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Invited Review

Cytoskeletal proteins connecting intermediate filaments to cytoplasmic and nuclear periphery

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Summary. Intermediate filaments (IFs), together with microtubules and microfilaments build up the cytoskeleton of most eukaryotic cells. Cytoplasmic IFs form a dense filament network radiating from the nucleus and extending to the plasma membrane. The association between the cytoplasmic and nuclear surfaces appears to provide a continuous link important for the organisation of the cytoplasm, for cellular communication, and possibly for the transport into and out of the nucleus. Cytoplasmic IFs approach the nuclear surface, thin fibrils seem to connect the IFs with the nuclear pore complexes and a direct interaction of cytoplasmic IFs with the nuclear lamin B has been observed by in vitro binding studies. However, none of the components that cross-link IFs to the nucleus has been unambiguously identified. Furthermore, if a direct interaction between cytoplasmic IFs and the nuclear lamin B occurs in vivo, the question of how cytoplasmic IFs get access to the nuclear interior remains to be resolved. The association of IFs with the plasma membranes involves different components, some of which are cell type specific. Two specialised complexes in epithelial cells: the desmosome and the hemidesmosome, serve as attachment sites for keratin filaments. Desmoplakin is considered as the cross-linking component of IFs to the desmosomal plaque, whereas BPAG1 (bullous pemphigoid antigen) would cross-link IFs at the hemidesmosomal plaque. In other cell types the modality of how IFs are anchored to the plasma membrane is less well understood. It involves different components such as the spectrin based membrane skeleton, ankyrin, myosin, plectin and certainly many other still unravelled partners. Association between the IFs and cellular membranes plays an important role in determining cell shape and tissue integrity. Thus, the identification and characterisation of the components involved in these interactions will be crucial for understanding the function of intermediate filaments.

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Introduction

Recent works demonstrate that intermediate filament (IF) networks contribute to maintain the structural integrity of the cell and provide mechanical strength for tissue (Steinert and Roop, 1988). Mutations in different keratin proteins have been identified as the cause of several tissue-specific human diseases (Fuchs, 1996; Fuchs and Cleveland, 1998). Such mutations resulted in a disorganised IF network that rendered cells very fragile and incapable of resisting any extracellular stress. The overall intracellular organisation of IFs with their interacting partners seems to be important for maintaining the shape and the plasticity of the cell.

Intermediate filaments (IFs) constitute major cytoskeletal components of the eukaryotic cytoplasm and of the nuclear lamina. Depending on cell type and developmental state, IFs can be assembled from single intermediate filament proteins (IFPs) or from a combination thereof. IFPs constitute a multigene family whose members can be grouped into six categories (I-VI) (Stewart, 1990; Fuchs and Weber, 1994). In contrast to the other two filament systems, IFs are built from a multitude of developmentally regulated and differentiation-specific subunits in such a way that the conserved α-helical rod domains of the subunits constitute the filament body, and the non conserved, non α-helical terminal polypeptide regions are largely exposed on the filament surface (Steinert and Roop, 1988). The surface regions vary in size and sequence and correspond to different biochemical properties, as well as to different interactions with components and structures characteristic of developing and terminally differentiated cells (Steinert et al., 1985).

In a cellular context, cytoplasmic IFs are radially distributed from the nuclear membrane towards the cell surface (Goldman et al., 1985). This implies site-specific recognition between IF subunits, and binding to specific proteins of the different cellular structures. Analysis of

IF function is difficult because of the complexity of its interaction partners. IFs have been shown to interact with the plasma membrane, nuclear envelope, mitochondria, microtubules (Georgatos and Maison, 1996), and actin filament bundles (Goldman et al., 1986). Interactions of IFs with the plasma membrane occur at specialised attachment sites, focal adhesions, desmosomes and hemidesmosomes. A new family named plakin consisting of desmoplakin, bullous pemphigoid antigen 1, envoplakin and plectin, is thought to mediate IF association with contact structures at the plasma membrane (Schmidt et al., 1994; Barradoti and Sonnenberg, 1996). Most of these proteins are expressed in different tissue and cell types, therefore suggesting different modes of attachment for the IFs depending on the subset of IFPs expressed in a cell type according to its developmental and differentiation stage.

IFs interact with microtubules and microfilaments

The IF-microtubule interaction was unravelled long ago. In most vimentin-containing cells, vimentin filaments form an extended network that stretches from the vicinity of the nucleus to the cellular periphery. This network of IFs appears to be established primarily through interaction with microtubules (Geiger and Singer, 1980). Depolymerization of the microtubule system leads to a collapse of the vimentin network into a dense coil near the cell centre (Blose et al., 1984). This phenomenon suggests a dependence of IF network organisation upon the integrity of the microtubule network, implying a physico-chemical linkage between the two systems. Structural evidence for the existence of cross-bridges between the two cytoskeletal elements has been shown for the neurofilament IFs where MAP2 and kinesin have been implicated (Hirokawa, 1982; Leterrier et al., 1982; Gyoeva and Gelfand, 1991). Interaction between IFs and microfilaments is generally apparent only upon drug disruption of the microtubule system. In the absence of microtubules, IFs undergo a microfilament-dependent centripetal collapse,which requires energy (Tint et al., 1991). Currently, we assume that IFs are dragged outward through interactions with microtubules, and pulled inward through interactions with the microfilament system. Recent electron microscopy studies have revealed 2 nm linking elements connecting all three cytoskeletal systems. Plectin was identified as a versatile cross-linker (Svitkina et al., 1996). Furthermore, plectin has a wide distribution among tissues and species, and is able to interact with different types of IFs such as vimentin, desmin, peripherin and neurofilament proteins, as well as with itself (Foisner and Wiche, 1991; Errante et al., 1994). In vitro interactions showed also that plectin binds to the α-helical rod domain of vimentin (Foisner et al., 1988). Interestingly, in cells lacking IFs, plectin is capable of cross-linking cytoskeletal structures independently of the IFs (Svitkina et al., 1996). Plectin may enable sufficient cross-linking of the cytoplasm to itself and therefore may play a key role in maintaining tissue integrity. This could explain the absence of obvious phenotypes in null vimentin mice (Colucci-Guyon et al., 1994).

Connections of the IFs to the nuclear envelope

The nuclear envelope comprises three distinct regions: the outer nuclear membrane, the inner nuclear membrane and the nuclear pore complexes. The nuclear pore complexes are inserted into the nuclear membranes where the inner and outer membranes merge to form a pore. The outer nuclear membrane is continuous with the endoplasmic reticulum. The inner membrane faces the nucleoplasm and is linked to the nuclear lamina (Aebi et al., 1986). The lamina is a thin fibrous structure composed of the nuclear lamins type A and B (Nigg, 1989) which appear to constitute a major structural framework for the nuclear envelope (Gerace and Blobel, 1980; Aebi et al., 1986). The nuclear lamins bind directly to chromosomes (Glass et al., 1993), polynucleosomes, matrix-associated DNA and core histones (Taniura et al., 1995). Several integral membrane proteins of the inner nuclear membrane are assumed to interact directly with the nuclear lamina (Georgatos et al., 1994): the lamin B receptor (LBR) (Worman et al., 1988) and the lamin-associated polypeptides (LAPs) (Foisner and Gerace, 1993). The LAPs (LAP1 A, LAP1 B, LAP1 C and LAP2) are typically integral membrane proteins with a single trans-membrane domain. LAP1 A and B represent splicing variants of LAP1 C (Martin et al., 1995). The LAPs bind directly to lamin para-crystals under in vivo conditions; LAP1 A and 1 B interact with all lamin types, while LAP2 associates exclusively with B-type lamins (Foisner and Gerace, 1993). LAP1 C binds to lamin A under in vivo conditions (Powell and Burke, 1990). The LBR is assumed to have eight membrane spanning segments, and a large positively charged amino-terminal domain facing the nucleoplasm that exhibits both lamin B and DNA binding activity (Worman et al., 1990). LBR associates with at least two other proteins: p18 and p34 (Georgatos et al., 1994).

There is one other group of nuclear envelope components with which the lamina might interact, namely the nuclear pore complexes. Structural studies as well as biochemical experiments clearly indicate such an association (Aebi et al., 1986; Goldberg and Allen, 1992). This latter association must involve, at the very minimum, B-type lamins, as these are the only members of the lamin family ubiquitously expressed in vertebrate somatic cells. However, no nuclear pore complex proteins which exhibit lamin-binding activity have been identified so far. Furthermore, several recent publications have reported the existence of lamins which are not associated with the envelope within the nuclear interior (Hozak et al., 1995).

Several electron microscopy studies refer to an association of the cytoplasmic IFs with the nuclear envelope in a variety of cells (Metuzals et al., 1988). The IFs are seen to loop and follow the nuclear periphery or

to connect with the nuclear pore-complex (Carmo-Fonseca et al., 1987). The fact that IFs are attached to the nucleus is further suggested by their persistence after nuclei isolation (Staufenbiel and Deppert, 1982). In vitro binding studies using isolated vimentin, desmin, and avian erythrocyte nuclear membrane, have revealed the existence of IF attachment sites along the nuclear envelope (Georgatos et al., 1987). The binding was localised to the carboxy-terminal tail domain of the type III IFs and the nuclear receptor was identified as lamin B (Georgatos and Blobel, 1987; Djabali et al., 1991). In addition, rabbits immunised with a synthetic peptide representing the proximal part of the carboxy-terminal region of peripherin, which is required for lamin B binding in vitro, produce anti-idiotypic antibodies that recognise lamin B (Djabali et al., 1991). These results suggest that lamin B is a physiological receptor for IFs at the nuclear envelope. However, there still is a topographical problem to be resolved in order to explain how cytoplasmic IFs associate with the nuclear lamins. Some IFs may traverse nuclear pore complexes since these are the only known channels between the nuclear interior and the cytoplasm. Ultrastructural studies at sites where IFs interact with the nuclear envelope will be required for us to understand how IFs are anchored. Immunofluorescence microscopy of cultured cells reveals a close association of plectin with the nuclear surface (Herrmann and Wiche, 1983). Solid phase binding assays show that plectin binds specifically to lamin B and not to lamin A and C. Phosphorylation of plectin and lamin B by different protein kinases significantly decreases the binding property between these two proteins (Foisner et al., 1991). These results may indicate that during mitosis, since lamins become hyperphosphorylated (Franke, 1987), the association between plectin and lamin B is disrupted which would suggest a highly dynamic mode of interaction between theses two partners. In addition to the binding between plectin and lamin B, binding between cytoplasmic IFs and lamin B, may also indicate an alternative link between cytoplasmic IFs and the nuclear lamin B. These different interactions are possible because plectin is a very elongated molecule self assembling into various shapes (Foisner and Wiche, 1987), and therefore may be able to pass through the nuclear pore and cross-link the IFs from the different compartments. It is noteworthy that cells lacking cytoplasmic IFs exhibit abnormal nuclear morphology (Serria et al., 1994). In these cells, the nucleus appears irregular with prominent folding or invaginations, while cells containing normal IF networks exhibit a uniform and smooth nucleus. These observations clearly imply an important role of the cytoplasmic IFs in positioning and maintaining the integrity of the nuclear envelope and, the shape of the nucleus.

Our current knowledge is compatible with four alternative ways in which IFs could be anchored to the nuclear envelope (Fig. 1). 1) Cytoplasmic IFs may interact directly with the nuclear lamin B located on the inner surface of the nuclear envelope. However, a topological problem will have to be resolved to understand how the cytoplasmic IFs can enter the nucleus in order to interact with the nuclear lamin B. Either the IFs somehow cross the nuclear envelope to get access to the inner surface of the nuclear envelope or they get access by entering through the nuclear pore complexes. 2) A crosslinking component, plectin, may mediate the interaction between cytoplasmic IFs and the nuclear envelope. 3) Components of the nuclear pore complex exposed to the cytoplasmic face may be responsible for the anchoring of IFs. So far, none of these elements has been reported apart from the existence of fibrils extending from the surface of the nuclear pore to the cytoplasm (Wiese and Wilson, 1993). 4) Another scenario could be the existence of IF associated-proteins localised on the extra-cellular nuclear membrane which would provide a direct link to the cytoplasmic IFs. Which of these four scenarios is true, remains to be established. Their respective research will certainly provide important insight into the function of IFPs.

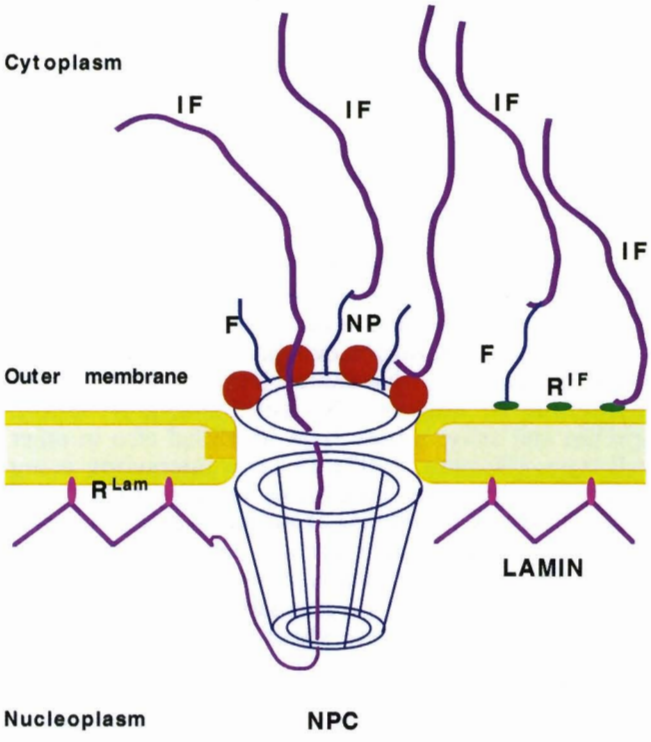

Fig. 1. Drawing of the nuclear envelope, sketching how IFs could connect the nucleus. IFs could interact with the nuclear envelope in four possible ways: 1) IF interacts with the lamin B by crossing the nuclear pore; 2) IF interacts with 2-nm fibrils exposed at the cytoplasmic surface of the nuclear pore; 3) IF interacts with cytoplasmic components of the nuclear pore complex; and 4) IFs are anchored to the nuclear membrane via specific interactions with components associated to the outer nuclear membrane. IF: intermediate filament; F: 2 nm fibrils; Rlam: receptor for lamins; RIF: receptor for IF; NP: cytoplasmic components of the nuclear pore; NPC: nuclear pore complex.

Connections between IFs and the cytoplasmic membrane

The identification of the modality of IF interactions to the plasma membrane is complex because it involves several cytoskeletal components: actin filaments, microtubules and other membrane-associated proteins such as integrins, spectrin, plectin, α-actinin (Fig. 2). Specific interactions between the cytoskeleton and membrane-associated components are observed when capping of cell surface receptors occurs, or when cells become adherent to a substratum and when cell-cell contacts are formed (Lee et al., 1988). The mechanism by which such special regions are organised indicates a highly dynamic process. A central role in assembly and maintenance of cell adhesions is attributed to the submembrane plaques of cell-cell and cell-extracellular contacts that link the adhesion receptors to the actin cytoskeleton (Bershadsky et al., 1995). These structures consist of protein complexes that are specific either to cell-extracellular matrix junctions (such as talin, paxillin) or to cell-cell junctions (α- and ß-catenin, and plakoglobin) or that are shared by both types of adhesion (vinculin, α-actinin, zyxin and tensin) (Ben-Ze'ev, 1997) . Thus, actin filaments appear to be a key element in linking IFs to the plasma membrane.

IF interactions in non-specialised areas of the cytoplasmic membrane

Studies of the erythrocyte membrane provided the first isolation of the putative element involved in linking IFs to cortical actin. The major constituents of the submembranous erythrocyte cytoskeleton are α- and ß-spectrin forming a filamentous lattice attached to the membrane by ankyrin (Bennett, 1985). Recently, spectrin and ankyrin have been identified also in other cell types (Bennett, 1992). In vitro interaction using inverted erythrocyte membrane vesicles shows that vimentin binds to ankyrin (Georgatos and Marchesi, 1985), and that the amino-terminal domains of vimentin and desmin specifically bind to ankyrin in overlay assays (Georgatos et al., 1987). Association of vimentin and desmin with erythrocyte spectrin is also observed (Langley and Cohen, 1987). Brain spectrin binds neurofilament proteins to a greater extent than does erythrocyte spectrin. Plectin is also reported to bind to spectrin (Herrmann and Wiche, 1987). Moreover, plectin interacts with several IF subunits (Foisner et al., 1988) and may in fact mediate the linkage of IFs to spectrin and thus to the actin cell cortex. This possibility is supported by previous experiments showing that microinjection of anti-spectrin antibodies into fibroblasts results in a collapse of the IF network (Mangeat and Burridge, 1984). In conclusion, the emerging picture is that interactions between IFs and submembrane cortex involve three molecules: ankyrin, spectrin and plectin.

IF associations at cell-cell junctions

Epithelial cells exhibit two major types of adhering junctions: adherens junctions anchoring actin microfilaments and desmosomes anchoring intermediate filaments to the plasma membrane (Koch and Franke, 1994; Schmidt et al., 1994). In adherens junctions, intracellular adhesion is caused by classical cadherins such as E- or N-cadherin (Geiger and Ayalon, 1992). Cadherins represent a distinct family of single-transmembrane domain glycoproteins. They form dimers in the plane of the membrane which in turn connect to those from opposing cells (Shapiro et al., 1995). The carboxyl-terminal cytoplasmic domain of cadherins interacts directly with the central region of the two related proteins plakoglobin and ß-catenin (Ozawa et al., 1989; Sacco et al., 1995). Furthermore, these proteins interact with α-catenin which then connects the membrane-associated complex to the actin cytoskeleton either directly or indirectly by association with α-actinin (another actin-binding protein) (Knudsen et al., 1995; Huber et al., 1997). IFs may be linked directly to the adhering junction, since actin can bind the vimentin tail domain, or indirectly by involving a cross-linking element such as plectin (Cary et al., 1994; Svitkina et al., 1996). However, the in situ localisation of the association between IFs and components of the adhering junction remains to be established.

IFs are anchored at desmosomes, which are constituted by desmosome-specific cadherins: desmocollins and desmogleins (Koch and Franke, 1994; Chidgey, 1997). Desmosomal cadherins are glycosylated type I transmembrane proteins. Their homology to classical cadherins is strongest in their extracellular domains. Intracellular plaques of both types of adhering junctions contain one common component: plakoglobin. In desmosomes, plakoglobin specifically interacts with desmocollin and desmoglein but not with α-catenin (Chitaev et al., 1996). Furthermore, desmocollin binds desmoplakins, which are cytoplasmic proteins of 220 kDa and 250 kDa derived from the same gene by alternative splicing. The carboxyl-terminal domain of desmoplakin interacts with the amino-terminal region of the basic keratins as well as with vimentin (Kouklis et al., 1994). Therefore, desmoplakin provides a direct link between the IFs and the desmosomal plaque. However, other components are probably also involved in the interaction with IFs since experiments based on the overexpression of the carboxyl-terminal domain of desmoplakin produced only a partial collapse of the IFs from the desmosome (Stappenbeck and Green, 1992). An additional linker to IFs may be Band 6 or plakophilin, which binds to keratin and interacts in a similar fashion as plakoglobin with the same central region of desmoglein (Hatzfeld et al., 1994; Marthur et al., 1994).

IF interactions to cell surface junctions

Hemidesmosomes are multiprotein complexes that

mediate the adhesion of epithelial cells to the underlying basement membrane and connect elements of the cytoskeleton to the extracellular matrix (Green and Jones, 1996). The keratin filaments are anchored to the cell surface at sites of the cytoplasmic plaques that are associated with hemidesmosomes. The plaque components involve at least two proteins: Bullous pemphigoid antigen 1 (BPAG1) (Stanley et al., 1988), and plectin (Foisner and Wiche, 1991). Studies demonstrate that both proteins are located at the cytoplasmic surface of the hemidesmosomes, where keratins are inserted (Jones et al., 1994). Furthermore, BPAG1 shares sequence homology with plectin and desmoplakin (Tanaka et al., 1991), and BPAG1 binds in vitro to keratin through their carboxyl-terminal domains (Yang et al., 1996). In conclusion, the interaction of keratins with hemidesmosomes is mediated by BPAG1 and plectin.

Connections of the neuronal intermediate filament proteins

In epidermal tissues, several studies have characterised specific sites of attachment for keratin IFs. However, little is known on how neurofilaments (NFs) are anchored to the cytoplasmic surface of neurons. The neuronal cytoskeleton provides a framework that defines, supports, and maintains the shape of neurons. A highly crosslinked network of microtubules, neurofilaments, and specific associated proteins forms the major cytoplasmic structural units of the neuronal cell body, the dendrites and the axons. The NFs are composed of three proteins: NF-L, NF-M, NF-H. These proteins appear to be synthesised in the perikaryon region of the cell and are slowly transported down to the peripheral axonal processes. The NF proteins assemble as obligate heteropolymers, in which NF-M and NF-H are anchored to a core of NF-L through their central domains (Hirokawa et al., 1984). The tail domains of the NF proteins are rich in charged amino acids, particularly glutamic acid. The charged tails of NF-M and NF-H protrude from the filament core and form at least a portion of the fibrous interconnections between parallel neurofilaments and microtubules in axons (Hirokawa et al., 1984). NF proteins are highly phosphorylated; the level of phosphorylation varies as they are transported from the cell body to the peripheral axon (Nixon et al., 1994). Studies using monoclonal antibodies have distinguished phosphorylated and non-phosphorylated epitopes on the NFs. When these antibodies were applied to sections of rat brain, it was shown that certain nerve cell bodies, their dendrites and the portion of the proximal axon possessed non phosphorylated neurofilaments, and that long fibers, including terminal axons, contained phosphorylated neurofilaments (Sternberger and Sternberger, 1983). In addition, the state of phosphorylation of NF proteins was directly correlated to the packing of the NFs and to the axonal caliber (Nixon et al., 1994).

Interaction between neurofilament proteins and the other cytoskeletal elements

In vitro studies have identified some putative components that could be implicated in the association between neuronal IFs and the cell periphery. Cross-linking components between NFs and microfilaments have recently been identified. In addition to plectin (Foisner and Wiche, 1991), another IF-microfilament cross-bridging protein has recently been revealed. The dystonin gene has been identified to be responsible for the mouse neurodegenerative disease dystonia musculorum (Brown et al., 1995) and as being an isoform of the epidermal BPAG1e. Furthermore, the ablation of the BPAG1 gene in mice produced rapid sensory neuron degeneration identical to the symptoms of dystonia musculorum (Guo et al., 1995). These observations lead to the conclusion that epidermal BPAG1e and its two neuronal variants BPAG1n1 and BPAG1n2 (also called dystonins) are alternatively spliced products of the same gene (Yang et al., 1996). The neuronal BPAG1 has the same sequence in the rod and carboxyl-terminal domain as the epidermal isoform, while the amino-terminal domain is more extended and contains an actin-binding site, absent in BPAG1e (Brown et al., 1995). Furthermore, immunohistochemistry shows that BPAG1n is found predominatly in axon termini, and not in the cell body of sensory neurons

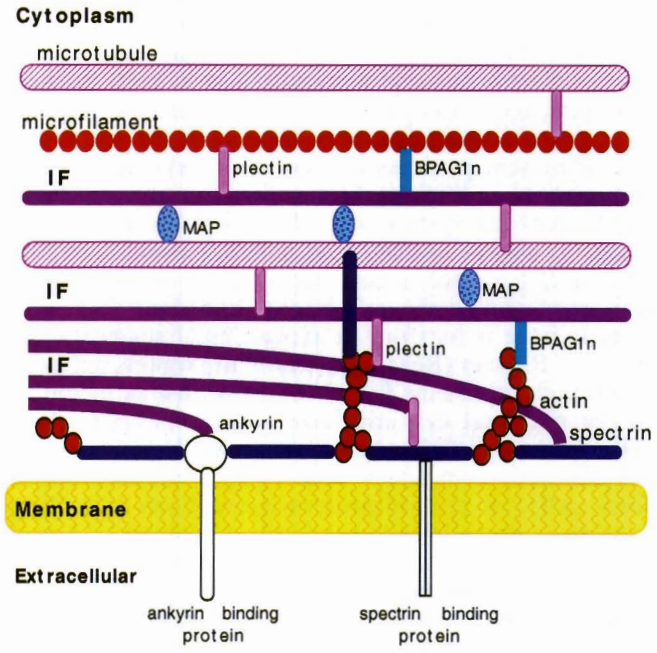

Fig. 2. Summary of several IF interactions in the neuronal context. IFs interact with the microtubule and microfilament systems. IFs can be anchored to the cell periphery by associations with different cytoskeletal components such as: 1) interaction with ankyrin; 2) interaction with spectrin; and 3) interaction with the cortical actin mediated by plectin or BPAG1n.

(Yang et al., 1996), whereas similar studies, in BPAG1n knockout mice revealed that NF networks were disorganised and that axonal degeneration was accompanied by a reduction of NFs, especially in areas close to the axonal membrane (Yang et al., 1996).

Possible candidates for linking NFs to the cell periphery

Brain spectrin has been localised and found associated to the plasmalemma in cell bodies, dendrites, axons, and nerve terminals (Kordeli et al., 1986). Spectrin is an essential component of the membrane-related cytoskeleton and links actin filaments to the plasma membrane. The spectrin cytoskeleton is attached to the plasma membrane by membrane proteins such as ankyrin (Bennett, 1992) or by association between several integral membrane proteins such as neuronal cell adhesion molecule (N-CAM 180) (Pollerberg et al., 1987). Furthermore, several in vitro studies using purified spectrin show the association of spectrin with F-actin, the microtubule-associated protein Tau, and with neurofilament protein NF-L (Carlier et al., 1984; Frappier et al., 1987). The multiplicity of membrane-associated domains in spectrin, together with the variability of spectrin ligands, may be responsible for selective targeting of spectrin to functionally distinct membrane domains and may therefore provide specific anchoring sites for the NFs to specialised domains of the peripheral membrane.

In vitro assays demonstrate that ankyrin binds specifically the amino-terminal domain of vimentin and desmin (Georgatos et al., 1987). Similar assays have provided evidence that ankyrin could as well interact with peripherin, a neuronal IF of the same type (Djabali et al., unpublished). However, binding of ankyrin to the neurofilament proteins has not been reported so far. Interestingly, multiple isoforms of ankyrin are expressed in the nervous system with diversity due to distinct genes, as well as to alternative splicing of mRNAs. The ankyrin B is located in neuronal processes while ankyrin R is confined to the cell bodies and dendrites; and ankyrin node is localised at axonal initial segments and nodes of Ranvier (Bennett, 1992). Furthermore, ankyrin-binding glycoproteins have been identified as members of the neuronal cell adhesion molecules (N-CAMs) (Davis et al., 1993). Associations of N-CAMs with ankyrin, and of ankyrin to NFs, may provide an example for a series of protein-protein interactions extending from the extracellular space to the cytoplasm and may therefore constitute a novel signalling pathway.

Figure 2 summarises different possible models of how NFs could be attached to the cell periphery. These models have been hypothesised based on the different components identified as cross-linking elements for IFs and the plasma membrane which have been essentially provided by studies of epithelial cells and erythrocyte membranes. Based on these findings, I propose three alternative ways that may be implicated in NF-cytoplasmic membrane interactions. 1) NFs could directly interact with spectrin which has been shown to be associated with the cortical cytoplasm of cell bodies, dendrites, and axons (Levin and Willard, 1981). Furthermore, spectrin could also cross-link the NFs between themselves, as well as to the microfilament system. 2) NFs could associate with ankyrin, a structural protein located on the cytoplasmic surface of the plasma membrane (Bennett, 1992). Moreover, ankyrin exhibits recognition sites for integral membrane proteins, which may contribute to specific regions of distribution in neurons and thereby, may contribute to the formation of specific multi-protein complexes for NF attachment. 3) Another alternative could be that NFs are indirectly linked to the plasma membrane. NFs could be cross-linked through associated proteins such as plectin and /or BPAG1n. These proteins have been shown to interact with NFPs (Foisner and Wiche, 1991; Yang et al., 1996). Furthermore, plectin and BPAG1n have been implicated in crosslinking NFs to the microfilament system.

Conclusion

The exact function of intermediate filaments still remains enigmatic. However, in the case of defective genes encoding some of the IFPs, it has been demonstrated that they are responsible for degenerative diseases (Fuchs, 1996). The intra-cytoarchitectural organisation of IFs and their mode of interaction with specific components may provide further insight into their function. For example, in epithelial cells, keratin proteins are connected to hemidesmosomes that are integrin-mediated adhesive junctions. Furthermore, the keratin IFs are also attached to desmosomes, that are cadherin-mediated cell-cell junctions. Both multi-protein complexes have been implicated in cell signalling events. These findings may suggest that IFs can act as signal transducers, relaying information from the extracellular matrix, or from cell-cell adhesion to the nucleus, which would indicate a new dynamic property of these IFPs. To date, several cross-linking proteins have been characterised, and some of them have been identified as important in the intracellular organisation of the IF network. It is now clear that an intact and well organised cytoplasmic IF is required for the cell to maintain its integrity. Identification and characterisation of the components involved in IF arrangement would probably help in the near future to unravel the real physiological role of the different IF subunits.

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References

Aebi U., Cohn J.B. and Gerace L.L. (1986). The nuclear lamina is a meshwork of intermediate-type filaments. Nature 323, 560-564.

Barradoti L. and Sonnenberg A. (1996). Hemidesmosomes: roles in adhesion, signaling and human diseases. Curr. Opin. Cell Biol. 8, 647-656.

Ben-Ze'ev A. (1997). Cytoskeletal adhesion proteins as tumor suppressors. Curr. Opin. Cell Biol. 9, 99-108.

Bennett V. (1985). The membrane skeleton of human erythrocyte and its implications for more complex cells. Annu. Rev. Biochem. 54, 273-304.

Bennett V. (1992). Ankyrins: adaptors between diverse plasma membrane proteins and cytoplasm. J. Biol. Chem. 267, 8703-8706.

Bershadsky A.D., Yehuda-Levenberg S. and Geiger B. (1995). Molecular interactions in the submembrane plaque of cell-cell and cell-matrix adhesions. Acta. Anat. 154, 46-62.

Blose S.H., Meltze D.I. and Feramisco J.R. (1984). 10-nm filaments are induced to collapse in living cells microinjected with monoclonal and polyclonal antibodies against tubulin. J. Cell Biol. 98, 847-859.

Brown A., Bernier G. and Mathieu M.R. and J. Kothary R. (1995). The mouse dystonia musculorum gene is a neuronal isoform of bullous pemphygoid antigen 1. Nature Gen. 10, 301-306.

Carlier M.F., Simon C., Cassoly R. and Pradel L.A. (1984). Interaction between microtubule-associated protein tau and spectrin. Biochim. 66, 305-311.

Carmo-Fonseca M., Cidadao A.J. and David-Ferreira D.F. (1987). Filamentous cross-bridges link intermediate filaments to the nuclear pore complexes. Eur. J. Cell Biol. 45, 282-290.

Cary R.B., Klymkowsky M.W., Evans R.M., Domingo A., Dent J.A. and Backhus L.E. (1994). Vimentin's tail interacts with actin-containing structures in vivo. J. Cell Sci. 107, 1609-1622.

Chidgey M.A.J. (1997). Desmosomes and disease. Histol. Histopathol. 12, 1159-1168.

Chitaev N.A., Leube R.E., Troyanovsky R.B., Eshkind L.G. and Franke W.W. (1996). The binding of plakoglobin to desmosomal cadherins: patterns of binding sites and topogenic potential. J. Cell Biol. 133, 359-369.

Colucci-Guyon E., Portier M.-M., Dunia I., Paulin D., Pournin S. and Babinet C. (1994). Mice lacking vimentin develop and reproduce without an obvious phenotype. Cell 79, 679-694.

Davis J.Q., McLaughlin T. and Bennett V. (1993). Ankyrin-binding proteins related to nervous system cell adhesion molecules: candidates to provide transmembrane and intercellular connections in adult brain. J. Cell Biol. 121, 121-133.

Djabali K., Portier M.-M., Gros F., Blobel G. and Georgatos S.D. (1991). Network antibodies identify nuclear lamin B as a physiological attachment site for peripherin intermediate filaments. Cell 64, 109-121.

Errante L.D., Wiche G. and Shaw G. (1994). Distribution of plectin, an intermediate filament-associated protein, in the adult rat central nervous system. J. Neurosci. Res. 37, 515-528.

Foisner R. and Gerace L. (1993). Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation. Cell 73, 1267-1279.

Foisner R. and Wiche G. (1987). Structure and hydrodynamic properties of plectin molecules. J. Mol. Biol. 198, 515-531.

Foisner R. and Wiche G. (1991). Intermediate filaments-associated proteins. Curr. Opin. Cell Biol. 3, 75-81.

Foisner R., Leichtrfried F.E., Herrman H., Small J.V., Lawson D. and Wiche G. (1988). Cytoskeletion-associated plectin: In situ localization, in vitro reconstitution, and binding to immobilized intermediate filament proteins. J. Cell Biol. 106, 723-733.

Foisner R., Traub P. and Wiche G. (1991). Protein kinase A- and protein Kinase C-regulated interaction of plectin with lamin B and vimentin. Proc. Natl. Acad. Sci. USA 88, 3812-3816.

Franke W.W. (1987). Nuclear lamins and cytoplasmic intermediate filament proteins: a growing multigene family. Cell 48, 3-4.

Frappier T., Regnouf F. and Pradel L.A. (1987). Binding of brain spectrin to the neurofilament subunit protein. Eur. J. Biochem. 169, 651-657.

Fuchs E. (1996). The cytoskeleton and disease: genetic disorders of intermediate filaments. Annu. Rev. Genet. 30, 197-231.

Fuchs E. and Cleveland D.W. (1998). A structural scaffolding of intermediate filaments in health and disease. Science 279, 514-519.

Fuchs E. and Weber K. (1994). Intermediate filaments: structure, dynamics, function, disease. Annu. Rev. Biochem. 63, 345-382.

Geiger B. and Ayalon O. (1992). Cadherins. Annu. Rev. Cell Biol. 8, 307-332.

Geiger B. and Singer S.J. (1980). Association of microtubules and intermediate filaments in chicken gizzard cells as detected by double immunofluorescence. Proc. Natl. Acad. Sci. USA 77, 4769-4773.

Georgatos S.D. and Blobel G. (1987). Lamin B constitutes an intermediate filament attachment site at the nuclear envelope. J. Cell Biol. 105, 117-125.

Georgatos S.D. and Maison C. (1996). Integration of intermediate filaments into cellular organelles. Int. Rev. Cytol. 164, 91-138.

Georgatos S.D. and Marchesi V.T. (1985). The binding of vimentin to human erythrocyte membranes: A model system for the study of intermdiate filament-membrane interactions. J. Cell Biol. 100, 1955-1961.

Georgatos S.D., Meier J. and Simons G. (1994). Lamins and lamin-associated proteins. Curr. Opin. Cell Biol. 6, 347-353.

Georgatos S.D., Weber K., Geisler N. and Blobel G. (1987). Binding of two desmin derivatives to the plasma membrane and the nuclear envelope of avian erythrocytes: Evidence for a conserved site-specificity in intermediate filament-membrane interactions. Proc. Natl. Acad. Sci. USA 84, 6780-6784.

Gerace L. and Blobel G. (1980). The nuclear envelope lamina is reversibly depolymerized during mitosis. Cell 19, 277-287.

Glass C.A., Glass J.R., Taniura H., Hasel K.W., Blevitt J.M. and Gerace L. (1993). The α-helical rod domain of human lamins A and C contains a chromatin binding site. EMBO J. 12, 4413-4423.

Goldberg M.W. and Allen T.D. (1992). High resolution scanning electron microscopy of the nuclear envelope: demonstration of a new, regular, fibrous lattice attached to the baskets of the nucleoplasmic face of the nuclear pores. J. Cell Biol. 119, 1429-1440.

Goldman R.D., Goldman A., Green K., Jones J., Lieska N. and Yang H.Y. (1985). Intermediate filaments: possible functions as cytoskeletal connecting links between the nucleus and the cell surface. Ann. NY Acad. Sci. 455, 1-17.

Goldman R.D., Goldman A., Green K., Jones J., Lieska N. and Yang H.Y. (1986). Intermediate filaments networks: organization and possible functions of a diverse group of cytoskeletal elements. J. Cell Sci. 5, 69-97.

Green K.J. and Jones J.C.R. (1996). Desmosomes and hemi-desmosomes: structure and function of molecular components. FASEB J. 10, 871-881.

Guo L., Degenstein L., Dowling J., Yu Q.C., Wollmann R., Perman B.

and Fuchs E. (1995). Gene targeting of BPAG1: abnormalities in mechanical strenght and cell migration in stratified squamous epithelia and severe neurologic degeneration. Cell 81, 233-243.

Gyoeva F.K. and Gelfand V.I. (1991). Coalignement of vimentin intermediate filaments with microtubules depends on kinesin. Nature 353, 445-448.

Hatzfeld M., Kristjansson G.I., Plessmann U. and Weber K. (1994). Band 6 protein, a major constituent of desmosomes from stratified epithelia, is a novel member of the armadillo multigen family. J. Cell Sci. 107, 2259-2270.

Herrmann H. and Wiche G. (1983). Specific in situ phosphorylation of plectin in detergent-resistant cytoskeletons from cultured chinese hamster ovary cells. J. Biol. Chem. 258, 14610-14618.

Herrmann H. and Wiche G. (1987). Plectin and IFAP- 300 K are homologous proteins binding to microtubule-associated proteins 1 and 2 and to the 240 kilodalton subunit of spectrin. J. Biol. Chem. 262, 1320-1325.

Hirokawa N. (1982). Cross-linker sytem between neurofilaments, microtubules and membranous organelles in frog axons revealed by the quick-freeze deep-etching method. J. Cell Biol. 94, 129-142.

Hirokawa N., Glicksman M.A. and Willard M.B. (1984). Organization of mammalian neurofilament polypeptides within the neuronal cytoskeleton. J. Cell Biol. 98, 1523-1536.

Hozak P., Sasseville A.M.J., Raymond Y. and Cook P.R. (1995). Lamin proteins form internal nucleoskeleton as well as a peripheral lamina in human cells. J. Cell Sci. 108, 635-644.

Huber O., Krohn M. and Kemler R. (1997). A specific domain in α-catenin mediates binding to ß-catenin or plakoglobin. J. Cell Sci. 110, 1759-1765.

Jones J.C.R., Asmuth J., Baker S.E., Langhofer M., Roth S.I. and Hopkinson S.B. (1994). Hemidesmosomes: extracellular matrix/ intermediate filament connectors. Exp. Cell Res. 213, 1-11.

Knudsen K.A., Soler A.P., Johnson K.R. and Wheelock M.J. (1995). Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. J. Cell Biol. 130, 67-79.

Koch P.J. and Franke W.W. (1994). Desmosomal cadherins: another growing multigene family of adhesion molecules. Curr. Opin. Cell Biol. 6, 682-687.

Kordeli E., Cartaud J., Nghiem H.O., Pradel L.A., Dubreuil C., Paulin D. and Changeux J.P. (1986). Evidence for a polarity in the distribution of proteins from the cytoskeleton in *Torpedo marmorata* electrocyte. J. Cell Biol. 102, 748-760.

Kouklis D.P., Hutton E. and Fuchs E. (1994). Making a connection: direct binding between keratin intermediate filaments and desmosomal proteins. J. Cell Biol. 127, 1049-1060.

Langley R.J.J. and Cohen C.M. (1987). Cell type-specific association between two types of spectrin and two types of intermediate filaments. Cell. Motil. Cytosk. 8, 165-173.

Lee J.K., Black J.D., Repasky E.A. and Bankert R.B. (1988). Activation induced a rapid reorganization of spectrin in lymphocytes. Cell 55, 807-816.

Leterrier J.F., Liem R.K. and Shelanski M. (1982). Interaction between neurofilaments and microtubule-associated proteins, a possible mechanism for interorganellar bridging. J. Cell Biol. 95, 982-986.

Levin J. and Willard M. (1981). Fodrin axonally transported polypeptides associated with the internal periphery of many cells. J. Cell Biol. 90, 631-643.

Mangeat P.H. and Burridge K. (1984). Immunoprecipitation of non-erythrocyte spectrin within live cells following microinjection of specific antibodies: relation to cytoskeletal structures. J. Cell Biol. 98, 1363-1377.

Marthur M., Goodwin L. and Cowin P. (1994). Interactions of a desmosomal cadherin, Dsg1, with plakoglobin. J. Biol. Chem. 269, 14075-14080.

Martin L., Crimaudo C. and Gerace L. (1995). cDNA cloning and characterization of lamina-associated polypeptide 1C (LAPC1C) an integral protein of the inner nuclear membrane. J. Biol. Chem. 270, 8822-8828.

Metuzals J., Robitaille Y., Houghton S., Gauthier S. and Leblanc R. (1988). Paired helical filaments and the cytoplasmic-nuclear interface in Alzheimer's disease. J. Neurocytol. 17, 827-833.

Nigg E.A. (1989). The nuclear envelope. Curr. Opin. Cell Biol. 1, 435-440.

Nixon R.A., Paskevich P.A., Sihag R.K. and Thayer C.Y. (1994). Phosphorylation on carboxyl terminus domains of neurofilament proteins in retinal ganglion cell neurons in vivo: influences on regional neurofilament accumulation, interneuronfilament spacing, and axon caliber. J. Cell Biol. 126, 1031-1046.

Ozawa M., Baribault H. and Kemler R. (1989). The cytoplasmic domain of the cell adhesion molecule uvomurilin associates with three independent proteins structurally related in different species. EMBO J. 8, 1711-1717.

Pollerberg G.E., Burridge K., Krebs K.E., Goodman S.R. and Schachner M. (1987). The 180 KD component of the neuronal cell adhesion molecule N-CAM is involved in cell-cell adhesion and cytoskeleton-membrane interactions. Cell Tissue Res. 250, 227-236.

Powell L. and Burke W.B. (1990). Internuclear exchange of an inner nuclear protein (p55) in heterokaryons: in vivo evidence for the interaction of p55 with the nuclear lamina. J. Cell Biol. 111, 2225-2234.

Sacco P.A., McGranahan M., Wheelock M.J. and Johnson K.R. (1995). Identification of plakoglobin domains required for association with N-cadherin and alpha-catenin. J. Biol. Chem. 270, 20201-20206.

Schmidt A., Heid H.W., Schafer S., Nuber U.A., Zimbelmann R. and Franke W.W. (1994). Desmosomes and cytoskeletal architecture in epithelial differentiation: cell type-specific plaque components and intermediate filament anchorage. Eur. J. Cell Biol. 65, 229-245.

Serria A.J., Lieber J.G., Nordeen S.K. and Evans R.M. (1994). The presence or absence of a vimentin-like intermediate filament network affects the shape of the nucleus in SW-13 cells. J. Cell Sci. 107, 1593-1607.

Shapiro L., Fannon A.M., Kwong P.D., Thompson A., Lehman M.S., Grubel G., Legrand J., Nielson A., Colman J. and Hendrickson W.A. (1995). Structural basis of cell-cell adhesion by cadherins. Nature 374, 327-336.

Stanley J.R., Tanaka T., Mueller S., Klaus-Kovtun V. and Roop D. (1988). Isolation of cDNA for bullous pemphigoid antigen by use of patients' autoantibodies. J. Clin. Invest. 82, 1864-1870.

Stappenbeck T.S. and Green K.J. (1992). The desmoplakin carboxyl terminus coaligns with and specifically disrupts intermediate filament networks when expressed in culture cells. J. Cell Biol. 116, 1197-1209.

Staufenbiel M. and Deppert W. (1982). Intermediate filament systems are collapsed onto the nuclear surface after isolation of nuclei from tissue culture cells. Exp. Cell Res. 138, 207-214.

Steinert P.M. and Roop D.R. (1988). Molecular and cellular biology of intermediate filaments. Annu. Rev. Biochem. 57, 593-625.

Steinert P.M., Steven A.C. and Roop D.R. (1985). The molecular

biology of intermediate filaments. Cell 42, 411-419.

Sternberger L.A. and Sternberger N.H. (1983). Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments in situ. Proc. Natl. Acad. Sci. USA 80, 6126-6130.

Stewart M. (1990). Intermediate filaments: structure, assembly and molecular interactions. Curr. Opin. Cell Biol. 2, 91-100.

Svitkina T.M., Verkhovsky A.B. and Borisy G.G. (1996). Plectin sidearms mediate intercation of intermediate filaments with microtubules and other components of the cytoskeleton. J. Cell Biol. 135, 991-1007.

Tanaka T., Parry D.A.D., Klaus-Kovtun V., Steinert P.M. and Stanley J.R. (1991). Comparison of molecularly cloned bullous pemphigoid antigen to desmoplakin I confirms that they define a new family of cell adhesion junction plaque proteins. J. Biol. Chem. 266, 12555-12559.

Taniura H., Glass C. and Gerace L. (1995). A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones. J. Cell Biol. 131, 33-44.

Tint I.S., Hollenbeck P.J., Verkhovsky A.B., Surgucheva I.G. and Bershadsky A.D. (1991). Evidence that intermediate filament reorganisation is induced by ATP-dependent contraction of actomyosin cortex in permeabilized fibroblast. J. Cell Sci. 98, 375-384.

Wiese C. and Wilson K.L. (1993). Nuclear membrane dynamics. Curr. Opin. Cell Biol. 5, 387-394.

Worman H.J., Evans C.D. and Blobel G. (1990). The lamin B receptor of the nuclear envelope inner membrane: a polytopic protein with eight potential transmembrane domains. J. Cell Biol. 111, 1535-1542.

Worman H.J., Yuan J., Blobel G. and Georgatos S.D. (1988). A lamin B receptor in the nuclear envelope. Proc. Natl. Acad. Sci. USA 85, 8531-8534.

Yang Y., Dowling J., Yu Q.C., Kouklis P., Cleveland D.W. and Fuchs E. (1996). An essential cytoskeletal linker protein connecting actin microfilaments to intermediate filaments. Cell 86, 655-665.

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