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THE CYTOSKELETON (1)

by

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SUMMARY

An intricate network of cytoplasmic filamentous structures determines the maintenance of shape and the motility of animal cells. Only some fibers are permanent features, most structural elements of the versatile cytoskeleton assembling and disassembling into building blocks. Tubulin microtubules, actin microfilaments, and the varied intermediate filaments may be ranked in terms of increasing stability of the polymer. Microtubules and microfilaments are intrinsically polarized and possess directional information, that is not shared by intermediate filaments. Tubulin, actin, and the five classes of intermediate filaments each consist of highly conserved molecules composing gene families probably derived from an original gene that was duplicated and modified during evolution. The specific properties of the cytoskeletal fibers according to species or cell type mainly result from their association with binding proteins which meet different requirements, like regulation of polymerization-depolymerization rate, positioning of the cytoskeletal fiber to other homologous or heterologous fibers as well as to plasma or internal membranes, or ATP-driven motor action. In the case of intermediate filaments, the several classes of cell-specific protein subunits are expressed in a developmental and histological pattern. The regulation of cytoskeletal functioning involves ATP or GTP binding, protein phosphorylations and local concentrations in calcium ions.

INTRODUCTION

Fascinating is the generation and maintenance of shape of animal cells, such as neurons with long axons and dendritic arborisation, enterocytes with microvilli, renal podocytes with pedicels, the Protists with pseudopodia or beating cilia. Even more fascinating is the cell motility referring to three basic forms of movement (WARRICK and SPUDICH, 1987). One is the migration of a cell across a substratum. The second is the variety of changes in shape, for example the furrowing that

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occurs when the cell divides. The third is the directed movement of organelles within the cell.

Both specific cell shape and cell motility involve a complex set of protein fibers : the cytoskeleton. This static term appears inappropriate if one refers to multicellular organisms, in which various organ systems participate in functions exerted by the cytoskeleton at the cell level. The cytoskeletal fibers and associated proteins would better correspond to cytobones, cytomuscles, cytonerves, and cytovessels within a cell.

Eukaryote cells contain three major classes of cytoskeletal fibers : 7 nm actin microfilaments, 24 nm microtubules, and 10 nm intermediate filaments. Some microtubules and microfilaments are permanent features of cells, as in flagella, cilia, or the contractile apparatus of muscle. These ordered systems allowed the acquisition of a great deal of information about the molecular structure and function of the cell cytoskeleton. However, I shall focus mainly on unspecialized forms which can be found in a generalized cell.

The Microtubules

Unlike the rigid image portrayed in electron micrographs, most microtubules are dynamic structures capable of being rapidly assembled and disassembled to adjust to an ever-changing cytoplasm.

Microtubules (Fig. 1) are linear polymers of tubulin dimers. Each dimer is a 100 kDa complex of closely related and highly conserved α- and β-tubulin polypeptides. Despite the multiplicity of genes encoding these tubulins in most eukaryotes, all are functionally equivalent. The 24 nm diameter cylindrical wall of the microtubule is formed by protofilaments of tubulin dimers, cytoplasmic and mitotic microtubules typically having 13 protofilaments. The head to tail arrangement of repeating αβ dimers in the tubulin lattice is polarized, giving the microtubule an intrinsic *structural polarity*. The two ends, (+) and (−) are not equivalent. They have specific structural and assembly characteristics and orient the direction of microtubule-associated transport. This is a crucial point.

The *dynamic instability* model (MITCHISON and KIRSCHNER, 1984, CASSIMERIS *et al.*, 1988) is the most accurate interpretation of the dynamic behavior of microtubules assembled from pure tubulin in vitro. According to this model, the ends of growing and shrinking microtubules are different. Each dimeric tubulin contains two bound GTP molecules. Microtubules grow preferentially by addition of GTP-bound tubulin to the (+) end of a preexisting nucleated microtubule. One GTP is then hydrolyzed to GDP during or just after incorporation of the dimer onto the elongating end of the microtubule. If the resulting end of the microtubule has a GDP cap, it is unstable and depolarizes rapidly. Conversely, if additional GTP-bound tubulin adds to the end before hydrolysis of GTP, that end is not only stable but continues to grow. This dynamic unstability is very sensitive to conditions at the ends of microtubules. A high concentration of free tubulin would favor continued growth, and a low concentration would allow a GDP cap to form, causing

depolymerization. Another condition affecting elongation or shrinkage is the rate of hydrolysis of GTP, the regulating factors remaining unknown.

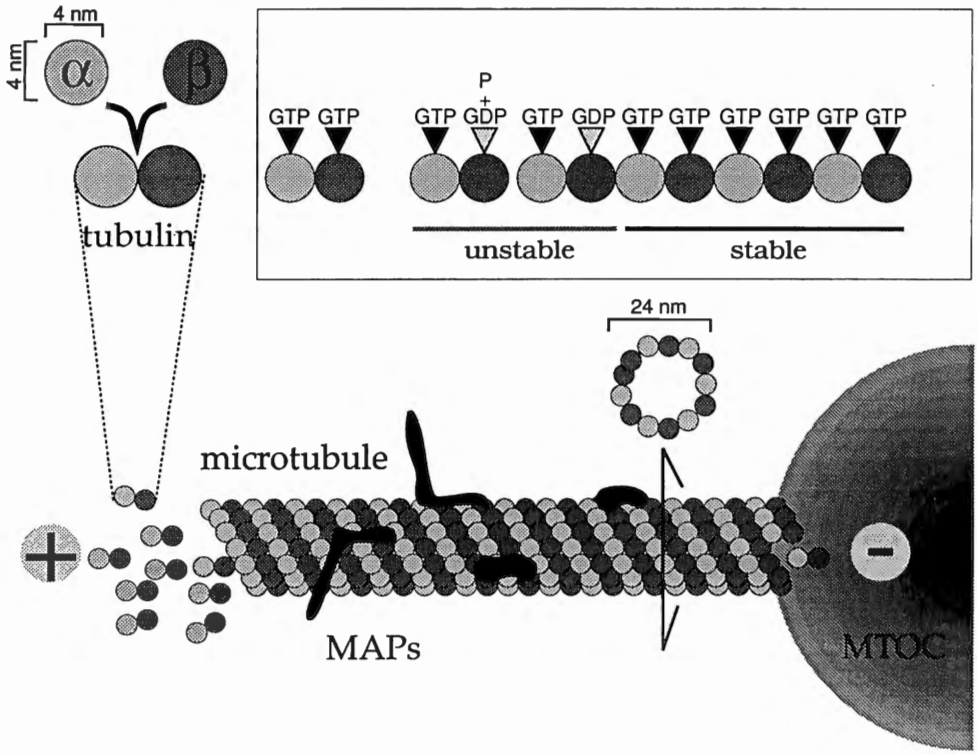

Fig. 1. — Organization of the tubulin microtubule, with microtubule-associated proteins (MAPs) and Microtubule-Organizing Center (MTOC). A scheme of the dynamic instability model is presented in the inset.

Microtubules are not arranged randomly in cells but are organized around one or more discrete foci named *Microtubule Organizing Centers* or MTOCs (see BRINKLEY, 1985). They are specific structural entities with varying forms and distribution, the major one being the centrosome. Due to unknown local conditions, these centers are preferred sites for the initiation, assembly, anchorage and stabilization of microtubules and associated proteins. As demonstrated by applying substances like colcemid and observing the progressive recovery of microtubules from soluble tubulin, these structural templates organize microtubules with defined polarity and distribution. Consequently, the expression of cell form-controlling microtubule patterns appears to involve the MTOCs, and these in turn appear to be controlled by signals arising from endogeneous and exogeneous sources.

Since all tubulins of the multigene family are functionally equivalent, the specific properties of microtubules are then based upon the presence of *Microtubule-Asso-*

ciated proteins or MAPs, a collection of varied molecules that have been defined on the basis of their binding and/or putative interaction with microtubules. In the absence of well-defined functions for most of the MAPs known to date, they are usually classified according to their size as determined by electrophoresis. A first group comprises proteins of very high Mr (>250 000) of which MAP1, MAP2 and dynein are the major components. Next is found a very heterogeneous group of MAPs having a Mr close to 200 000. Other major MAPs include kinesin (Mr 110 000-134 000), STOPs (Mr 72 000-145 000) and chartins (Mr 69 000-80 000). The last set is tau proteins (Mr 55 000-62 000) (see OLMSTED, 1986 ; WICHE, 1989).

If the precise role of most of the MAPs remains speculative, their molecular shape and affinity for other cell components may suggest at least three kinds of functions. First, the smaller MAPs would serve to stabilize, or destabilize, individual microtubules by binding to adjacent tubulin molecules. Second, the larger MAP1 and MAP2 have been visualized at the ultrastructural level as filamentous arms extending from the surface of microtubules. The region protruding from the microtubule may have binding affinities for other microtubules, intermediate filaments, actin microfilaments, serving then as linkers between the other two major cytoskeletal systems. A third function exerted by kinesin- and dynein-like molecules has been recently demonstrated experimentally. Allen Video-Enhanced Contrast microscopy (ALLEN, 1987) can visualize individual microtubules gliding in vitro on the surface of glass slides, or show the bidirectional movement of vesicular organelles along a single microtubule. The addition of MAPs to purified tubulin in this system featured the existence of microtubule-based motors having ATPase activity (MCINTOSH and PORTER, 1989). *Kinesin*-like molecules (300-600 kDa) contain several polypeptides (VALE, 1987). One end of the kinesin molecule can bind to a microtubule, and the other end, to a receptor inserted for example in the membrane of a vesicle. In the presence of ATP, the kinesin molecule moves along the microtubule from the (−) to the (+) end, transporting the bound vesicle with it. In the specific case of nerve cells, this would correspond to anterograde motility for an axonal organelle. On the other hand, the cytoplasmic *dynein* (or MAP 1C, about 350 kDa) is similar in structure and function to dynein in flagella. This large molecule is also composed of several polypeptides. The two globular heads appear to translocate along microtubules in the presence of ATP, and the other end can bind to a vesicle (VALE, 1987, PASCHAL and VALLEE, 1987, SCHNAPP and REESE, 1989). Contrary to kinesin, dynein moves from the (+) to the (−) end of the microtubule, generating then for example retrograde axonal transport. The identification of such molecules provides an answer to one of the older questions in cell biology : how things move from one part of a cell to another ? What is not yet clear is the specificity of the process (DARNEL *et al.*, 1990). How do organelles know whether to bind a protein like kinesin, jump onto a microtubule, and then move ? Some organelles, like pigment granules in melanophores, can alternate their direction of movement along microtubules. They must then contain both receptors for centripetal and centrifugal motors, but only one is active at time. The directionality of movement could be affected by phosphorylation of these receptors or proteins associated with the motor proteins themselves.

The Microfilaments

Several genes code for actin molecules. At least six have been identified in higher vertebrates. Four α-actins are found in muscle cells : one unique to striated muscle, one to cardiac muscle, one to vascular smooth muscle, and one to enteric smooth muscle. Two others called β and γ-actins are found in the cytoplasm of all cells. Clearly, actin genes have evolved from a common precursor and most of the sequence is highly conserved. Regardless of source and species, all actins form the same type of polymer and can copolymerize with all other actins. They probably differ in their affinities for actin-binding proteins and other proteins, although this has not been demonstrated experimentally.

Globular G-actin (42 kDa) is a dumbbell-shaped molecule with dimensions of 6.7, 4, 4 nm (Fig. 2). Microfilaments of F-actin are polymers where a single chain of G-actin monomers forms a helical filament, as deduced from computerized image reconstruction (BULLITT *et al.*, 1988). Since each actin subunit has a defined polarity and the subunits polymerize head to tail, the filaments also have a defined (+)(−) polarity (DARNELL *et al.*, 1990).

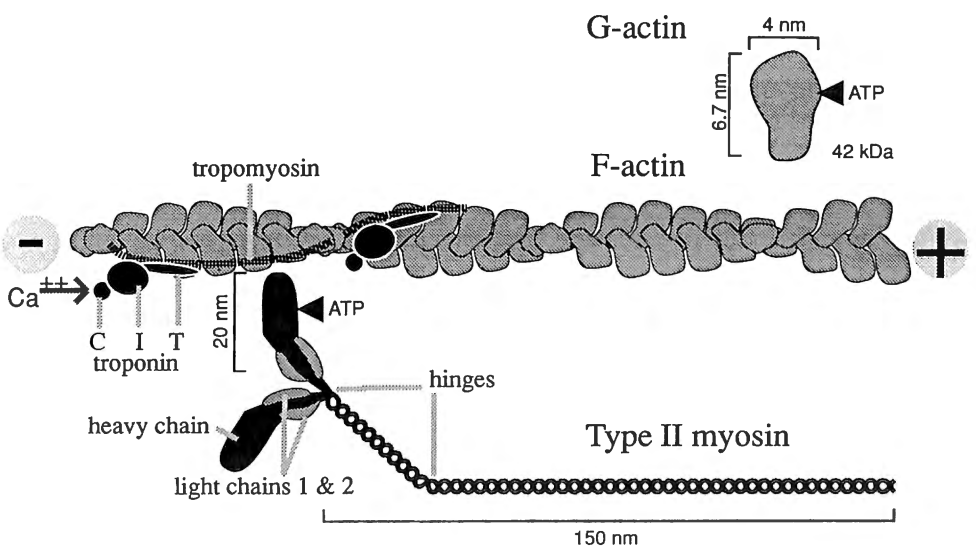

Fig. 2. — Organization of the actin microfilament, and its interaction with type I myosin, as found in the striated muscle cell.

Like microtubules, microfilaments grow by addition of actin subunits to both ends, the rate of growth being 5-10 times faster at the (+) than at the (−) ends. In addition, G-actin molecules contain a special site for ATP or ADP binding. An ATP-actin adds much faster to the microfilament than an ADP-actin. Upon polymerization, the ATP is hydrolyzed, and the resulting ADP remains bound to the F-actin. This hydrolysis does not seem essential for polymerization to occur, but

ADP would have to be displaced by ATP before the structure can be depolymerized (KORN *et al.*, 1987).

Like microtubules again, actin filaments are in equilibrium with monomers, under steady-state conditions. However, two important differences emerge when the assembly-disassembly kinetics are compared between microfilaments and microtubules. First, the polymerization of G-actin is induced by Mg^{2+} and by K^+ and Na^+ at concentrations normally found in the cytosol. In consequence, the dissociation rate of subunits from actin filaments is slow ; they are more stable than microtubules. Second, the critical concentration for assembly of actin monomers into microfilaments is very low, so that the vast bulk of actin should be polymerized within cells (DARNELL *et al.*, 1990).

Since all actins are equivalent, the specific properties of microfilaments according to cell type or cell function result from actin-binding proteins, as was the case for MAPs and microtubules. These actin-binding proteins are quite varied.

The most extensively studied *type II* myosin has been identified in muscle and non-muscle cells (WARRICK and SPUDICH, 1987, SPUDICH, 1989). It is a hexamer composed of one pair of heavy chains (230 kDa) each consisting of a N-terminal globular head 20 nm long, which binds ATP and actin, and a long coiled-coil α helical tail of about 150 nm, which is involved in thick filament formation. Associated to each head are two different light chains, of about 20 kDa. There are two, perhaps three, flexible joints in the molecule. In muscle cells, the hydrophobic tails of 300-400 myosins pack together to form a bipolar aggregate, the thick filament 15-20 nm in diameter and from 1.4 to more than 5 μm long. We shall not consider in detail this specific arrangement, but only recall the interaction of actomyosin.

In addition to actin, the thin filament in striated muscle contains two protein complexes. *Tropomyosin* is a coiled-coil of two α-helical polypeptides, 41 nm long, extending over seven actin subunits. It is bound to actin helix and lies in the groove of the microfilament. Tropomyosin also possesses a specific site for binding another protein complex : *troponin*. Calcium can bind to the C-subunit of troponin, inducing a conformational change to the I-subunit that is bound to actin, and then to the T-subunit bound to tropomyosin. Tropomyosin shifts from the groove in actin filament and exposes a region of the actin monomers to which the myosin heads can bind. Myosin energized by ATPase activity pivots the head to 45°, causing actin to move.

Vertebrate smooth muscle and invertebrate muscle contain tropomyosin, but are devoid of the troponin complex. One of the two light-chain pairs associated with the myosin heads inhibits the interaction of these heads with actin microfilaments. Binding of calcium to the light chain releases this inhibition and activates myosin ATPase activity. Calcium regulation of contraction may also involve the stimulation of activity of a kinase, which phosphorylates one pair of light chains, triggers assembly of bipolar myosin thick filaments and myosin-actin binding (SELLERS and ADELSTEIN, 1987).

The role of phosphorylations at multiple sites of the non-muscle Type II myosin remains unclear at present. The C-terminal portion (the tail) of the heavy chain can

be phosphorylated, which appears to decrease the ability of the myosins to form filaments and lowers their actin-activated ATPase activity. The regulatory myosin light chain can be reversibly phosphorylated as well, by a calcium-calmodulin-dependent myosin light chain kinase, which allows actomyosin interaction. These phosphorylations seem to make non-muscle myosin versatile motors which can adapt to the changing requirement of non-muscle cells, as for exocytosis, cytokinesis, cytoplasmic streaming (CITI and KENDRICK-JONES, 1987, KORN and HAMMER, 1988).

Type I myosin or minimyosin is a 110 kDa protein that has one actin-binding head and a short tail that binds to phospholipids in membranes. It exists namely in filopodia and microvilli, where it might determine retraction or positioning of the microfilaments. It is also thought to be able to move vesicles along actin (WARRICK and SPUDICH, 1987).

A large number of actin-binding proteins (Fig. 3) are known in addition to the myosin motors described hereabove (POLLARD and COOPER, 1986).

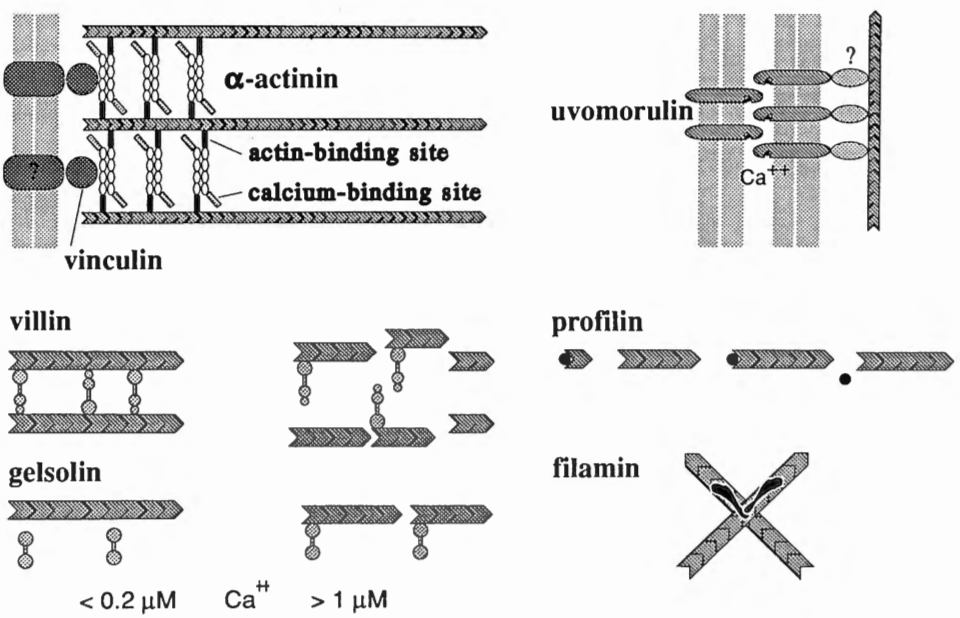

Fig. 3. — Some actin-binding proteins (modified from DARNELL *et al.*, 1990). See text for comments.

Some serve as cross-linking and attachment proteins for actin filaments, such as *α-actinin, dystrophin and spectrin.* They form antiparallel dimers or tetramers, where the N-terminal domains form the actin-binding site, and the C-terminal domains contain calcium binding sites. These two sections are highly conserved. The middle sections consist of repeats of triple helical segments. The antiparallel feature of

these proteins exposes actin binding sites at each extremity of the molecule, which both can bind actin filaments.

With the help of other proteins, the attachment proteins may also participate in anchoring actin filaments to membranes. At intercalated disks in cardiac muscle, or dense plaques of smooth muscles, *vinculin* is thought to bind to an integral membrane protein and to α-actinin, which cross-links actin filaments and attaches them to the membrane. Similarly, in epithelial cells, the trans-membrane *uvomorulin* (or E-cadherin) is concentrated in the junctional region called the belt desmosome, where microfilaments encircle the cell under the plasma membrane. Uvomorulin links the plasma membranes of adjacent cells together in a calcium-dependent manner, and on the other hand, anchors the sides of actin filaments to the membrane via a set of actin-binding proteins that are not yet clearly identified.

In brush-border cells, in addition to *minimyosin* linked by the tail to the plasma membrane and positioning or retracting bundles of microfilaments of the microvilli, other actin-binding proteins are found. In the core of the microvillus, *fimbrin* (68 kDa, 1 molecule per 10 actin monomers), tightly packs parallel actin bundles. *Fodrin,* a long protein similar to spectrin in its structure and function, links adjacent actin bundles, as well as actin to integral proteins in the plasma membrane. The cross-linking *villin,* found in the core has a dual function as being also a regulator of actin filament stability. Villin (95 kDa) contains three domains : both N- and C-termini can bind actin, serving then as cross-link at low calcium concentration (< 0.2 μM), but at high calcium concentration (> 1 μM), the conformational change of this protein gives severing properties towards microfilaments. The N-terminus of villin remains bound to the (+) end of each actin fragment at the severed site.

Gelsolin is another protein causing disassembly of actin networks. It ressembles villin, but has only one actin binding site at the N-terminal domain. Thus, it cannot cross-link filaments. It is only an actin-severing protein, which binds to actin at micromolar concentrations of calcium, cuts the filament, and remains attached to the (+) end of the fragment. In the same line of thinking, *profilin* (15 kDa) can bind to actin monomers forming profilactin, that can attach only to the (+) end of a filament. However, the (+) end of many existing filaments is already capped by other proteins (cap Z, α-actinin, villin, gelsolin, ...). Profilin decreases then the polymerization rate, and if all filaments are capped, blocks all actin polymerization.

A last actin-binding protein to be evoked is *filamin* (270 kDa), a stabilizing one. This long (160 nm) and flexible molecule possess two actin-binding sites near its ends. Associated dimers of filamin can connect actin filaments by forming crossed branches, causing then the formation of a three dimensional network.

The Intermediate Filaments

The third class of cytoplasmic fibers are the 8 to 12 nm diameter intermediate filaments (IF, Fig. 4). Since some cell types appear not to contain IF, it seems unlikely that IF have fundamentally important « housekeeping » functions, but it is probable that they are involved in specialized functions related to the differentiation

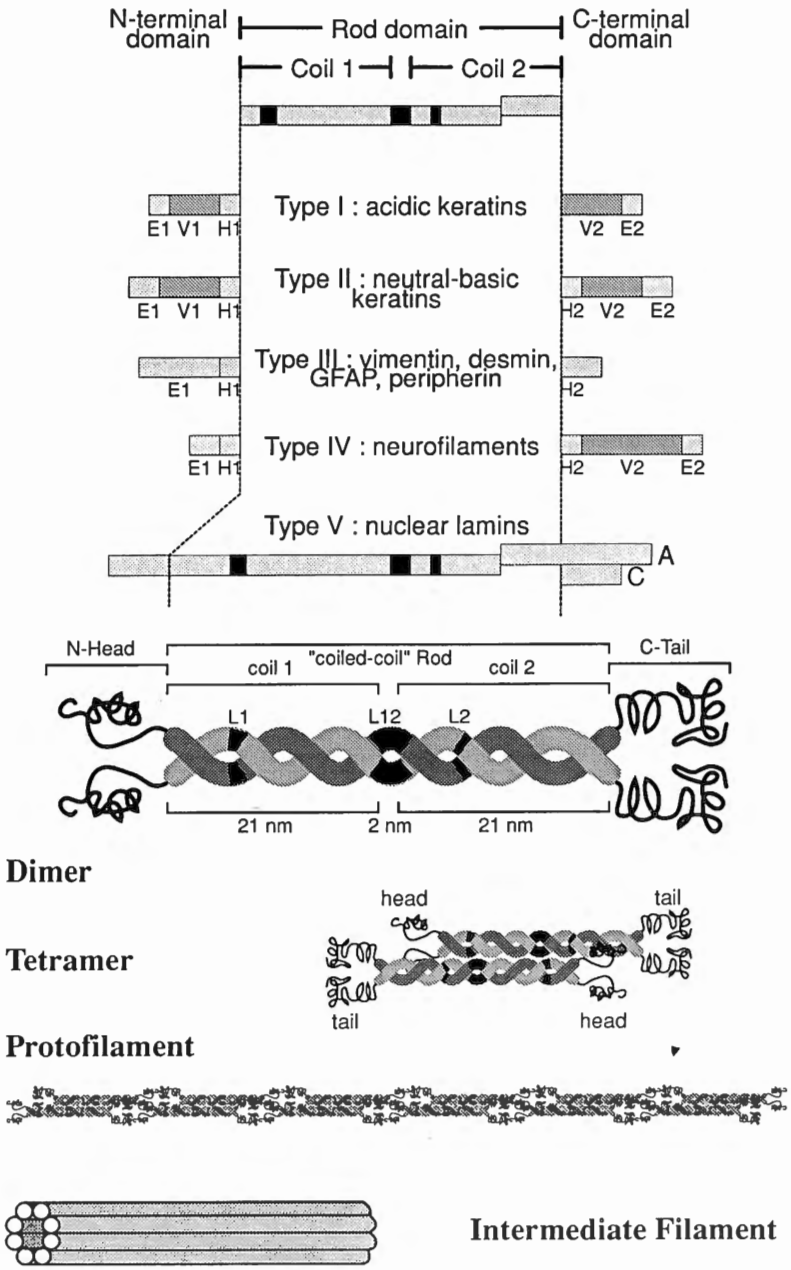

Fig. 4. — Organization of the intermediate filaments (IF) and the chemical classification of IF and nuclear lamins (modified from STEINERT and ROOP, 1988). E : end sequences ; V : variable sequences ; H : homologous sequences ; L1, L2, L12 : linkers. A scheme of IF polymerization is also presented.

state of the cell, including mechanical coordination of the cytoskeleton, information transport, and signal transduction (STEINERT and ROOP, 1988). IF proteins constitute an extremely heterogeneous multigene family (30 or more per mammalian species), being then far more complex than other major classes of cytoskeletal fibers, actin microfilaments and tubulin microtubules. IF proteins are expressed in a developmental and histological pattern. Although the typical differentiated cell expresses only one type of IF, coexpression has been repeatedly described, and it occurs also during development, as cells switch from one IF type to another and some cells seem to remain stuck at a point of coexpression (OSBORN and WEBER, 1983, TORELLI *et al.*, 1989).

The *vimentin* gene is expressed typically in mesenchymal cells such as fibroblasts or endothelium. The fibers often terminate at the nuclear envelope and at the plasma membrane, suggesting the continuity of the IF network in cells by connecting the plasma membrane cytoskeleton to the nuclear lamina or karyoskeleton. They may also function to keep organelles like lipid droplets in a defined place within the cell. The *desmin* gene is expressed predominantly in myogenic cells. The fibers can be arranged like vimentin IF, and also determine the specific arrangement of the contractile apparatus of actomyosin. The Glial fibrillary acidic protein (*GFAP*) is the product of one gene expressed in glial cells and astrocytes. These three first types of monomers, of about 50 kDa, form homopolymeric filaments.

In contrast, neurons express at least three different proteins of increasing molecular weight, which copolymerize into neurofilaments. They are named *NF-L* (60-70 kDa), *NF-M* (105-110 kDa), and *NF-H* (135-150 kDa). A fourth gene has been identified more recently, which is expressed in neurons of the peripheral nervous system, hence the name *peripherin* (57 kDa). These neurofilaments complexed to microtubules formed in the cell body move down the axon allowing strength and rigidity.

More complicated are the cytokeratins expressed in epithelial cells. Some 30 of these are divided into two classes : types I (acidic, 40-60 kDa) and II (neutral/basic, 50-70 kDa). Both type I and type II keratins are required for 10-nm filament formation at the heterodimer level ; they are obligate copolymers. Each type of epithelium has its characteristic complement of multiple cytokeratins, found in tightly packed bundles known as tonofilaments distributed throughout the cytoplasm and forming an elaborate cage around the nucleus. They are associated with desmosomal plaques in regions of cell-cell contacts.

Recently, a new type of IF has been added to the list, the lamins forming the nuclear skeleton on the inner nuclear surface of the nuclear membrane. The fibers are arranged in a quasi-tetragonal meshlike lattice, and consist of at least four proteins (60-75 kDa) in vertebrates.

Amino acid sequences show all intermediate filament peptides to contain a highly conserved central rod domain, approximately 40-nm long. This domain exhibits repeated heptad of amino acids with hydrophobic residues in positions a and d. These heptad repeats are typical of a coiled-coil configuration between two molecules. The rod is interrupted by three non-conforming helical regions, the spacers.

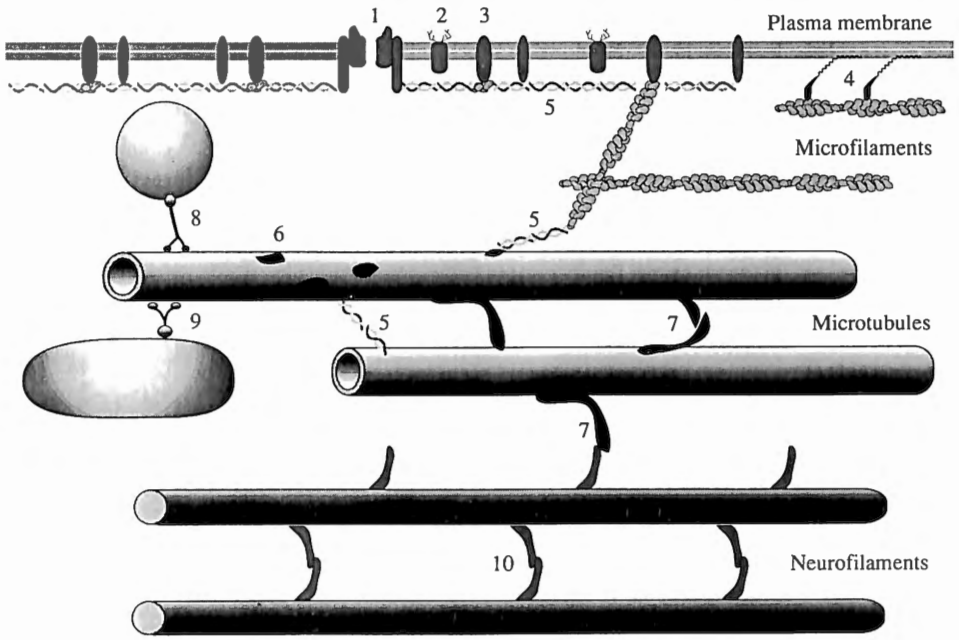

Fig. 5. — Schematic representation of interactions between cytoskeletal components in mature neurons (modified from RIEDERER, 1990). The plasma membrane contains ion channels (1), integral membrane glycoproteins (2) and anchoring proteins like ankyrin (3). Type I myosin (4) is also associated with the membrane. Spectrin (5) forms a submembrane network anchored on ankyrin, coats microtubules, may form crossbridges between microtubules, microfilaments, and is involved in the interaction between microtubules and microfilaments. Microtubules are stabilized by MAP tau (6) and are cross-linked via MAP 2 (7). Microtubules are also tracks for anterograde and retrograde transport of membraneous organelles by kinesin (8) and dynein (9). Intermediate filaments are cross-linked by extensions of the IF molecules or IF-associated proteins (10).

At the ends of the central rod domain are a N-terminal head and a C-terminal tail that vary between the individual intermediate filament peptides, and which are responsible for the differences in molecular weight and biochemical properties.

The assembly of the two monomers in the dimer is parallel, but a tetramer is formed by antiparallel, staggered side-by-side aggregation of two dimers, so that it is no longer polarized. This will distinguish IF from both microfilaments and microtubules which each possess a clear structural directionality. Tetramers aggregate end-to-end to form a protofilament, and eight protofilaments form a cylindrical 10 nm thick filament. Unlike what would be expected from stable polymers as are known IF, they are dynamic structures that exchange subunits with a small soluble pool (ANGELIDES *et al.*, 1989 ; MILLER *et al.*, 1991)

There is evidence that the head plays a role in the assembly of filaments by means of phosphorylations, and at least in neurofilaments, phosphorylations of the tail control the cross-linking state and space-filling properties (MATUS, 1988).

A growing list emerges of minor proteins that frequently coisolate and/or associate with the major IF structural proteins, and that are termed IF-associated proteins (IFAPs). It seems possible to assign these IFAPs into different functional classes that may also correlate with their size (STEINERT and ROOP, 1988). (1) IFAPs of low Mr (10-45 kDa) that bind IF laterally into tight macrofilament aggregates, as filaggrin or high-sulfur and high tyrosine-glycine protein families ; (2) IFAPs of high Mr that cross-link IF into loose networks, like paranemin, synemin, plectin, ... ; (3) IFAPs that function as « capping » proteins, like ankyrin, spectrin, desmoplakin, lamin B ; (4) several IFAPs that do not appear to conform to this scheme, such as epinemin and internexins.

Conclusions

The concluding remarks can be illustrated by presenting possible interactions of cytoskeletal elements in an axon (Fig. 5). Most structural elements of the versatile cytoskeleton assemble and disassemble into building blocks. In terms of increasing stability of the polymer, are found tubulin microtubules, actin microfilaments, and the varied intermediate filaments. Each group consists of highly conserved molecules composing gene families probably derived from an original gene in a primordial eukaryotic cell, that was duplicated and modified during evolution. Due to the polarization of the monomer and the head-to-tail polymerization, microfilaments and microtubules are intrinsically polarized with (+) and (−) ends conferring directional information. This is not shared by intermediate filaments. The specific properties of the cytoskeletal fiber according to species or cell type result from two different aspects. In the case of intermediate filaments, several classes of cell-specific protein subunits exist. In the case of microfilaments and microtubules, the widely distributed actin or tubulin molecules can associate with binding proteins. Intermediate filaments also possess associated proteins. These diverse binding proteins meet different requirements. They participate to the regulation of polymerization-depolymerization rate, in addition to GTP or ATP bound to the subunits and the monomer concentration. They ensure positioning of the cytoskeletal fiber to other homologous or heterologous fibers and to plasma or internal membranes. They exert ATP-driven motor action, like type I and II myosins, kinesin, and dynein. Several aspects of cytoskeleton functioning including the association with binding proteins, cross-linking, space-filling properties, and polymerization, are headed up by local concentration in calcium and other ions, or protein phosphorylation.

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