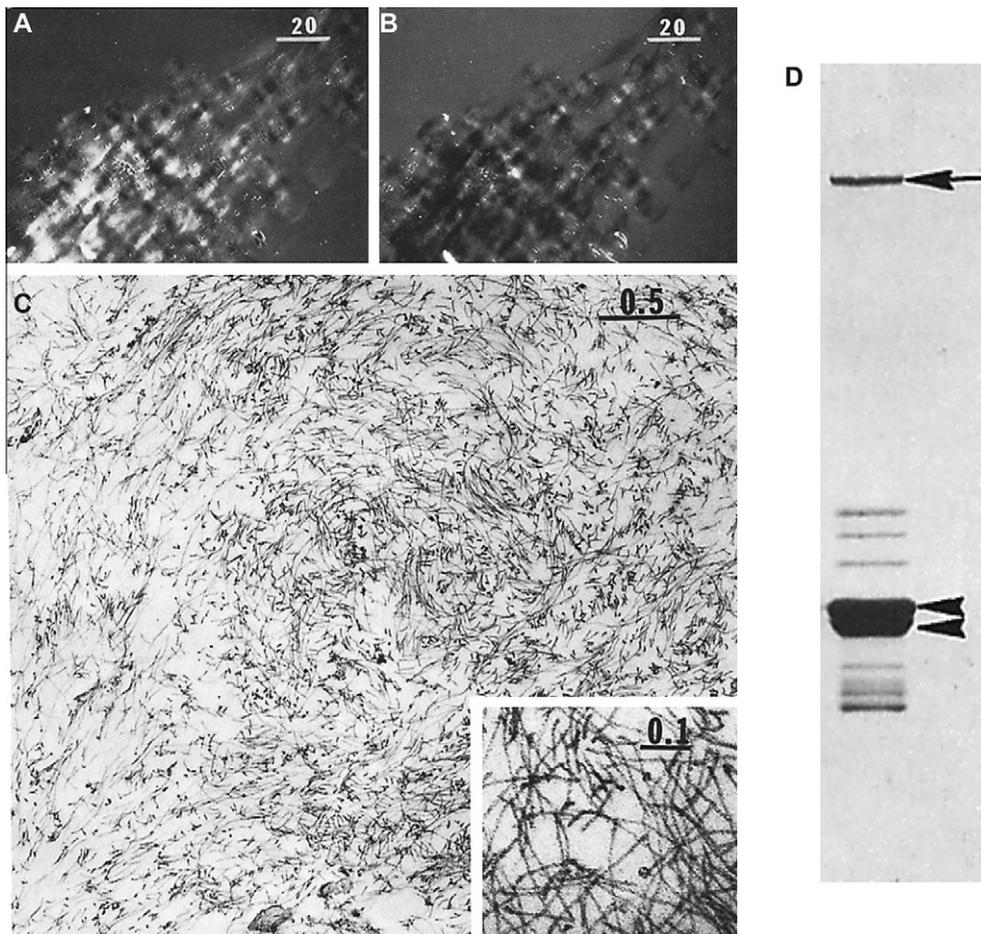


Fig. 4. Panels (A and B) show pellets of isolated birefringent juxtannuclear caps at opposite compensator settings; panel (C) is an electron micrograph of a thin section of a similar pellet showing large numbers of IF. Panel D shows a Coomassie stained SDS-gel of intact IF obtained from the caps. From reference Starger et al. (1978).

that the 10 nm filaments isolated from BHK-21 cells and keratin IF had similar structures as determined using optical rotary dispersion (ORD) and wide angle X-ray diffraction analyses. Specifically the results showed that both IF types contained ~42% α -helix arranged in a coiled-coil configuration, features now known to typify all cytoskeletal IF (Steinert et al., 1978).

5. In vitro assembly of IF

The isolation of the juxtannuclear IF caps to obtain enriched preparations of their constituent proteins was soon replaced with a method for preparing “IF-enriched cytoskeletons”. This method was rapid and simple. It involved extracting BHK-21 cells in high salt/detergent containing solutions, followed by digestion with DNAase I to remove chromatin and also to depolymerize filamentous actin (see above). Under these conditions greatly enriched preparations of polymerized IF were obtained in milligram quantities in the absence of tubulin and actin. Further purification was achieved by the disassembly of these IF-enriched cytoskeletal preparations in low ionic strength buffers followed by reassembly under physiological conditions. After several cycles of disassembly and reassembly in vitro, minor “contaminating” proteins disappeared from the IF preparations. We developed a combined turbidimetric/negative staining assay by which we determined that the critical concentration required for the in vitro polymerization of the IF proteins (i.e., vimentin and desmin) was in the range of 0.05–0.15 mg/ml. The overall results of these studies showed that IF assembly in vitro involved a two-step nucleation–condensation reaction in which short IF form first, followed by elongation (Zackroff and Goldman, 1979).

Based upon our results with isolating and reassembling BHK-21 IF, we went on to develop a common method for preparing IF-enriched cytoskeletons from a wide variety of cell types (e.g., HeLa and PC12 cells). Incorporating the use of 8 M urea as a universal solvent, a technique developed by Peter Steinert for epidermal keratins (Steinert et al., 1976), we were able to disassemble and reassemble different members of the IF family (e.g., see (Aynardi et al., 1984; Parysek and Goldman, 1987)). We also developed methods for the isolation and in vitro reconstitution of neuronal IF obtained from squid giant axons and also bovine spinal cords. In all cases the overall assembly properties were similar to those found for BHK-21 IF, except that neurofilaments typically contained 3–4 different IF proteins which formed complex heteropolymers. In addition we found that the exact conditions for solubilization/disassembly and reassembly of neurofilaments differed for each system (Zackroff and Goldman, 1980; Zackroff et al., 1982). These early studies on the identification of IF protein subunits and their in vitro assembly properties were followed by much more sophisticated structural analyses carried out in the laboratories of Ueli Aebi and Harald Herrmann. In a series of elegant studies, Ueli and Harald defined the steps involved in the polymerization of vimentin and desmin. The long term collaboration between these two groups led to the discovery of the unit length filament (ULF) which is an essential building block of cytoskeletal IF. Their work represents the cornerstone of our knowledge of the roles of the different subdomains of the protein chains in IF assembly mechanisms (Herrmann and Aebi, 1998, 2004; Herrmann et al., 1996, 2007; Meier et al., 2009; Nicolet et al., 2010; Plodinec et al., 2011; Portet et al., 2009; Strelkov et al., 2001, 2002, 2003).

6. Early insights into the dynamic properties of IF

For many years cytoskeletal IF were considered to be static, “space filling” elements of the cytoplasm of non-muscle vertebrate cells. Historically this was primarily based upon the biochemical

findings that they could be isolated intact as 10 nm filaments from cells (Starger et al., 1978) and there was little evidence for soluble pools of IF subunits (Soellner et al., 1985). These biochemical properties suggested that once IF proteins were synthesized and polymerized in cells, there was little subunit exchange. Indeed, this view is consistent with in vitro studies which demonstrate that there is hardly any exchange of subunits among filaments, even after 2 days of incubation (Winheim et al., 2011). These early studies suggested that the steady state for IF was regulated mainly by protein synthesis and degradation or post-translational modifications. These properties contrasted with those known for the other cytoskeletal systems, microtubules and actin/microfilaments, which depended on large pools of soluble subunits for regulating subunit exchange and their disassembly/reassembly. For these reasons the roles of IF in various cellular functions, such as motility, were largely ignored.

However, our early studies showing that IF were reorganized during spreading and shape formation in cultured cells led us to believe that they were, indeed, dynamic in living cells. Therefore we developed methods to determine the subunit exchange properties of IF in live cells. Initially this involved the use of soluble, biotinylated vimentin which was microinjected into BHK-21 cells. In order to obtain sufficient quantities of purified vimentin for tagging with biotin (this was the period prior to the introduction of bacterial expression systems), we used fresh cow lenses obtained from the slaughter house (Lieska et al., 1980). Importantly, the in vitro assembly properties of vimentin were preserved if the biotin was added during the polymerization reaction. The biotinylated vimentin was then solubilized and microinjected into live BHK-21 cells which were fixed at different time intervals following injection. The results showed, by double label immunofluorescence, that the exogenous cow lens protein was incorporated into endogenous IF within 30 min. This demonstrated that IF were not static structures and provided early clues that they had dynamic properties in living cells (Vikstrom et al., 1989). Similar experiments were carried out on the keratin IF networks in epithelial cells. These studies involved a different part of the bovine anatomy, the cow's tongue (also obtained at the slaughter house), which provided a source of keratin Types I and II. The major keratins were separated by ion-exchange chromatography and then biotinylated as they formed the obligate heteropolymers required for 10 nm filaments to assemble in vitro. Following assembly, the two types of keratin were separated and only the biotinylated Type I (which was soluble) was injected into live epithelial cells. Following fixation and processing for double label immunofluorescence at time intervals post injection, we found that within minutes small aggregates or particles of the injected keratin formed in close association with the endogenous bundles of keratin IF (tonofibrils). After 4 h, the exogenous keratin was fully integrated into the endogenous network (Miller et al., 1991) (Fig. 5). In a subsequent study we showed that the non-filamentous aggregates of biotinylated Type I keratin, which formed immediately following microinjection, also contained endogenous Type II keratin IF. Importantly, injections of significantly higher concentrations of the Type I protein resulted in the disassembly of the endogenous IF network. These results suggested that keratin IF were also regulated by an equilibrium between polymerized and non-polymerized subunits and suggested that heteropolymer keratin IF are dynamic cytoskeletal components (Miller et al., 1993). Other experiments shedding light on the dynamic properties of keratin IF involved expressing mutant forms of epidermal keratin 14 (K14) in simple epithelial cells (Albers and Fuchs, 1987, 1989).

Further insights into the dynamic properties of vimentin IF were derived from fluorescence recovery after photobleaching (FRAP) analyses. Initially this involved the microinjection of x-rhodamine-conjugated vimentin which was used to monitor vimentin

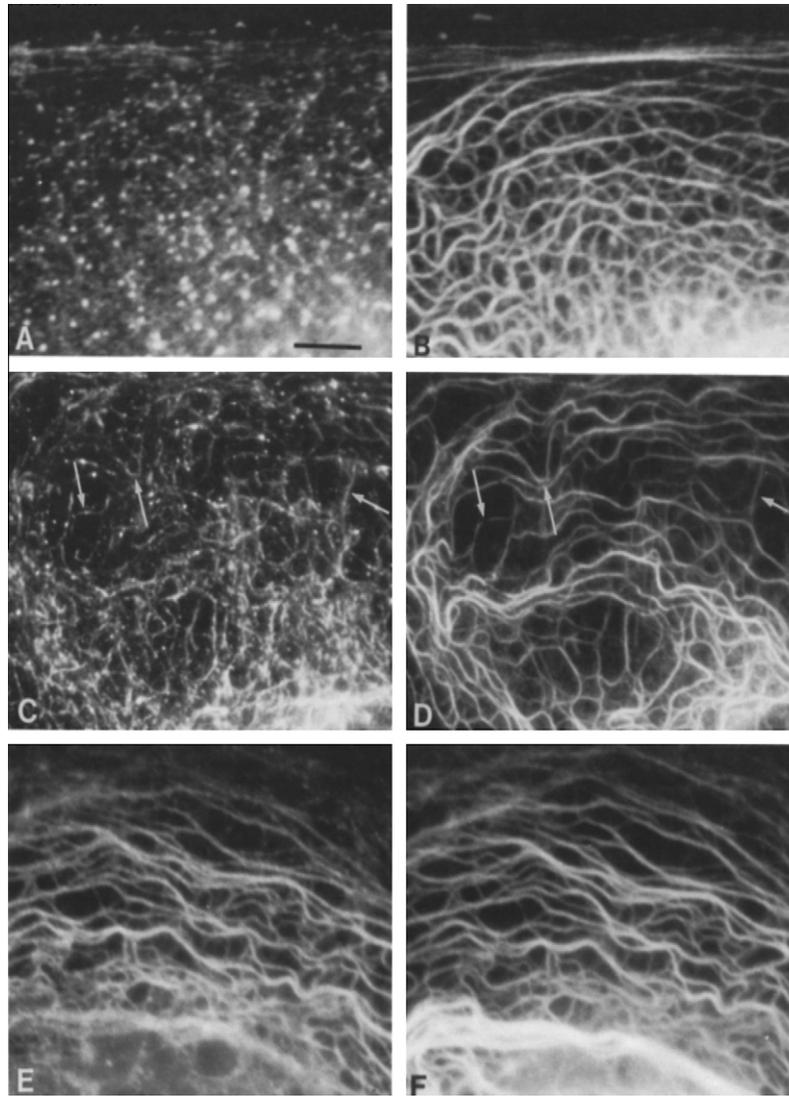


Fig. 5. Purified biotinylated Type I keratin was microinjected into PtK2 cells and cells were fixed and processed for immunofluorescence with anti-biotin (A, C and E) and anti-keratin (B, D and F) at different time intervals (20 min (A and B), 1 h (C and D) and 4 h (E and F) post-microinjection). Taken from reference Miller et al. (1991).

IF behavior and dynamics in live cells. Following microinjection the cells were incubated for ~ 8 h to ensure that a steady state had been achieved. At this time all of the exogenous protein was incorporated into the endogenous IF network. After photobleaching bars across single or multiple filaments, recovery of fluorescence was seen to take place equally all along the length of the bleach zone. Importantly these observations showed that IF were apolar in vivo and were capable of incorporating subunits anywhere along their long axes. Although it was difficult to determine the $t_{1/2}$ for full recovery due to background photobleaching effects, we did determine that significant recovery of fluorescence required more time than could be accounted for by simple diffusion (Vikstrom et al., 1992). Several years later we carried out similar FRAP analyses on cells expressing GFP-vimentin. This permitted us to determine that the $t_{1/2}$ for full recovery of vimentin was ~ 6 min (Yoon et al., 1998) (Fig. 6).

Another benefit of expressing GFP-tagged IF proteins was that cells could be observed by time lapse imaging for longer periods. The results of such observations demonstrated that IF constantly alter their shape and that individual fibrils which are in close proximity can move in opposite directions. These movements required energy and most types of movements were dependent

on microtubules or microfilaments. These live imaging studies also revealed that there were three distinct forms of vimentin in cells which we termed non-filamentous particles, short IF or squiggles and long IF. These three forms appeared to be interconvertible and most likely represented the different assembly states of IF in vivo and in fixed stained cells (Fig. 7). Importantly we showed that both the particles and squiggles were capable of moving at high rates of speed in cells. These rapid movements ($0.55 \pm 0.24 \mu\text{m/s}$) were bidirectional, typically tracking along microtubules. We also determined that these rapid movements required both kinesin and dynein (Helfand et al., 2002; Prahlad et al., 1998, 2000). These findings led us to propose that there is a mechanism for rapidly delivering and targeting IF precursors such as particles and squiggles to different regions of the cell for the assembly of IF networks. We speculated that this system might be responsible for regulating the structural and mechanical properties of different regions of cells (Helfand et al., 2003a). We were also able to show that a significant fraction of the non-filamentous particles of IF are formed co-translationally in a process we termed dynamic co-translation. This latter study demonstrated that multiple peripherin mRNAs (peripherin is a Type III IF protein) are clustered, typically at sites where peripherin is being synthesized. The

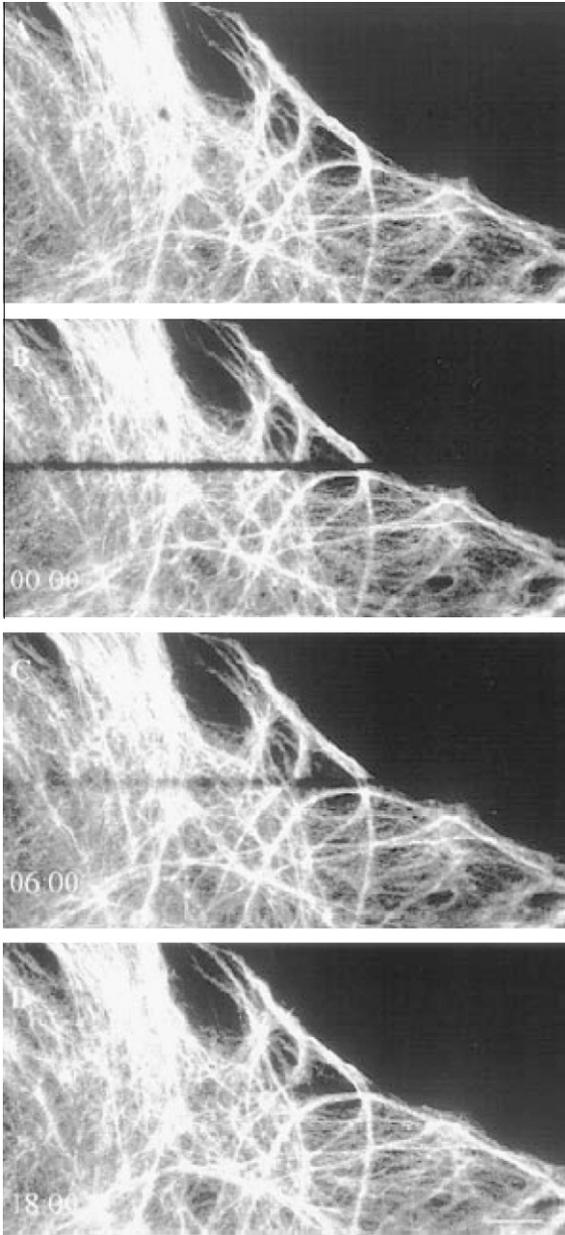


Fig. 6. A region of a BHK-21 cell expressing GFP-vimentin before (top) and immediately following photobleaching (00); 6 min after bleaching (06) and 18 min following bleaching (18). Figure is from reference Yoon et al. (1998).

synchronous synthesis of peripherin protein chains most likely reflects a mechanism for the formation of in-parallel and in-register coiled-coil dimers, the essential building blocks of IF (Chang et al., 2006).

7. The roles of IF in cell shape determination and maintenance

We have been interested in the roles of IF in cell shape determination and maintenance since the early 1970s. One of the most obvious experimental approaches for determining the role of IF in cell shape is to drive their disassembly *in vivo*. Unfortunately there were, and still are, no drugs available which specifically disassemble IF *in vivo*. Drugs such as colchicine and nocodazole for microtubules and cytochalasin B for microfilaments are not yet available for IF. This led us to explore a variety of alternative methods for causing their disassembly in cells. In collaboration with Peter Steinert we developed the use of a mimetic peptide derived

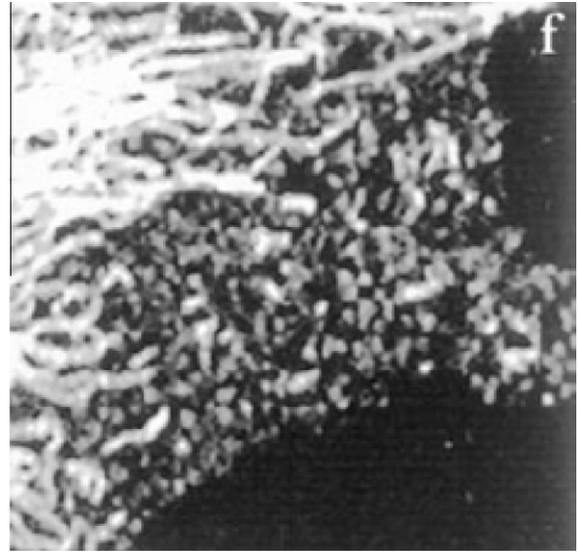


Fig. 7. A BHK-21 cell fixed and processed for immunofluorescence with anti-vimentin. Shown are different structural forms of IF including vimentin particles which give rise to squiggles or short IF and, through end domain interactions, form long IF. From reference Prahlad et al. (1998).

from the helix initiation region of the vimentin central rod domain, termed 1A. *In vitro* this 1A peptide drives the disassembly of IF into small oligomers and monomers most likely by binding to the same 1A sequences in the intact IF causing destabilization of the IF structure. Furthermore we showed that the 1A peptide has no impact on the assembly state of microtubules and actin filaments. When the 1A peptide is microinjected into live cells, it rapidly causes the disassembly of IF and coincident with this disassembly, fibroblasts dramatically alter their shape as they retract their processes and completely round up. The cells do not die, and within a few hours IF networks begin to reform and cells re-spread into the elongated shapes of fibroblasts. These experimental results support the central role of IF in maintaining the shape and mechanical integrity of cells (Goldman et al., 1996). A significant role for neuronal IF proteins in maintaining cell shape was also demonstrated by silencing expression of peripherin (Helfand et al., 2003b) and the Type III IF glial fibrillary acidic protein (GFAP), which showed that this IF system was required for the formation of astrocytic processes (Weinstein et al., 1991).

The cells of most adult tissues maintain well-defined shapes and positions with respect to their neighbors, and normally do not exhibit gross movements. Epithelial cells, for example, have specialized junctions holding them in tight apposition to form an effective barrier against mechanical stress. The keratin IF characteristic of these cells assembles into a network that is closely associated with specialized cellular adhesion sites (e.g., desmosomes, hemidesmosomes and focal adhesions) (Green and Goldman, 1986), and interacts with specific components of these structures (Bhattacharya et al., 2009; Gonzales et al., 2001). This network of keratin IF and cell–cell junctions contributes to the overall strength and stability of epithelial tissues (Fuchs, 1995; McLean and Lane, 1995). In contrast, various stages of morphogenesis are characterized by dramatic changes in the shape and motility of cells (Hay, 2005). This process, the epithelial to mesenchymal transition (EMT), involves a switch from expressing keratin IF to vimentin IF. Indeed vimentin expression in epithelial cells is a hallmark of the EMTs that take place both during development and in metastasis (Hay, 2005). We have long been intrigued by the expression of vimentin in epithelial cells and recently initiated studies to determine more precisely the functional significance of its expression in

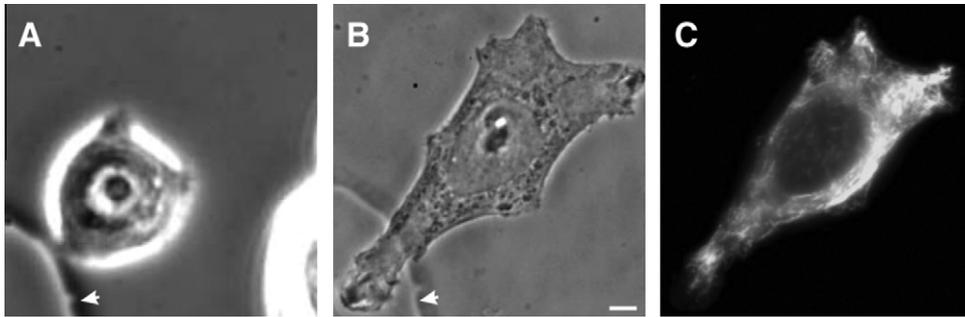


Fig. 8. An MCF-7 cell prior to (A) and following the microinjection of soluble vimentin. The cell was fixed and processed for immunofluorescence with vimentin antibody at 5 h. (A and B) are phase contrast images and (C) is the same cell as (B), but observed with fluorescence optics. From reference Mendez et al. (2010).

breast epithelial cells such as MCF-7 which express only keratin IF. We have found that the microinjection of vimentin into MCF-7 cells (or the expression of vimentin cDNA) caused rapid changes in cell shape (Fig. 8). Specifically, the more rounded epithelial cells became highly motile and mesenchymal in shape. This latter shape change involved the formation of a leading edge lamellipodium which typifies a locomoting mesenchymal cell (e.g., a motile fibroblast) (Mendez et al., 2010). When MCF-7 cells growing in colonies were transfected with vimentin, there was a rapid loss of desmosomes, a dramatic increase in focal adhesion dynamics and motility, as their epithelial morphology was converted into the typical leading-trailing edge polarity of mesenchymal cells. Conversely, silencing vimentin expression or altering the organization of VIF by the expression of a dominant negative mutant of vimentin in mesenchymal cells caused them to assume a more epithelial shape. These results demonstrate that the expression of vimentin IF in epithelial cells is much more than an epiphenomenon, but in fact helps to explain many of the morphological and behavioral changes seen during the EMT (Mendez et al., 2010).

8. Insights into the role of vimentin IF in cell motility

Based upon our insights into the role of vimentin IF in the EMT, we have recently initiated studies to delve more deeply into their specific roles in cell locomotion. These studies have been carried out in a close collaboration with the laboratories of Ueli Aebi and Harald Herrmann. The results to date demonstrate that vimentin IF play an important role in the formation of the lamellipodium, the locomotory structure located at the leading edge of migrating mesenchymal cells (e.g., fibroblasts). In non-motile or serum starved fibroblasts in which there are no lamellipodia, vimentin IF networks extend to and are closely associated with the cell membrane. Following serum addition vimentin is phosphorylated (e.g., Ser38 in the N-terminal domain) within seconds and this is accompanied by the retraction and disassembly of IF networks only at sites where lamellipodia form (Helfand et al., 2011). Lamellipod formation can also be induced by the local activation of a photoactivatable form of Rac1 in serum deprived cells (PA-Rac1 (Wu et al., 2009)). This local activation of PA-Rac1 results in the

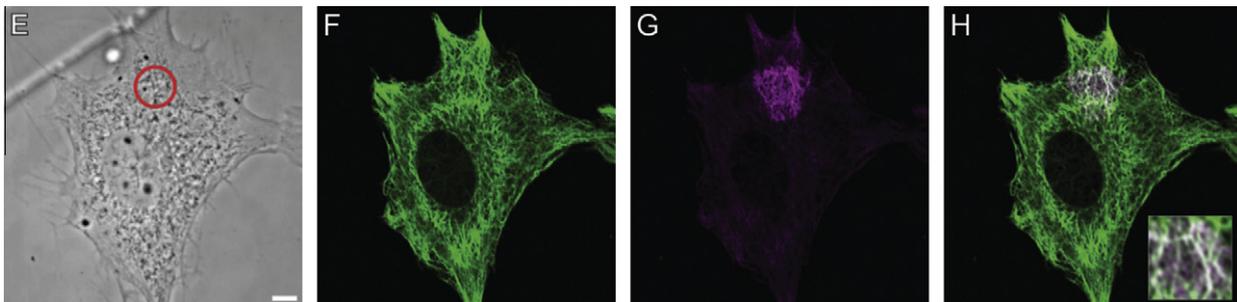


Fig. 9. A mouse embryo fibroblast (mEF) expressing PA-Rac1 before (E) and fixed within 5 s after spot irradiation. Subsequently this cell was processed for double label immunofluorescence with vimentin (green) and vimentin pSer38 (magenta) antibodies. Insert shows that this latter antibody stains vimentin IF only in the irradiated spot). Taken from reference Helfand et al. (2011).

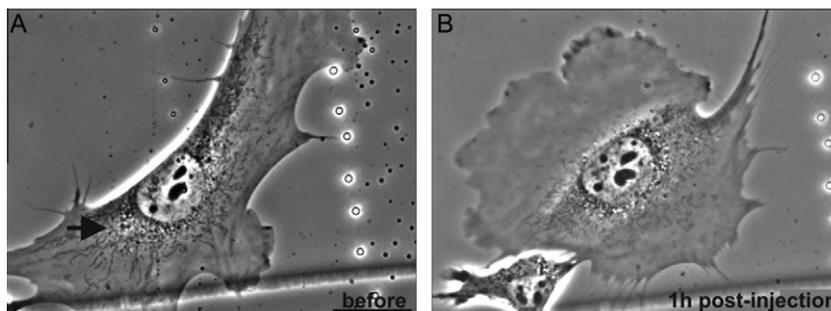


Fig. 10. Serum deprived live mEF before (A) and at 1 h following microinjection of peptide 2B2. The arrow shows site of injection where a lamellipodium formed (upper left). Taken from reference Helfand et al. (2011).

rapid (i.e., within 5 s) increase in vimentin phosphorylation only at the irradiation site (Fig. 9). Subsequently this leads to the local disassembly of vimentin IF where lamellipodia form (Helfand et al., 2011). We have also shown that the microinjection of a vimentin mimetic peptide (2B2) into serum deprived fibroblasts results in the local disassembly of vimentin IF, most likely into ULF, at sites of microinjection. Subsequently lamellipodia form at these same sites (Fig. 10). In addition, fibroblasts null for vimentin expression cannot engage in locomotory activity, despite the existence of lamellipodia over their entire surfaces (Helfand et al., 2011). Taken together these studies suggest that fully assembled VIF networks act as a ‘brake’ to inhibit membrane ruffling and the formation of lamellipodia. Conversely the disassembly of VIF appears to be required for the formation of lamellipodia.

9. Phosphorylation and the assembly states of vimentin IF

Our interest in the role of phosphorylation in regulating vimentin IF assembly and organization came from studies of BHK-21 cells undergoing mitosis. In this cell type the extensive networks of VIF present during prophase disassemble into aggregates or particles as cells transition from late prophase to metaphase. Coincident with this disassembly vimentin is hyperphosphorylated (Chou et al., 1989; Skalli et al., 1992). This phosphorylation is attributable to cdk1 (Chou et al., 1990) which phosphorylates vimentin at Ser55 (Chou et al., 1991). Interestingly, disassembly of the phosphorylated vimentin is dependent upon the presence of the intermediate filament protein nestin, which co-assembles with the vimentin (Chou et al., 2003). During interphase the turnover rate of vimentin phosphorylation appears to be very rapid since treating human fibroblasts or BHK-21 cells with phosphatase inhibitors such as okadaic acid, calyculin A and dinophsistoxin 1 (35-methylokadaic acid) results in a rapid increase in vimentin phosphorylation (Eriksson et al., 1992, 2004; Yatsunami et al., 1991). This is most likely attributable to the many kinases and phosphatases known to phosphorylate and dephosphorylate vimentin at numerous sites. A combination of in vivo and in vitro experiments have identified a large number of kinases including Protein Kinase A, Protein Kinase C, Calcium Calmodulin Kinase, p21-activated Kinase, Cdc2 Kinase, Rho-Kinase, Aurora-B, Polo-like Kinase and AKT Kinase which phosphorylate one or more of the 41 sites in vimentin identified to date (Eriksson et al., 2004; Goto et al., 1998; Izawa and Inagaki, 2006; Sihag et al., 2007).

In general, it appears that phosphorylation of vimentin subunits increases the off-rate and drives disassembly of IF, while dephosphorylation increases the on-rate and favors assembly of IF (Eriksson et al., 2004). The observed in vivo phosphorylation involving large numbers of sites provides for enormous complexity and very specific fine-tuning of the assembly states of vimentin. Likewise the kinases responsible for phosphorylation of vimentin are highly regulated and very sensitive to external chemical and mechanical stimuli like growth factors and shear stress (see below).

10. Mechanical properties of IF

The IF cytoskeleton plays an important role in the mechanical stability of cells and tissues that are subjected to external mechanical forces. We and our colleagues have studied the response of IF to shear stress in vascular endothelial and alveolar epithelial cells. Vascular endothelial cells modulate the physiological response to local changes in blood flow, or hemodynamic shear stress (Helmke, 2005; Helmke et al., 2000). Live cell imaging of GFP-vimentin expressing endothelial cells subjected to a shear stress of 12 dynes/cm² revealed that within 3 min the movement or displacement of vimentin IF significantly increased, suggesting a role

in intracellular mechanotransduction (Helmke et al., 2000, 2001). We also found that keratin IF exhibit significant displacements following the onset of shear stress in PtK2 cells (Yoon et al., 2001). In alveolar epithelial cells, the mesh size delineated by the keratin IF network is smallest near the nucleus and largest at the cell perimeter. After subjecting these cells to moderate shear stress, the peripheral keratin IF network mesh size decreases to approximate that seen in the perinuclear region. Particle tracking microrheology (PTM) measurements before and after shear stress showed that there was a significant increase in the stiffness of the KIF network following shear stress, especially in the peripheral regions of the cytoplasm (Sivaramakrishnan et al., 2008). We also found that shear stress caused non-filamentous keratin particles to become incorporated into IF, resulting in the formation of thicker bundles (tonofibrils) and that this assembly was also regulated by phosphorylation (Flitney et al., 2009). These results showed that the keratin IF networks function as an “intracellular structural scaffold” which provides cells with a mechanism to rapidly respond to and withstand external forces. IF are uniquely suited to this mechanical role, as it has been shown that their rheological properties are distinctly different from microtubules and microfilaments. For example, IF are much more flexible than either actin filaments or microtubules, but at the same time they are also the only cytoskeletal system capable of withstanding strains of greater than 100%, which is similar to those experienced by soft tissue cells during the deformations accompanying mechanically induced stress (Janmey et al., 1991). Moreover, as the mechanical stress is increased, IF exhibit strain stiffening properties. Elegant work by Aebi and colleagues has extended our understanding of these special mechanical properties of IF down to the level of individual filaments. Using atomic force microscopy they showed that a single filament could be stretched up to 2.5–3.5 times its resting length, and that this is accompanied by a decrease in the filaments width of >60% (Kreplak and Fudge, 2007; Kreplak et al., 2008). These findings suggest that IF are major determinants of the mechanical properties of cells.

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