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*Review*

# WATER AND THE CYTOSKELETON

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**Abstract** - The diffusion of intracellular fluid and solutes is mainly limited by the density and the geometry of crossbridges between cytoskeletal polymers mediating the formation of an integrated cytoplasmic scaffold. Evidence for specific relationships between water and cytoskeletal polymers arises from the effect of heavy water on their polymerization process *in vitro* and on the cytoskeleton of living cells. The hydration of cytoskeletal subunits is modified through polymerization, a mechanism which may be involved in the direct contribution of the cytoskeleton to the osmotic properties of cells together with changes of hydration of polymers within networks. The dynamic properties of the hydration layer of cytoskeletal polymers may reflect the repetitive distribution of the surface charges of subunits within the polymer lattice, thus inducing a local and long range ordering of the diffusion flows of water and solutes inside polymer networks. The interactions between subunits in protofilaments and between protofilaments determine the specific viscoelastic properties of each type of polymer, regulated by associated proteins, and the mechanical properties of the cell through the formation of bundles and gels. Individual polymers are interconnected into dynamic networks through crossbridging by structural associated proteins and molecular motors, the activity of which involves cooperative interactions with the polymer lattice and likely the occurence of coordinated modifications of the hydration layer of the polymer surface. The cytoskeletal polymers are polyelectrolytes which constitute a large intracellular surface of condensed anionic charges and form a buffering structure for the sequestration of cations involved in the regulation of intracellular events. This property allows also the association of cytoplasmic enzymes and multimolecular complexes with the cytoskeleton, facilitating metabolic channelling and the localization of these complexes in specific subdomains of the cytoplasm. The consequences of interactions between membranes and the cytoskeleton in all cellular compartments range from the local immobilization and clustering of lipids and membrane proteins to the regulation of water and ion flows by the association of cytoskeletal subunits or polymers with transmembrane channels. The possibility that the polyelectrolyte properties of the cytoskeletal polymers contribute to the modulation of membrane potentials supports the hypothesis of a direct involvement of the cytoskeleton in intercellular communications.

**Key words**: Cytoskeleton, water, hydration, polyelectrolytes, viscoelasticity, interactions

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**Note**: This review uses a selection of articles from a huge literature on the many fields of research related to the cytoskeleton and to the interactions of water with cellular components and compartments. Many of these references are from original reports and reviews published in the last 5 years, in order to provide the reader with an up to date overview of this broad subject and an insight into the directions of future investigations. However, this choice resulted in the arbitrarly rejection of older papers of high value, of which the lack of citation does not mean that they are no more valid. Thus, the reader is invited to find these funding articles from the last few decades in the reference list of more recent reviews and articles.

## INTRODUCTION

*What is the difference between a random concentrated mixture and the living milieu that is a cytoplasm? Dynamic changes in molecular ordering is the answer*

The cytoplasm of all cells appears as a heterogenous viscous fluid, enclosed in the cytoplasmic membrane, containing many types of subcellular structures that are either in direct contact with the intracellular medium (polysomes, centrioles, fibrous polymers of the cytoskeleton) or delimited by lipid membranes (nucleus, mitochondria, Golgi and reticulum membranes, secretion or capture vesicles). In contrast with the random diffusion of suspensions of particles in a fluid medium, the instantaneous distribution and relative organization of all components of the cytoplasm is precisely controlled, and this organization is constantly under active remodeling for adaptating the cell to extracellular signals and contacts by coordinated modifications of the molecular composition, the structure and the dynamics of local cytoplasmic domains. This highly plastic adaptability suggests an integrated coordination between all cytoplasmic compartments for achieving the physical modifications of the cell that are functionally appropriate to a modified situation. A central contribution of the cytoskeleton to this complex process involves the mechanical and biochemical properties of individual polymers forming three-dimensional dynamic networks interacting together and with the other subcellular compartments through specialized associated proteins. This functional organization of the cytoskeleton as a scaffold implies likely the existence of microdomains of the cytoplasmic space in which the free diffusion of molecules (ions, nucleotides, soluble proteins) is limited by physical and chemical barriers. The very high protein density of the cytoplasm, usually described as macromolecular crowding, is thought to induce aqueous phase separation (315). In particular, the association of water molecules to cytoskeleton polymers may determine an unusual structure of the solvent which could be part of some of the physicochemical properties unique to the cytoplasm of living organisms.

*Presentation of the ordering partners. The cytoskeletal polymer families and their associates*

Three polymer species, microtubules (MTs), intermediate filaments (IFs) and actin filaments or microfilaments (F-actin or MFs) form the cytoskeleton in the majority of eukaryotic cells with the exception of some cell types such as red blood cells. MTs are rigid hollow polymers (diameter 25 nm) formed by the assembly of α and β tubulins into 11 to 15 protofilaments associated laterally to each other (average of 13 protofilaments) (67). F-actin consists of two actin protofilaments associated together in a helical fashion (7 to 10 nm diameter) (31,118,192). However, recent models of F-actin involve motion domains of actin molecules responsible for dynamic conformational changes of the filament between ribbon-like and helical structures (154,237,272). IFs (11 nm diameter) are formed by a complex family of subunits of which the cellular expression is specific of differentiated tissues (77,243). The building block of IFs is made of antiparallel homo- or hetero-dimers, which assemble longitudinally into protofilaments. The association of two protofilaments forms a protofibril and the final IF structure results from the coalignment of 4 protofibrils, a structure that is responsible for the unique mechanical resistence of IFs. All three types of polymers are at steady state in cells between freely soluble subunits and polymers, following distinct mechanisms: in contrast to the polarized MTs and MFs polymers in which a plus end favors the addition of subunits and a minus end favors the depolymerization by loss of subunits, IFs are non-polar and subunit exchange occurs along their whole length (113,308).

Structural proteins are associated to MTs, MFs and IFs polymers, whose functions range from the catalytic stabilization or the fragmentation of each type of labile polymer (MTs et MFs) to the organization of polymers into networks and their anchorage to various membranous compartments (46,53,59,112,200,254,313). A second group of cytoskeleton-associated proteins is constitued by molecular motors of the dynein (MTs), kinesin (MTs) and myosin (MFs) superfamilies (115,218). These molecules share with the structural cytoskeleton-associated proteins an organization into distinct functional domains, but their major characteristic is an energy-dependent conformational change of their secondary structure which generates their active motion along cytoskeleton polymers.

The cellular distribution of MTs, IFs, and MFs reflects their functions. MTs form the mitotic apparatus along which the active segregation of chromosomes occurs by the action of several molecular motors, the structure of centrioles and that of cilia and flagella beating based on a dynein-dependent lateral gliding of adjacent MTs (146,202). In interphasic differenciated cells, a MT network seeded in one or several MT organisation centers (MTOC: nuclear membrane and specialized domains underneath the plasmalemma in plants, pericentriolar region of animal cells) extends in the cytoplasm following a decreasing density gradient, the distal MTs being rarely in direct contact with the plasma membrane (146,202). MT-associated proteins allow the anchorage of many organelles to MTs in a static (structural MT-associated proteins: MAPs) or a dynamic (molecular

motors) fashion. In addition to this function of MTs as a rigid mechanical support for the dynamic distribution of membranous compartments, the polymerization-depolymerization mechanism of MTs determines rapid modifications of the cytoplasmic space. This phenomenon is controlled both by the molecular motors and structural stabilization factors of MTs (MAPs) (200,313). IF networks are frequently organized in a cytoplasmic pattern similar to that of MTs as a consequence of local interactions between IFs and MTs via molecular motors and associated proteins, but are also associated to the plasma membrane by anchorage to intercellular junctions and to the nuclear membrane of which the frame consists of a specific type of IFs, the lamins (77,98,112,243). The interconnection between these two cytoskeletal networks contributes in a cooperative manner to the spatial organization of the cytoplasm (MTs) and its mechanical stability (IFs). MFs form a dense network in physical contact with the plasma membrane where they participate directly into the organization of transmembrane proteins (32,93). This distribution of MFs reflects their role in cell motility (ameboïd motion) and in rapid changes of the plasma membrane associated with ruffling, phagocytosis and pinocytosis, which imply specific molecular motors (26,93,333). Such movements occur also through gelation-solation of actin filaments which is controlled by a large family of actin-binding proteins, and by the anchorage sites of MFs on the plasma membrane in contact with the extracellular matrix (26,59). MFs are also present inside the cytoplasm where they contribute to the motility of membranous organelles and multimolecular complexes in cooperation with the MT-dependent traffic (93).

The three cytoskeletal networks are interconnected through their associated proteins, thus inducing a functional cooperation between MTs, MFs and IFs. This integrated dynamic cytoskeleton forms a composite cellular structure in physical continuity between the plasma membrane and the extracellular matrix, and the nuclear membrane. A major direct consequence of the dynamic properties and the interdependance of the three cytoskeletal networks is that the cytoplasm of living cells is a highly ordered plastic medium, in which the local distribution of subcellular elements and macromolecules is in constant cooperation and control in space and time.

## WATER IN THE CYTOPLASM

→ Does water in cells behave as an anonymous isotropic solvent, as a tight tango dancing dress to macromolecules, or as a composite set of dynamic but ordered states depending on its partners? All of this together, likely.

*Viscosity of the cytoplasm*

→ One way to approach water intimity with proteins is global rather than local. Another is to count molecules where they are.

The apparent viscosity of the fluid phase of the cytoplasm in between the supramolecular structures is slightly higher than that of water (81,193,253,336). Measurements of the diffusion coefficient of a variety of inert molecules in the cytoplasm of living cells showed that the diffusion of macromolecules of size up to 500 kDa is nearly similar to that in water-based buffers (13,104,274). However, observations of the limited diffusion of molecules of size around or above 50 nm suggested that a meshwork of macromolecular structures determines local obstruction to passive diffusion (140,289). The probable contribution of the cytoskeleton to percolation and sieving processes opposing the diffusion of soluble molecules is suggested by evidence that viscosity and diffusion of particles vary within distinct cytoplasmic subdomains such as the leading and trailing regions of a moving cell, in actin-rich domains underneath the plasma membrane or in the growing processes of neurons, in which diffusion is affected by cytoskeleton drugs (140,250,289,340). From experimental data and their theoretical counterpart, the cytoplasm is generally described as a composite milieu in which percolation of soluble metabolites and proteins through the cytomatrix is the main limit to diffusion (13,24,104,270, 274). However, since many of these studies were performed using inert labelled dextrans, the current values for diffusion constants do not reflect the consequences of weak or strong interactions between diffusing soluble molecules and immobilized macromolecular structures of the cytoplasm.

Water in the cytoplasm is a free solvent, or, either mildly or tightly bound to macromolecules. NMR studies indicated that the state of water in cells differs from that of bulk water, and varies between subcellular compartments or during cell cycle events (41,110,121,281,348). A relatively large portion of cellular water is partially bound to membranes and macromolecules as hydration water (42,105,128,131). However, recent investigations in dilute protein solutions suggest that with the exception of a few stably bound water molecules, the average residence time of hydration water molecules associated with the surface of proteins is small (18,37,82,153,216). These indications support the hypothesis that water-protein interactions are much more dynamic than previously thought. Thus, the model of long-living stable organizations of water surrounding the surface of macromolecules proposed earlier (323) may be

inconsistent with these data, although more experiments are needed in living cells for understanding the dynamic behavior of water molecules in cytoplasm crowded with high concentrations of proteins and macromolecular structures, limited by the plasma membrane. It is possible that the high frequency of contacts of water molecules with the surface of proteins imposed by the cytoplasmic crowding results in the permanent modifications of water diffusion parameters observed in whole cells, however including a very dynamic component to the apparent stable hydration layers of proteins (42,105,128,131). Furthermore, specific aminoacid sequences of proteins may induce an unusual structure of water molecules at their contact, such as with antifreeze proteins that exhibit an ice-binding surface (190). This indication opens room for the possible existence of unexpected hydration properties of concentrated solutions/suspensions of many types of cellular proteins with a large variety of specialized distribution of charged and hydrophobic domains on their solvent-exposed surfaces (217). The important contribution of the cytoskeleton proteins to the total mass of cytoplasmic molecules and their organization in a physical frame make this system a probable target for privileged relationships with water molecules in living cells.

*Osmotic properties of the cytoskeleton*

➔ Water goes in and out of cells which swell or shrink. The cytoskeleton frame seems to capture water molecules and control their release by changes of its structure.

An experimental approach to the contribution of the cytoskeleton to the behavior of water and solutes in cells is based on osmotic actions on cell structure and physiology (257). The primary involvement of the F-actin network underneath the plasma membrane in the response of cells to changes in osmotic variations of the extracellular medium has been established in several reports (43,69,103,168,176, 209,220), while other data suggest in addition the coordinated contribution of MTs to this process (49,189,221,278). A few indications suggest that the physical and biochemical properties of the cytoskeleton are directly affected in the response of the cell to the osmotic shock: a direct involvement of the polymerization-depolymerization of actin in cell volume regulation has been evidenced (68,349), while other reports pointed at the importance in osmotic functions of the actin cytoskeleton of crossbridging between actin filaments or/and the phosphorylation of actin-associated proteins (43,150,168,220). In addition, some types of cells exhibit a strong resistence to swelling in hypotonic conditions, which may be the consequence of an extensive cytoskeleton-plasma membrane interaction (1,228). Altogether, the available indications on the direc contribution of the cytoskeleton to regulation of cell volum and exchanges of water, metabolites and ions with th extracellular medium led to the hypothesis that the whol actin network acts as an osmotic sensor, alone or i cooperation with MTs (43,68,257,278).

The molecular basis of such a property of cytoskeleto polymers is not well known. Indirect evidence was obtaine in model systems, suggesting that polymerizatio mechanisms of macromolecules and their organization int networks may be directly related to their osmotic propertie (57,100,158,185,273), which raises the possibility tha interactions between water molecules and protein domain of the cytoskeleton subunits are key factors in the propertie of cytoskeleton toward the behavior of water compartment in the cytoplasm. The changes of actin hydration during th transition of G-actin to F-actin may be part of the majo involvement of actin in osmotic properties of cell (78,130,287). However, other mechanisms of interaction c cytoskeletal polymers with water are likely required for the contribution to the osmotic balance of cells. A more effectiv component of this function of cytoskeleton in water contr could be the hydration level of polymers and of crossbridge polymers in cytoskeletal networks of cells, as reflected b the changes in compressibility associated wit polymerization and network formation, consecutive to modification of the area of solvent exclusion of polyme (257,312). The shrinkage of F-actin under osmotic pressur (273), the bundling of F-actin induced by osmotic stre (288), and the inhibition of osmotically driven water flow by actin filaments, regulated by the crossbridging prote ABP (129,130), suggest strongly that the immobilization c cytoskeletal polymers into crossbridged networks affe directly the mobility of water in cells. The control of osmot pressure through the water release or adsorbtion by th cytoskeleton may depend primarly on the physic organization of cytoskeletal polymers together into dense c loose networks, as suggested by the regulation of ce volume by the reversible polymerization of F-actin (349 Another element of the cell volume regulation by th cytoskeleton results from the direct control of ion channe by cytoskeletal proteins and polymers (3,124,209,221,257 The involvement of actin networks in the regulation c stretch-activated channels (90,133,334) further showed th the dynamic organization of F-actin underneath the plasm membrane is a major effector of the exchange of ion between the cell and the extracellular medium. In th perspective, the hypothesis was recently raised that th regulation of cell volume occurs through the modulation c the life time of crossbridges between filaments, affectin

consecutively the rigidity of the membrane (258).

## CONTRIBUTION OF WATER TO CYTOSKELETON PROPERTIES

➔ The structure of water molecules in contact with long cytoskeletal polymers is a crucial question, yet unsolved. The options are: a large scale clathrate-like sheet of immobilized water molecules covering the polymer; a layer of rapidly jumping water molecules at the polymer surface between bound and free states resulting in a constant flow oriented by the polymer properties; no water molecules bound to the surface of polymers but few ones buried inside the polymer structure. Indications for selecting a correct model are obtained from indirect evidence ranging from the study of the polymerization mechanisms, of the effects of $D_2O$ versus $H_2O$ on the cytoskeleton in cells and *in vitro*, and of the behavior of ions in the presence of cytoskeletal polymers. All data point to the existence of specific relationships between water and cytoskeletal structures in addition to the interactions between water and polypeptides during folding and conformational changes common to all proteins. Thus, an appropriate model of polymer hydration would assume that there exists a particular water domain surrounding polymers, the size and the properties of which are determined by the average ionic density of the polymer surface and the precise topography of hydrophobic domains and positive/negative charges specific of the surface of each type of cytoskeletal polymer. Whether water in such domains is more or less stable or highly dynamic may vary with the surface topography of the polymers. Then, the repetition of exposed motives of subunits within the polymer lattice should create a similar repetitive although dynamic structure of the surrounding water, which should be strongly affected by the binding of cytoskeleton-associated proteins as well as by post-translational modifications (i.e. phosphorylation) of both subunits and associated proteins.

*Direct involvement of water in the polymerization mechanisms of cytoskeleton subunits*

➔ Less water associated with the polymer than with its subunits may constitute an exquisite process to control cell water flows and states, since the relative size and mass of all cytoskeletal polymers together in the cytoplasmic space is very large.

The understanding of the association of water molecules to cytoskeleton monomers and during polymerization remains incomplete. Hydrophobic interactions accompagned by exclusion of hydration water are likely to contribute to many mechanisms of interaction between biological macromolecules (27,50,79). Similarly, the conformational changes of proteins and enzymatic activities involve modifications of the number of hydration water molecules (244,256,304). The polymerization of tubulin, IF subunits and actin seem to follow this principle: The direct involvement of water in the polymerization processes of actin has been demonstrated (78,287), and may contribute to the formation of hydrophobic interactions between the two actin strands (31). The polymerization of the vimentin IFs occurs through the exclusion of hydrophobic domains from exposure to water (66). The tubulin polymerization process is likewise accompagnied by water release from monomers (175,309), which is probably involved in the hydrophobic contribution to lateral interactions between MT protofilaments (227). The hydration of polymers, estimated from analysis of sound velocity in suspensions of increasing protein concentrations, differs strongly between MTs and F-actin with a much larger contribution of water to the packing density of F-actin than for MTs (312). These hydration characteristics of cytoskeletal polymers contribute directly to their elastic behavior (312).

*Heavy water has spectacular influence on the cytoskeleton*

➔ Although clearly demonstrated, the effects of $D_2O$ on the cytoskeleton remain largely mysterious: a key to new findings through more investigations? Coordinated hydration changes during muscle contraction may show the way.

Indirect evidence for a strong influence of water on cytoskeleton properties was obtained from observations of the effects of heavy water ($D_2O$) on polymerization and stability of MTs and actin filaments *in vitro* and in living cells. $D_2O$ enhances the rate of polymerization of both actin and tubulin *in vitro* without affecting their polymerization equilibrium (47,232,240). When added to cellular media, $D_2O$ determines the abnormal stabilization and cytoplasmic distribution of both actin- and tubulin-dependent structures *in vivo*, resulting often in toxic situations (162,170,171,232, 347). These observations first support the general concept that the equilibria between monomers and polymers are critical for the function of the cytoskeleton. The stabilization of MTs and F-actin by $D_2O$ in cells occurs likely through polymerization-independent mechanisms involving $D_2O$-enhanced stabilization of polymers by associated proteins. Second, these findings suggest that the differences in the physical properties of $H_2O$ and $D_2O$ molecules have direct consequences on the association between tubulin or actin monomers into polymers. A hypothesis on the molecular mechanisms of action of $D_2O$ on MTs is that it may enhance hydrophobic interactions between monomers through the

stabilization of some critical conformational steps and/or modify the strength of hydrogen bounds by substitution of deuterium for hydrogen (47,240). Using *in vitro* gelation assays of actin filaments in the presence of the actin-binding protein ABP which crosslinks individual filaments, it was shown that the presence of $D_2O$ allowed a denser gel than that obtained in $H_2O$ buffers (232), suggesting that the interaction between F-actin and associated proteins involves also water-protein relationships. Similar conclusions were reached by studies of interactions between actin and the S1 fragment of muscle myosin (22,286), leading to the hypothesis that the controlled expulsion of water from the hydration layers of actin and myosin is a major element in myofibril contraction (234,300).

*Cytoskeletal polymers as polyelectrolytes*

→ Ion clouds captured in a restricted volume, self-organization and bundling of cytoskeletal polymers, and binding of wandering molecules are unexpected consequences of monotonous polymerization of subunits.

A structure-dependent property of cytoskeleton polymers resulting from the association between subunits is the constitution of linear polyelectrolytes in the cytoplasm, the total surface of which is estimated to be much larger than that of all membranes of the cell (86,133). This situation offers binding surfaces for soluble molecules, which can be transiently or permanently bound to the polymers, thus adding another degree of ordering of the cytoplasmic medium to that of the limited diffusion of macromolecules resulting from inert hindrance to Brownian motion by the cytoskeleton scaffold. The study of mixtures of polymers and soluble globular proteins at concentrations close to that of the cytoplasm has shown that the bundling and segregation of polymers from soluble components occurs spontaneously (164). This entropy-driven phenomenon has been suggested to be based on repulsion between anionic polymers of persistence length much larger than that of globular proteins, and a similar process drives the formation of liquid crystals in a polymer length- and concentration-dependent manner (80,116,152,283,285). Although this self-organization of fibers in crowded solutions is thought to be a component of the bundling mechanism of cytoskeletal elements in cells, further regulated by associated proteins, another distinct mechanism of bundling and interaction between cytoskeletal polymers and soluble components based on their linear polyelectrolyte nature has been explored recently (292,293,335). These studies revealed that the high density of anionic charges along all types of cytoskeleton polymers allow their bundling by a large variety of cationic molecules, and the bundles obtained are characterized by properties distinct from those of liquid crystals (292,293,335). Evidence for the bundling of polymers by metallic cations, homopolymers of basic aminoacids or cytoskeleton-associated proteins and their inhibition by phosphorylated nucleotides (96,133,292,293,294) which result from numerous weak interactions along lengthy polymers, suggests how such electrostatic interactions may contribute to the organization and the regulation of the cytoskeleton in living cells by ions and nucleotides. Alternatively, the transient sequestration of cations on the large intracellular surfaces formed by the cytoskeletal polymers may limit or/and control their diffusion in the cytoplasm, thus providing a highly plastic store for these ions involved in regulation of many intracellular events (133). For example, the very important mass of cytoskeletal polymers in myofibrils of muscle cells constitutes a large anionic domain of the cytoplasm from which anions are excluded in contrast to cations (210), the bulk of $K^+$ ions being completely bound to the myofibrillar structure (187,188). The implication of the polyelectrolyte nature of cytoskeletal polymers in binding soluble enzymes (133) and other macromolecular complexes of the cytoplasm, will be analyzed later (page 911).

## POLARIZATION AND COMPARTMENTATION OF THE CYTOPLASM

→ The large scale relationships between the cytoskeleton, water and solutes in living cells occur in space delimited by membranes, in which geometry is important, and where no cytoplasmic subdomain is equivalent to another. This is a major property of living matter, which depends on the existence of molecular processes that organize the cell space and impose in each subdomain a particular polarity, viscosity, and mobility of the crowded populations of macromolecules and organelles. The cytoskeleton is a main agent of this active segregation process following simple rules: once polymerized cytoskeletal polymers form reversible three-dimensional networks which resists bulk solvent flow, thus maintaining constant the concentration of soluble proteins and small molecules inside their web. The ways to form and dissociate networks are numerous, as are their resulting structures and physical properties. Due to the length and relative stiffness of individual polymers, the resulting networks are more or less elastic, i.e. they store mechanical and chemical energy in global deformations which can be recovered by coordinated mechanical actions. In all steps of these molecular crosstalk from which arise life, a local and long range order is generated which is imposed to the water-based medium of

the cell, and where water molecules seem to play a central role.

The cytoskeleton polymers bind a very large variety of proteins. Among these are the families of specific structural MT-, F-actin- and IF-associated proteins, which were most often identified by their physical linkage to polymers during purification or their effect on polymerization equilibriums (46,53,59,112,200,254,313). The binding of these proteins to polymers is based on sequence-specific domains of the associated proteins in which the contribution of both hydrophobic and ionic interactions are involved (160).

Three major types of structural changes in the networks formed by cytoskeletal polymers affect the diffusion of soluble molecules in the cytoplasm: 1/ increase in the stiffness and the stability of polymers leading to bundling (73,127); 2/ severing, fragmentation of polymers and sequestration of monomers (53,313), and 3/ crossbridging and reticulation between polymers of similar or different types (9,10,112,147,148,254,263,326,342).

These modifications of the organization of the cytoskeleton by interactions with their associated-proteins induce important changes in the local viscosity of the cytoplasm, and determine the mechanical properties of the polymers and of the whole cell.

*Viscoelasticity of living cells*

→ A number of elegant methods have recently emerged that allow measurement of whole cell rheology. Why rheology? Because it tells of mechanical behavior of cells in action and motion, and shows how distinct cytoplasmic subdomains collaborate in a global performance. This is like ultrasound scanning of embryos *in utero*: life in action.

An integrative approach to the contribution of cytoskeleton to cell mechanics has been made by rheological studies of living cells under various experimental conditions. The viscosity of the cytoplasm depends of F-actin and MTs in distinct manners: upon addition of the F-actin depolymerizing drug cytochalasin-D, a decrease of cell viscosity was observed, while an opposite result was obtained by the stabilisation of MTs with taxol; the effect of the depolymerizing drugs colcemid or nocodazole varies with the cell type (151,317,332,337). Measurements of viscoelastic parameters showed the stiffening of spreading cells as compared to resting cells (56,298) This change in cell mechanics during spreading is likely to result from reorganization of the F-actin cytoskeleton in the cytoplasm in association with myosin II in the anterior region of the moving cell (246). The regulation of myosin light chain phosphorylation via small G-proteins affects directly the stiffness of cells (40). In addition, mutant cells lacking IFs or the actin-associated protein vinculin involved with F-actin in focal cell adhesions showed lower stiffness than wild type cells, demonstrating the integrated contribution of all cytoskeleton systems in the cell mechanical properties (91,318). Similarly, significant alteration of cell elasticity was found in mutants lacking the F-actin crossbridging protein α-actinin and/or the ABP 120 gelation factor (70).

*Viscoelasticity of cytoskeletal networks in vitro*

→ If the mechanisms of life were to be reproduced *in vitro*, one primary requirement would be to create order in a soup of molecules and structures. In this aim, a beginning is to form simple networks of cytoskeletal polymers, then increase step by step the number of players, to reach structures resembling dynamic polarized cellular networks. At the present time, this approach has demonstrated several unexpected properties of cytoskeletal networks which can explain many of their cellular functions. More is to come in future, with the use of new powerful tools neither invasive nor destructive of the delicate scaffolding of the soft matter that is based on cytoskeleton gels. In these systems, a direct access for the control of the behavior of water molecules is possible, and shows promising insights into the reality of the living cytoplasm.

The study of the viscoelastic properties of networks formed *in vitro* with pure cytoskeletal polymers is an indirect but powerful approach to the understanding of the molecular mechanisms underlying the physical properties of the cytoskeleton and their consequences on the dynamic organization of the living cells. The three types of cytoskeletal polymers exhibit distinct intrinsic physical properties: MTs are stiff rodlike structures, F-actin filaments are semi-flexible and IFs are flexible polymers (152).

– *Stiffness*: → the collective behaviors of polymers together is primarly based on the physical properties of the polymer lattice, characterized by the filament size, the number and arrangement of protofilaments, and the mechanical articulation between subunits within a polymer. Bending freedom determines stiffness level, which determines elasticity of the network.

Stiffness of MTs is increased by the binding of associated proteins, in parallel with stabilization (73,165), and identical conclusions were drawn from studies of F-actin stabilization by tropomyosin (127). Similarly, the absence of the two high molecular weight neurofilament subunits NF-H and NF-M in reassembled filaments from NF-L alone leads to mechanical instability in the polymers (36). Furthermore, the involvement in modulation of MTs and F-actin stiffness by the bound nucleotides and their hydrolysis rate (GTP/GDP-$P_i$ and ATP/ADP-$P_i$) suggest important conformational

changes in the subunits which could store energy as mechanical component in the protein lattice within the polymer (127,219,303,306). The impact of hydration water on these molecular processes has not been investigated extensively, but nucleotide hydrolysis in cytoskeletal proteins is coupled with hydration changes (286,309,330).

When analyzed by rheological methods, suspensions of MTs, F-actin and IFs reveal different viscoelastic properties (132,134,138).

– *Viscoelasticity of F-actin*: → a very elastic, plastic and dynamic type of network, but which breaks easily. Cells use it to achieve rapid deformations of their cytoplasm. They may be the muscle equivalent of the cell skeleton.

F-actin gels, the most studied structure, are characterized by a slow increase in elastic modulus after initiating polymerization, related to the progressive increase in filament length which ultimately dominates the rheologic properties of F-actin (135,136,137). The elastic modulus of F-actin at polymerization steady state is high, compared to IFs and MTs, but exhibits little resistence to increasing strains, which induce the breaking of the gel structure (132,134,138). However, the elasticity of F-actin suspensions decreases more than that of MT with increasing protein concentration, as a result of different hydration of the two types of polymers (312). The individual filaments inside actin gels are not stably associated together and their diffusion occurs in worm-like tube domains delimited by the neighbouring filaments (135,152). Adding actin binding proteins to pure F-actin gels result in strong modifications of their viscoelastic response to deformations: the ABP crosslinking protein forms a much more resistent gel to strain which does not creep under stress in contrast with control F-actin alone (136,310). Similar results were obtained studying the effects of α-actinin and ABP120 from *Dictyostelium discoideum* with pure F-actin, adding further informations on the regulation by Ca ions of the crosslinking activity of α-actinin using point mutations (142). Interestingly, cells lacking these two proteins have reduced viscoelasticity (70), which bring together with *in vitro* measurements the first comparative study of the contribution of well identified associated proteins to the physical characteristics of F-actin networks in model systems *in vitro* and in the living cells. Furthermore, major differences were found in the viscoelastic properties between F-actin made of the three actin isotypes, which are consistent with their presumed functions in cells (7). At the present time, the available data on actin networks and their regulation by associated proteins constitute the more advanced approach to the biophysical mechanisms by which one type of cytoskeletal polymer organizes the cytoplasmic space.

– *Viscoelasticity of IFs*: → flexible, resistent, stable, the are the bones of the cytoskeleton, withstanding stron stresses.

In contrast to F-actin, IFs are less elastic at low strain, bu exhibit a strain hardening under increasing strains, which i consistent with their presumed role in stabilizing the ce integrity against strong deformations. This property is share by all types of the large IF family tested (134,138,180,19 with a particularly high resistence to large strains specific fo the unusal NF polymers which are unique in havin numerous interfilament contacts through the latera projections of their HMW subunits that induce the formatio of semi parallel aligments of these flexibl IFs similar to that of NF bundles in neurons (180). Althoug several IF-associated proteins have been identified whic were shown to mediate interactions between IFs and othe cytoplasmic structures (112), no studies similar to those o F-actin have been performed yet on their effect o IF viscoelasticity, with the exception of preliminary evidenc that small heat shock proteins interacting with type III IFs i cells may strongly interfere with IF gelation *in vitro* (63 Nevertheless, the comparison between the physical strengt of gels made of NFs and vimentin or desmin IFs suggest that the presence of NF sidearms is a major determinant o the mechanical properties of NFs in axons, and raises th possibility that similar effects may be achieved by IF associated proteins in other cell types.

– *Viscoelasticity of MTs*: → lonely, stiff, however ofte bundled. Are they the brain of the cytoskeleton, organizin without direct involvement in action? Their MAPs an motors do the work.

Rheological studies of MTs are rare, and suggest tha they exhibit little viscoelasticity under small strains whic does not increase with larger strains that induce further th breaking of the sample (39,132,134,138,268,312). Thes data are consistent with the fact that MTs are stiff polyme not interacting with each others, and raise in addition th hypothesis that their associated proteins (MAPs) do no mediate significant interactions between MTs. Howeve studies of viscosity changes in high concentrations of MT purified from brain with saturated amounts of MAP showed that the weak viscosity of MT suspensions i affected by nucleotides, and by cytoplasmic organelles suc as mitochondria which bind to MTs via MAPs sidearm (179). Although preliminary, these indications support th hypothesis that weak interactions between MAPs-bearin MTs may occur, which remain to be analyzed in furthe work, such as with non-destructive methods recently devise (239,312). No evidence of neuronal MAPs-mediate crossbridging of MTs have been obtained *in vitro*, althoug

antiparallel dimers of MAPs form spontaneously (204,327). Thus, the molecular mechanisms of MT bundling induced naturally by MAPs in neurons and in non-neuronal cells by transfection with various constructs of MAPs remains elusive. Two non-exclusive hypotheses are currently evoked, which suggest either the contribution of weak interactions between MAPs through their projection domain (38,147,148), or the possibility that bundling is the indirect consequence of stabilization of MTs by MAPs following a process analogous to the segregation of long polymers which drives the formation of liquid crystals in concentrated suspensions (74,194). In contrast, the crossbridging or bundling of MTs *in vitro* for a variety of proteins including the synaptic vesicle anchoring protein synapsin, certain types of MAPs, and molecular motors of the dynein and kinesin families has been reported (9,21,122,126,149).

– *Fate of water inside cytoskeletal gels*: ➔ the presumed role of crossbridging associated proteins in packing density and water retention.

Analyses of viscoelasticity of networks formed by F-actin, IFs or MTs do not include studies of the behavior of water during gelation, or following strain-induced deformations, creeping, or hardening of gels. A few data exist, however, derived from comparison of solutions of highly viscous but non-gelling molecules with gelling mixtures: No polymer-concentration dependent change in the diffusion coefficient of water was observed in highly viscous but non-gelling solutions of guar galactomannan (34). In contrast, a low water diffusion coefficient was found in a gelling mixture of pectin and sucrose, depending on the formation of the network and which decreases with increasing concentrations of the two constituents (34). Similarly, the diffusion of water decreases in $K^+$/dextran gels as compared to that in non-gelling solutions of dextran alone (321). From both studies, a common conclusion is that the diffusion of water is reduced in direct relationship with true gel formation, in contrast to a constant diffusion coefficient of water molecules, similar to that of bulk water, in highly viscous but non-gelling solutions. This situation may result from the direct contribution of water molecules to the structure of the gelling polymers and the binding of crossbridging molecules, as shown with the gellan gum in the presence of $K^+$ ions (48). Thus, similar structure-dependent alterations can be expected for the behavior of water molecules inside biological gels, which might have important consequences for the mobility of water molecules in cells following reorganization of cytokeletal networks under mechanical stress or during the morphological adaptation of cells to extracellular signals. Evidence that diffusion of small tracers in ABP cross-linked F-actin gels is smaller than that in control F-actin gels but is not modified by another cross-linking agent (145) suggested further that the geometry of the formation of interconnected networks of cytoskeletal polymers has important consequences with regard to diffusion properties of solutes inside the gel. In addition, the ionic charges of the cytoskeleton-associated proteins, involved or not in crossbridging, interfere with the polyelectrolyte nature of the polymer surface, thus adding another element of regulation of local interactions between the network and molecules of water, ions and solutes. For example, the phosphorylation level of NF sidearms involved in regulating inter-NF interactions controls the structure of sidearms as well as NF bundling (96,180). It is likely that these effects are the consequence of interactions between water molecules and bivalent cations with the numerous phosphate groups of repetitive phosphorylation sequences of NF sidearms, resulting in a strong conformational change of the lampbrush-like structure of NF lateral projection domain (96). Similarly, intriguing alterations in the structure of MAPs sidearms were identified from the increasing packing density of sedimented MTs with aging (178), which strongly suggest that the structural function of MAPs sidearms in mediating the spacing between MTs and adjacent structures is a conformation-dependent mechanical property. These structural aspects of the regulation of cytoskeleton - crossbridging or -associated proteins remain to be analyzed with regard to their interactions with water molecules in order to appreciate their contribution to the osmotic and hydration properties of the polymers and their effect in regulating the diffusional properties of cytosolic components inside interconnected networks.

*Interactions among the three types of polymers and dynamic organization of cytoskeletal elements*

➔ Complexity is achieved by simple means. Self-organization in various patterns depends on few partners, but shows intriguing properties. Do MTs store information in their structure? And what is the mysterious hollow core of MTs: a special water store?

In addition to specific mechanical properties of each type of cytoskeletal polymer regulated by their associated proteins, a cooperative dynamic organization of the cytoskeleton in the cytoplasm is attained through functional interactions between the F-actin, MT and IF networks. These interactions are mediated by both conventional and new types of associated proteins, and the molecular motors. Interactions between MTs and F-actin are mediated by some of the MAPs family members, which can crosslink MTs with and bundle F-actin, while cell adhesion induces interactions between the F-actin crosslinking protein fimbrin

and the IFs vimentin (55,61,245,269,299,339) and crossbridges between MTs and the IFs vimentin are mediated by kinesin (98,184). Furthermore, the plakin family, containing the ubiquitous plectin molecule (263,326), consists of proteins with IF-, F-actin- and MT-binding domains allowing physical connections between the three polymers and their anchorage to cell-cell or cell-matrix junctions (112,342). The existence of inter-connections among all three cytoskeleton systems and with focal adhesion complexes of the plasma membrane, involved together in the intracellular propagation of extracellular signals, have motivated the tensegrity hypothesis, in which mechanotransduction is thought to occur through a hard-wired cytoskeleton (125). However, a direct test of the tensegrity model in living fibroblasts showed instead that the cell response to applied forces is not global: the reaction of a cell to a deformation of the plasma membrane induced by a glass needle involves exclusively the local reoganization of MF and MT cytoskeletal networks underneath the same membrane domain (108). This suggests that the rapid coordinated reorganization of the interacting cytoskeletal and membrane elements in limited cell domains is at the basis of the mechanical response of cells to local tension, instead of an integrated reaction of a tense global cytoskeleton frame predicted by the tensegrity model (108,125).

The involvement of molecular motors in controlling the respective organization of cytoskeletal polymers is a recent concept which emerged from intracellular transport studies showing that F-actin and MT-dependent motors cooperate frequently, if not systematically, for the induction and the maintenance of dynamic cytoplasmic structures and the positionning of organelles (93,322). Recent findings demonstrated that IFs are actively transported in cells by kinesin along MTs (251,343), while bidirectional saltatory motion of axonal NFs in association with MTs suggest the alternate contribution of retrograde and anterograde molecular motors (262,275,316). Saltatory motion is a common characteristic of many intracellular movements (4,161,163,236), thus suggesting the implication of several molecular motors organized together in functional units (20,123). A major consequence of the findings that motors are involved in interactions between cytoskeletal polymers is that their action might contribute to determine the dynamic geometry and the spatial organization of cytoskeleton-based cytoplasmic structures such as the spindle body of dividing cells or the dendritic and axonal MTs and MT-NF bundles and the MT-F-actin complex in the growth cone of elongating neurites (2,203,259,265,276, 277,307,344,345). This possibility has been adressed with *in vitro* models in which both the presence of motors and the geometry of th limiting compartment determine the morphology of th cytoskeleton arrays (107,120,223). The self-organization c cytoskeletal polymers in the presence of a limited number c associated proteins may thus be a major principle of th pattern formation of cytoskeleton arrangements in livin cells involved in morphogenesis or differentiated ce specialization. Furthermore, evidence that gravity dependent reaction-diffusion limited MT patterns occur i suspensions *in vitro* (52,241,290,291) and *in vivo* (242 showed that MTs are self-organizing dissipative structures In addition, theoretical propositions for coherent mechanica ordering within the MT structure through interactions o tubulin monomers with the water molecules of their hollo core (143,211) bring support to a theory of informatio processing in MT structure (248). The intriguing recen finding that a motion of gold-coupled antibodies on the M surface occurs in the absence of direct binding to the moto kinesin or dynein but as a consequence of these moto dependent motions of MTs, adds experimental support fo such hypotheses, since these data suggest that coheren conformational changes of tubulin drive this lateral diffusio of bound proteins, a process which could be also at the origi of the diffusion-dependent sliding of the STOP protein o MTs (201,311).

From these attractive hypotheses, the possibility is likel that the physicochemical properties of cytoskeletal polymer determine several levels of molecular ordering of th cytoplasm, from the controlled diffusion of ions and solub proteins resulting from their organization as an anion cytoplasmic scaffold, to sophisticated topograph integration of signals inside their three-dimension structures. This latter level may also involve the stron cooperativity between monomers, inducing conformation transitions during the polymerization mechanism, or insi polymers following the binding of associated protei (72,252). Similarly, the binding of associated proteins or t activity of cytoskeleton-associated motors exhil cooperativity, which may result in a discontinuous (patch distribution of associated proteins along the polym (45,101,197,314,320) or induce long range conformation changes of the polymer subunits such as in actomyosin which a change in the twist of F-actin is thought to produ the contraction force (177,272).

*Molecular motors*

→ Molecules that move on their own, provided son fuel: a great discovery of the last 20 years. But the rail seen to do as much work as the engine. How water molecul covering the MTs or F-actin cytoskeletal rail escape or ha

the moving machine remains to be found. Are all motors working as water-jets?

The MTs or F-actin-dependent motion of the molecular motors of the myosin, dynein, and kinesin families are spectacular illustrations of directional active translocation of macromolecules, a behavior in strong contrast to random diffusion. This result is achieved by the precise temporal coordination between several cooperative events which have been explored in detail with kinesin molecules: The binding and hydrolysis of ATP within one of the motor domains bound to the MT wall results in its strong conformational change which affects the binding to tubulin and is transmitted to the neck region outside the motor domain pointing toward the direction of motion. The neck acts as a force amplifier through another conformational modification and transfers it to the unbound motor domain, bringing it closer to the next tubulin dimer. Once both motor heads are bound to MTs, the exchange between ADP and ATP in the forward motor head brings the neck back to its previous conformation thus allowing the detachment of the first motor head (266). Depending on the structure of neck domain specific for kinesin subtypes, motion occurs toward the $^{+}$ or the $^{-}$ end of the MT (71,111,266). Distinct roles of the head and the neck domains of myosins have been established, although with a different function of the neck or lever arm in regulating myosin velocity than with kinesin, but its orientation seems also to determine directionality of the movement (324,341). An interesting feature of the mechanism of the motion of motors is their variable processivity, which seems to depend essentially upon nucleotide-dependent interactions with the actin or the tubulin substrate, and can be regulated by specific motor-associated proteins (155,214,279). Some data suggest conformational modifications of tubulin induced by the binding of kinesin, and the direct involvement of the C-terminal region of tubulin in motor processivity has been demonstrated (117,319,331). These elements support the hypothesis of a biased diffusional Brownian model of the mechanism of motion, in which both the motor molecule and the subunits of the polymer substrate are active partners of ATP-modulated interactions (14,16,261). Such models are particularly consistent for explaining the motion of monomeric motor molecules (214,230,341). Due to the stoichiometry of interaction with the polymer lattice, the resulting motion driven by molecular motors consists of multiples of minimal quanta of displacement, which appear to be an universal characteristic of biological motile events (25,58,249,280,341). The contribution of water molecules to the submolecular mechanisms of motility is not established. However, it is likely that important changes in the hydration water of both motors and the F-actin or the MT protofilaments take place during the coordinated conformational modifications of interactions during the motile quantal steps, and may be particularly involved in processivity. From comparison between all types of motions analyzed, including the F0-F1 ATPase, the bacterial flagellar rotor, nucleic acid-dependent motors, the linear motors and their induced F-actin and MT rotations (65,156,157,191, 272), the hypothesis was raised that a common mechanism of motion for all molecular motors involve water jets (15,235).

*Functional association of enzymes and multi-enzymatic machines with the cytoskeleton*

→ The existence of a large intracellular surface offered by the cytoskeleton allows nesting opportunities for many birds: enzymes and multi-enzymatic complexes. Some are permanent hosts, others bind transiently and go. All profit of the reduced Brownian agitation compared to the bulk water medium to accomplish their tasks.

The cytoskeletal frame established through interactions between subunits, associated proteins and molecular motors forms a three-dimensional dynamic scaffold on which several macromolecular systems are anchored. These include signalling enzymes (29,30,133), glycolytic enzymes (222,238), mRNA and polyribosomes (17,23,99, 141,199,231) and multiprotein complexes of the folding and degradation machineries (11,12,64,83,106,144,183). Of particular interest with regard to the role of water in the efficiency of the activity of such cytoskeleton-bound complexes is the situation of glycolytic enzymes which are normally soluble molecules but, when bound to the cytoskeleton, are thought to determine the metabolic channelling of substrates passing from one enzyme to the other, thus increasing the efficiency of the chain of reactions and its regulation (5,95,159). The anchoring of macromolecular complexes to the cytoskeleton is expected to result either in increasing their global enzymatic activity (altering and changing the control mechanisms of glycolytic enzymes), or in the creation of an integrated reactive device as suggested by the relation of the cytoskeleton to cellular signalling, achieved by specific associations between many transduction enzymes (kinases, phosphatases) with their cytoskeletal substrates (29,30,133). In this line, the association between aggresomes and the proteasome core with the cytoskeleton, are equally thought to produce significant functional associations between enzymatic machineries and the cytoskeletal proteins which they fold and degrade, as well as to provide a dynamic distribution of these multimolecular enzymatic particles for substrates other

than cytoskeletal proteins present in different regions of the cytoplasm. The binding of subpopulations of mRNA and polysomes to the cytoskeleton through specific molecular mechanisms is likely related to similar requirements for the proper delivery of newly synthesized polypeptides to their subcellular targets, beside the obvious structural advantage of the production of nascent polymer subunits in close proximity to their incorporation site into the preexisting cytoskeletal frame such as for β-actin (102,255)

Altogether, the increasing evidence for the existence of integrated and dynamic complexes between cytoskeleton networks and previously unrelated subcellular structures is a strong indication for the generalisation of this concept to the organization of all cellular compartments (30). Following this line of reasoning, the possibility exists that very few molecules diffuse freely throughout the whole cytoplasmic medium, but instead, that diffusion is the exception in trajectories of molecules from one binding structure to the next. In such a scheme, the role of water molecules would be that of a solvation medium (bulk water) carrying molecules between spatially close hydration layers surrounding macromolecular structures in which the organization, possibly the stability and residential time, of water molecules might differ following the aminoacid architecture of proteins that is exposed to the solvent (217), as suggested by the sequence-dependent properties of antifreeze proteins (190).

*Particularities of cytoskeleton-membrane interactions*

→ At the limit of the intracellular world is a closed border guarded by a hydrophobic line. Exchanging with the outer world implies special partners with bodies crossing the border, and special arrangements with channelling factors. Regulating two-dimensional topography favors crosstalks, and modulation of water and ion fluxes makes a possible language. Does the cytoskeleton allow a body language of the cell?

The cytoskeleton is anchored to the plasma membrane through non-specialized and through focal adhesion domains (59), and other specialized structures involved in intercellular contacts (226,229). Static or/and dynamic links between the cytoskeleton and intracellular membranous organelles involve specific members of the large family of molecular motors, structural crossbridging membranes or cytoskeleton-associated proteins, and integral membrane proteins which contribute together to the morphology, intracellular positioning and motility specific of each type of cytoplasmic membranous compartment following a complex sorting process (94,115,260).

A common feature of these membrane-cytoskeleton multimolecular complexes is the presence of lipid layers in or to which partner proteins are embedded, bound or weak associated. The bidimensional mobility of transmembra proteins in lipid bilayers is directly or indirectly affected the membrane skeleton and the cytoskeleton, as a result fro the strength of interactions between proteins of the tw structures (28,139,166,264,267,271,282,328). The physiologi consequences of this topographic constraint are numerou the tension-induced reinforcement of integrin-bas adhesion sites is the result of clustering of integrin molecul through the recruitment of integrin- and actin- bindi proteins in the immediate submembranous cytoplasm domain (59,133), and the cytoskeleton-dependent clusteri of many plasma membrane proteins such as N-CAI neuroreceptors, Na/K ATPase and other ion channels is a k event in their physiological function at specific locations the cell surface (28,60,114,167,205,215,325,328). Simil conclusions were reached when studying intracellul membranous compartments such as tubulovesicul reticulum membranes or mitochondria in which clusters membrane proteins are induced by interactions with t cytoskeleton (6,181).

These functional relationships between cytoskelet elements and membranes might involve particul interactions of the cytoskeletal polymers with wat molecules at the vicinity of lipid bilayers, where lipid-bou water molecules contribute to dipole potentials membranes (33,85). Model systems suggested that t interaction between proteins and lipid membranes affe both the organization of lipid molecules and the activity the protein, and that the hydration water plays a key role these interactions (85,87,284). Detailed indications for su rearrangements were obtained from studies of the binding the protein kinase C (PKC) to membranes, which occu through a higher hydration of the enzyme (88). The bindi to membranes promotes the activity of PKC in dire correlation with the phase of the lipids (89). Thus, t activation of PKC in cell membranes by its activat diacylglycerol could be the consequence of the induction l diacylglycerol of the transition of the bilayer to hexagonal cubic phases (92). Similar mechanisms might be at the hea of interactions between cytoskeletal proteins an membranes. The binding of actin to lipid bilayers throug tight interaction with few lipid molecules induc conformational change of the protein (87,329). The insertio of tubulin into biological membranes involv postraductional modifications of the tubulin polypepti (acetylation, palmytoylation) and the interaction of t modified tubulin subunit with transmembrane protei (8,346). The binding of the vimentin IF subunit to lip bilayers involves both electrostatic interactions of t

N-terminal domain of the protein with acidic phospholipids and hydrophobic interactions of the a helical rod domain of the molecule with neutral lipids, a finding that is in support of priviledged associations between IFs and membranes in most cell types (247). Actin-binding proteins, the MT-associated proteins MAP1b and MAP2 and the molecular motor dynein were shown also to bind directly to phospholipids through electrostatic interactions (75,109, 139,169,338). The direct involvement of the surface organization of phospholipids in driving the polymerization of cortical actin filaments in interphase cells and cytokinesis of late telophase of dividing cells (302) and the actin-dependent organization of cell surface protein complexes with lipid rafts (119) indicate further the existence of reciprocal interactions between the cytoskeleton and specific lipid components of the mosaic structure of the membrane. Since the relative organization of lipid species in membranes is strongly influenced by their hydration level (84), the interdependent topography of membrane and cytoskeleton results likely in local alterations of the dynamic structure of the higher water density in the vicinity of the membrane layer (225).

Numerous reports demonstrate the regulation of transmembrane receptor proteins and ion channels by interaction with cytoskeletal proteins, and cytoskeleton-mediated membrane tension changes affect the opening of ion channels (114,133,167,198,205). In addition, broader scale physiological interactions between cytoskeletal polymers and the membrane potential are presumed from studies of the influence on cytoskeleton of electric fields, which may derive directly from the polyelectrolyte nature of the cytoskeleton polymers (174). The direction of motility of cells in culture is affected by the application of electric fields (19,35,54,195,233), which are known to enhance neurite growth and axonal regeneration of neurons (212,224). Direct evidence for the possible involvement of MTs and F-actin in such cellular behaviors were obtained with pure polymers *in vitro*: MTs orient in parallel arrays under electric and magnetic fields, from which the production of electric currents in MTs was suggested (301,305), and F-actin carries electric currents following stimulation by electric fields, likely as a consequence of the displacement of the counterions bound to the polyanionic surface of the polymers (44,186). These observations support the suggestion that electric fields determine the orientation of MT and F-actin polymers in cells (19,51,195,213), and may form a basis for the hypothesis that reciprocal interactions occur between membrane potentials and cytoskeleton arrays underneath the plasma membrane such as in axons in which MTs were shown to participate directly in the generation of Na currents, through interactions with the Na channels (206,207,208,297). The occurence of a rapid change in birefringence in response to electric stimulation of axons has been frequently assumed to result exclusively from a coherent and transient reorientation of Na channel polypeptides in the membrane associated with an increase in axonal water (172,295,296). However, the possible contribution of MTs to this birefringent response cannot be excluded, since it is reduced by colchicine, an observation which is consistent with the effect of colchicine on Na currents in isolated squid giant axons (173,208). Taken together, these data suggest a highly cooperative interaction between cytoskeleton and membrane in the generation of action potentials, through electric and physical coupling between MT and F-actin polymers and membrane channels. The direct change in water content in nerves correlated with the transient birefringent changes in axonal membrane (and cytoskeleton?) during electric stimulation is indicative of such coordinated events between the two structures, in which the polyelectrolyte and osmotic properties of the cytoskeletal polymers are likely involved. The possibility has been suggested that the membrane channels may self-organize in a dissipative structure (76,182). Thus, in the perspective of integrated functional coordination between membrane channels and membrane-associated cytoskeleton, the dissipative structures of membrane channels and MTs (52,241,242,290,291) could constitute together a highly sensitive membrane-cytoskeletal organization for the generation of subcellular patterns involved in memory and plasticity of neurons. While most of the investigations on interactions between cytoskeleton and membrane potentials following the stimulation of axons were made over 10 years ago, the recent findings on the polyelectrolyte properties of cytoskeleton polymers might bring new avenues for the exploration of this critical aspect of cellular communication.

## PROBLEMS AND PERSPECTIVES

The implication of water in interactions between macromolecules of the cytoskeleton together and with their subcellular partners is presently a puzzle of single pieces of knowledge remaining to be assembled at the scale of cellular vital functions. The indications for the involvement of water molecules in biochemical and biophysical properties of cytoskeletal polymers reviewed here point at several lines of developing research areas which could drive to a much deeper understanding of the cellular functions of water in mediating communications between the thousands of molecular species in the crowded, although highly ordered

cytoplasm. Among the most obvious evidence for a specific role of water molecules in the properties of subunits and polymers of the cytoskeletal proteins arises from the effects of heavy water on these structures *in vivo* and *in vitro*. The current practical experience of using $D_2O$-containing buffers with fragile structures such as MTs and NFs *in vitro* demonstrate that their most sensitive physicochemical properties such as multiple cycles of temperature-dependent polymerization of MTs, the bundling of NFs in the presence of Mg ions over cycles of gelation and disruption by mechanical shearing (180), and the dynein- and kinesins-mediated bidirectional motility of NF fragments along MTs (275), are preserved for days (even months for NF properties) in buffers containing 50-80% $D_2O$. Thus, it seems that $D_2O$ allows *in vitro* what $H_2O$ does *in vivo*. This mystery is likely at the heart of specific properties of cell water molecules which are not appreciated yet. Although subjective, these observations could prepare the ground for systematic analyses of the reversible conformational dynamics of cytoskeletal subunits between their soluble and polymerized states which are likely underlying this behavior of cytoskeletal structures in $D_2O$ buffers.

Similarly, unraveling the controlled involvement of water in the mechanisms of motion of molecular motors along their polymer substrates is likely a key to future investigations of a possible unified principle of directed motion at the basis of intracellular traffic. Of particular interest with respect to hydration as a significant molecular property of proteins is the concept that the motion of motors results from coordinated conformational modifications of both the motor molecule and the subunits of the polymer on which it binds at a given time. The existence of a subclass of the kinesin family [Kin I proteins (266)] which, instead of moving on MTs, destabilizes and depolymerizes MTs, is a strong indication for the importance of local alterations in the structure of the polymer lattice in response to the binding of associated proteins and motors, a notion that is also at the heart of several models of myosin-induced changes in the twist of F-actin in the course of muscle contraction (272). An extension of the idea that discrete and local conformational changes occur at the surface of polymers, which could be propagated by subunit cooperativity, is found in studies of cooperative binding of MAPs to MTs and the cooperative modification of F-actin following the binding of associated proteins, and may explain the diffusional translocation of proteins bound to MT polymers in the absence of a direct contribution of molecular motors. The fact that the C-terminal domain of tubulin exposed at the surface of MTs which binds motors and determines their processivity, has not been fully defined in crystal structure analysis of MTs (a consequence of the disorder induced by the charge density the variable region of the last 10-18 aminoacids (é supports further the possibility that this domain (and hydration state) plays a crucial role in the dialogue betwe MTs and their numerous binding partners.

The supramolecular organization of subunits i cytoskeletal polymers determines the emergence of n phenomena, which are most clearly represented by polyelectrolyte nature of all polymers and the se organization of concentrated suspensions following distil processes such as liquid crystal arrays, motor- crossbridging protein-dependent bundles, asters and ge and dissipative energy structures. The probability for spontaneous organization of cytoskeletal polymers i liquid crystal arrays in living cells is high: In cytoplasn subdomains such as axons and dendrites of neurons, concentration of MTs is close to 200 µM (estimated fr their density in electron microscopic sections compared that of sedimented MTs); F-actin is concentrated in grow cones of neurons, in the front lamella of moving cells or muscle cells (were F-actin concentration is about 10 tim the concentration of 60 µM that is required for the nema phase shift). These properties of cytoskeletal structures ha profound consequences on the structure of water and solu in the cytoplasm. Whether a hydration layer of polymers i stable, metastable or highly labile organization of wa molecules on the surface of the polymer remains to established. Nevertheless, evidence for the dir consequences of crossbridging between polymers on t diffusional characteristics of water is emerging. If transpos to the real situation of the cytoskeleton in cells, th indications suggest that all cellular events involving chang in the stability, the stiffness and the relative organization cytoskeletal elements through associated and crossbridgi proteins, likely result in strong modifications of the lo diffusion of water and solutes. The numerous observatio of the contribution of crossbridging and polymerization the cytoskeleton to the osmotic properties of living cells a in direct support of this hypothesis. The importance of t polyelectrolyte function of cytoskeletal polymers in th spatial organization together and the association of ma cellular proteins, including non-cytoskeleton molecules, the cytoskeleton scaffold, has been reviewed recently (13 Moreover, another consequence of this property to consid with respect to the presence of a cloud of counterio surrounding the polymer structure, is the channelling of i transport along the cytoskeleton (44,186), possibly relation with membrane potentials and electric fields. W new developments of this approach in the next years, o can expect that the studies of functional interactions betwe

the plasma membrane and the cytoskeleton might expand to investigate the possibility of reciprocal modulation processes between the two systems. The importance of cytoskeleton-dependent clustering of transmembrane proteins in their cellular functions has been established for years. However, the possibility that the same principle applies to the distribution of ion channels has not been yet explored to its full development in terms of modulation of electrophysiological signals, although the local accumulation of Na/K channels at the axon hillock through interactions with the cytoskeleton is known to create a retrograde potential barrier for the axonal depolarization wave (328). Thus, it is possible that the cytoskeleton-dependent topography of ion channels on the surface of all membrane compartments of neurons is involved in modulating the intensity and frequency of potentials. If this hypothesis is validated by experiments, the cooperative interactions between the cytoskeleton and ion channels and other transmembrane proteins migh be part of a coding process allowing the reciprocal communication of information between the organization state of the cytoskeleton and that of the membrane surface which migh be translated into the firing pattern of neurons. Information processing in the cytoskeleton structure was frequently suggested, with a particular focus on the MT lattice (62,143,211,248). The experimental findings supporting such a hypothesis are rare, but the evidence that MTs are dissipative structures is a major one. Thus, future explorations of the complex dynamic behavior of membrane-cytoskeleton interactions in the control of fluxes of ions and water by osmotic and polyelectrolyte properties of cytoskeletal polymers as well as by direct protein-protein interactions may bring clues to this critical question.

The protein density in the cytoplasm is very high. Such a molecular crowding is thought to drive competition for water between proteins and may be at the origin of many multimolecular complexes (30). This situation is far from the conditions usually adopted for experimental approaches of biochemical and biophysical interactions between enzymes or cytoskeletal proteins *in vitro*. Protein densities such as those *in situ* are accessible *in vitro* when using sedimentation forces for the packing of cytoskeletal polymers, in which the spacing distance between MTs, IFs or F-actin can be compared with similar bundles *in vivo* by electron microscopy. From such situations, unsolved problems arise for the transposition of information obtained *in vitro* with relatively dilute suspensions to their possible significance in living cells. For example, packing NFs by centrifugation to densities as high as in axon bundles requires centrifugation forces which cause denaturation of the proteins. The comparison of inter-NF spacing in semi-parallel arrays formed in NF gels *in vitro* (3-8 mg/ml) with that of axonal NF bundles suggests that NF concentration in axons is higher than 15-20 mg/ml, a situation which cannot be reached *in vitro*. Furthermore, NFs are transported along MTs through molecular motors in neurons (262,316), and similar studies of MT-dependent motion of NFs *in vitro* can be analyzed only in very dilute conditions, since higher NF concentrations would result in gel formation and a total immobilization of all polymers together (275). In order to study the behavior of cytoskeletal polymers and their regulation at the molecular and submolecular levels under experimental conditions close to that of cells, a complete rethinking of the design of experiments *in vitro* is required. Such a line of investigations would be based on the use of concentrated solutions of proteins in buffers that remain to be defined, following a deeper understanding of the structure of water-protein complexes in living cells, and possibly inside membrane-limited compartments (97).

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## REFERENCES

1. Aitken, P.G., Borgdorff, A.J., Juta, A.J.A., Kiehart, D.P., Somjen, G.G. and Wadman, W.J., Volume changes induced by osmotic stress in freshly isolated rat hippocampal neurons. *Pflugers Arch.* 1998, **436**: 991-998.
2. Ahmad, F.J., Hughey, J., Wittmann, T., Hyman, A., Greaser, M. and Baas, P.W., Motor proteins regulate force interactions between microtubules and microfilaments in the axon. *Nat. Cell Biol.* 2000, **2**: 276-280.
3. Ahmed, N., Ramjeesingh, M., Wong, S., Varga, A., Garami, E. and Bear, C.E., Chloride channel activity of ClC-2 is modified by the actin cytoskeleton. *Biochem. J.* 2000, **352**: 789-794.
4. Alexander, S.P. and Rieder, C.L., Chromosome motion during attachment to the vertebrate spindle: initial saltatory-like behavior of chromosomes and quantitative analysis of force production by nascent kinetochore fibers. *J. Cell Biol.* 1991, **113**: 805-815.
5. Al-Habori, M., Microcompartmentation, metabolic channelling and carbohydrate metabolism. *Int. J. Biochem. Cell Biol.* 1995, **27**: 123-132.
6. Allan, V. and Vale, R., Movement of membrane tubules along microtubules *in vitro*: evidence for specialized sites of motor attachment. *J. Cell Sci.* 1994, **107**: 1885-1897.
7. Allen, P.G., Shuster, C.B., Kas, J., Chaponnier, C., Janmey, P.A. and Herman, I.M., Phalloidin binding and rheological differences among actin isoforms. *Biochemistry* 1996, **35**: 14062-14069.
8. Alonso, A.C., Nunez-Fernandez, M., Beltramo, D.M., Casale, C.H. and Barra, H.S., $Na^+,K^+$-ATPase was found to be the membrane component responsible for the hydrophobic behavior of the brain

membrane tubulin. *Biochem. Biophys. Res. Commun.* 1998, 253: 824-827.
9. Amos, L.A., Brain dynein crossbridges microtubules into bundles. *J. Cell Sci.* 1989, **93**: 19-28.
10. Andrews, S.B., Gallant, P.E., Leapman, R.D., Schnapp, B.J. and Reese, T.S., Single kinesin molecules crossbridge microtubules *in vitro*. *Proc. Natl. Acad. Sci. USA* 1993, **90**: 6503-6507.
11. Arai, H. and Atomi, Y., Chaperone activity of α B-crystallin suppresses tubulin aggregation through complex formation. *Cell Struct. Funct.* 1997, **22**: 539-544.
12. Arcangeletti, C., Sutterlin, R., Aebi, U., De Conto, F., Missorini, S., Chezzi, C. and Sherret, K., Visualization of prosomes (MCP-proteasomes), intermediate filaments and actin networks by "instantaneous fixation" preserving the cytoskeleton. *J. Struct. Biol.* 1997, **119**: 35-58.
13. Arrio-Dupont, M., Cribier, S., Foucault, G., Devaux, P.F. and d'Albis, A., Diffusion of fluorescently labelled macromolecules in cultured muscle cells. *Biophys. J.* 1996, **70**: 2327-2332.
14. Astumian, R.D., The role of thermal activation in motion and force generation by molecular motors. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 2000, **355**: 511-522.
15. Astumian, R.D. and Derenyi, I., Fluctuation driven transport and models of molecular motors and pumps. *Eur. Biophys. J.* 1998, 27: 474-489.
16. Astumian, R.D. and Derenyi, I., A chemically reversible Brownian motor: application to kinesin and ncd. *Biophys. J.* 1999, 77: 993-1002.
17. Bassell, G. and Singer, R.H., mRNA and cytoskeletal filaments. *Curr. Opin. Cell Biol.* 1997, **9**: 109-115.
18. Belton, P.S., Nuclear magnetic resonance studies of the hydration of proteins and DNA. *Cell. Mol. Life Sci.* 2000, 57: 993-998.
19. Bereiter-Hahn, J. and Luers, H., Subcellular tension fields and mechanical resistence of the lamella front related to the direction of locomotion. *Cell Biochem. Biophys.* 1998, **29**: 243-262.
20. Beningo, K.A., Lillie, S.H. and Brown, S.S., The yeast kinesin-related protein Smy1p exerts its effects on the class V myosin Myo2p via a physical interaction. *Mol. Biol. Cell* 2000, **11**: 697-702.
21. Bennett, A.F. and Baines, A.J., Bundling of microtubules by synapsin 1. Characterization of bundling and interaction of distinct sites in synapsin 1 head and tail domains with different sites in tubulin. *Eur. J. Biochem.* 1992, **206**: 783-792.
22. Bertrand, R., Derancourt, J. and Kassab, R., Probing the hydrophobic interactions in the skeletal actomyosin subfragment 1 and its nucleotide complexes by zero-length cross-linking with a nickel-peptide chelate. *Biochemistry* 1997, **36**: 9703-9714.
23. Bloom, K. and Beach, D.L., mRNA localization: motile RNA, asymmetric anchors. *Curr. Opin. Microbiol.* 1999, 2: 604-609.
24. Blum, J.J., Lawler, G., Reed, M. and Shin, I., Effect of cytoskeletal geometry on intracellular diffusion. *Biophys. J.* 1989, **56**: 995-1005.
25. Blyakhman, F., Tourovskaya, A. and Pollack, G.H., Intact connecting filaments change lenght in 2.3-nm quanta. *Adv. Exp. Med. Biol.* 2000, **481**: 305-315.
26. Borisy, G.G. and Svitkina, T.M., Actin machinery: pushing the envelope. *Curr. Opin. Cell Biol.* 2000, **12**: 104-112.
27. Brandsdal, B.O. and Smalas, A.O., Evaluation of protein-protein association energies by free energy perturbation calculations. *Prot. Eng.* 2000, **14**: 239-245.
28. Braun, N., Schikorski, T. and Zimmermann, H., Cytoplasmic segregation and cytoskeletal organization in the electric catfish giant electromotoneuron with special reference to the axon hillock region. *Neuroscience* 1993, **52**: 745-756.
29. Bray, D., Protein molecules as computational elements in living cells. *Nature* 1995, **376**: 307-312.
30. Bray, D., Signaling complexes: biophysical constraints on intracellular communications. *Annu. Rev. Biophys. Biomol. Struct.* 1998, 27: 59-75.
31. Bremer, A., Henn, C., Goldie, K.N., Engel, A., Smith, P.R., Aebi, U., Toward atomic interpretation of F-actin filament three-dimensi reconstructions. *J. Mol. Biol.* 1994, **242**: 683-700.
32. Bretscher, A., Regulation of cortical structure by the ezrin-radi moezin protein family. *Curr. Opin. Cell Biol.* 1999, **11**: 109-116.
33. Brockman, H., Dipole potentials of lipid membranes., *Chem. F Lipids* 1994, **73**: 57-79.
34. Brosio, E., D'Ubaldo, A. and Verzegnassi, B., Pulsed field grad spin-echo NMR measurement of water diffusion coefficien thickening and gelling agents: guar galactomannan solutions pectin gels. *Cell. Mol. Biol.* 1994, **40**: 569-573.
35. Brown, M.J. and Loew, L.M., Electric field-directed fibrobl locomotion involves cell surface molecular reorganization an calcium independent. *J. Cell Biol.* 1994, **127**: 117-128.
36. Brown, H.G., Troncoso, J.C. and Hoh, J.H., Neurofilamen homopolymers are less mechanically stable than na neurofilaments. *J. Microsc.* 1998, **191**: 229-237.
37. Bryant, R.G., The dynamics of water-protein interactions. *Annu. I Biophys. Biomol. Struct.* 1996, **25**: 29-53.
38. Burgin, K.E., Ludin, B., Ferralli, J. and Matus, A., Bundling microtubules in transfected cells does not involve an autonom dimerization site on the MAP2 molecule. *Mol. Biol. Cell* 1994, 5: 5 517.
39. Buxbaum, R.E., Dennerll, T., Weiss, S. and Heidemann, S.R., F-a and microtubule suspensions as indeterminate fluids. *Science* 1 **235**: 1511-1514.
40. Cai, S., Pestic-Dragovitch, L., O'Donnell, M.E., Wang, N., Ingber, Elson, E. and De Lanerolle, P., Regulation of cytoskeletal mechan and cell growth by myosin light chain phosphorylation. *Am. J. Physi* 1998, 275: 1349-1356.
41. Callahan, D.E., Deamond, S.F., Creasey, D.C., Trapane, T.L., Bru S.A., Ts'o, P.O. and Kan, L.S., NMR studies of intracellular wate 300 MHz: T2-specific relaxation mechanisms in synchronized EGF-stimulated cells. *Magn. Reson. Med.* 1991, **22**: 68-80.
42. Cameron, I.L., Kanal, K.M., Keener, C.R. and Fullerton, G.D., mechanistic view of the non-ideal osmotic and motional behavior intracellular water. *Cell Biol. Int.* 1997, **21**: 99-113.
43. Cantiello, H.F., Role of actin filament organization in cell volume a ion channel regulation. *J. Exp. Zool.* 1997, 279: 425-435.
44. Cantiello, H.F., Patenaude, C. and Zaner, K., Osmotically induc electrical signals from actin filaments. *Biophys. J.* 1991, **59**: 12 1289.
45. Case, R.B., Rice, S., Hart, C.L., Ly, B. and Vale, R.D., Role of kinesin neck linker and catalytic core in the microtubule-bas motility. *Curr. Biol.* 2000, **10**: 157-160.
46. Cassimeris, L., Accessory proteins regulation of microtubu dynamics throughout the cell cycle. *Curr. Opin. Cell Biol.* 1998, 134-141.
47. Chakrabarti, G., Kim, S., Gupta, M.L.Jr., Barton, J.S. and Himes, R. Stabilization of tubulin by deuterium oxide. *Biochemistry* 1999, 3067-3072.
48. Chandrasekaran, R., Interactions of ordered water and cations in gel-forming polysaccharide gellan gum. *Adv. Exp. Med. Biol.* 19 **302**: 773-784.
49. Chen, J.G. and Kempson, S.A., Osmoregulation of neutral aminoa transport. *Proc. Soc. Exp. Biol. Med.* 1995, **210**: 1-6.
50. Cheng, Y.K. and Rossky, P.J., Surface topography dependence biomolecular hydrophobic hydration. *Nature* 1998, **392**: 696-699.
51. Cho, M.R., Thatte, H.S., Lee, R.C. and Golan, D.E., Reorganization microfilament structure induced by ac electric fields. *FASEB J.* 19 **10**: 1552-1558.
52. Chou, K.C., Zhang, C.T. and Maggiora, G.M., Solitary wa dynamics as a mechanism for explaining the internal motion duri microtubule growth. *Biopolymers* 1994, **34**: 143-153.
53. Cooper, J.A. and Schafer, D.A., Control of actin assembly a disassembly at filament ends. *Curr. Opin. Cell Biol.* 2000, **12**: 97-10

54. Cooper, M.S. and Schliwa, M., Electrical and ionic controls of tissue cell locomotion in DC electric fields. *J. Neurosci. Res.* 1985, **13**: 223-244.
55. Correia, K., Chu. D., Chou, Y.-H., Goldman, R.D. and Matsudaira, P., Integrating the actin and vimentin cytoskeletons: adhesion-dependent formation of fimbrin-vimentin complexes in macrophages. *J. Cell Biol.* 1999, **146**: 831-842.
56. Coughlin, M.F. and Stamenovic, D., A tensegrity model of the cytoskeleton in spread and round cells. *J. Biomech. Eng.* 1998, **120**: 770-777.
57. Courtenay, E.S., Capp, M.W., Anderson, C.F. and Record, M.T. Jr., Vapor pressure osmometry studies of osmolyte-protein interactions: implications for the action of osmoprotectants *in vivo* and for the interpretation of "osmotic stress" experiments *in vitro*. *Biochemistry* 2000, **39**: 4455-4471.
58. Coy, D.L., Wagenbach, M. and Howard, J., Kinesin takes one 8-nm step for each ATP that it hydrolyzes. *J. Biol. Chem.* 1999, **274**: 3667-3671.
59. Critchley, D.R., Focal adhesions - the cytoskeletal connection. *Curr. Opin. Cell Biol.* 2000, **12**: 133-139.
60. Crossin, K.L. and Krushel, L.A., Cellular signaling by neural cell adhesion molecules of the immunoglobulin superfamily. *Dev. Dyn.* 2000, **218**: 260-279.
61. Cunningham, C.C., Leclerc, N., Flanagan, L.A., Lu, M., Janmey, P.A. and Kosik, K.S., Microtubule-associated protein 2c reorganizes both microtubules and microfilaments into distinct cytological structures in an actin-binding protein-280-deficient melanoma cell line. *J. Cell Biol.* 1997, **136**: 845-857.
62. Dayhoff, J., Hameroff, S., Lahoz-Beltra, R. and Swenberg, C.E., Cytoskeletal involvement in neuronal learning: a review. *Eur. Biophys. J.* 1994, **23**: 79-93.
63. Der Perng, M., Cairns, L., van den Ijssel, P., Prescott, A., Hutcheson, A.M. and Quinlan, R.A., Intermediate filament interactions can be altered by HSP27 and α B-crystallin. *J. Cell Sci.* 1999, **112**: 2099-2112.
64. Djabali, K., de Nechaud, B., Landon, F. and Portier, M.M., Alpha B-crystallin interacts with intermediate filaments in response to stress. *J. Cell Sci.* 1997, **110**: 2759-2769.
65. Doering, C., Ermentrout, B. and Oster, G., Rotary DNA motors. *Biophys. J.* 1995, **69**: 2256-2267.
66. Downing, D.T., Molecular modeling of vimentin filament assembly. *Proteins* 1996, **26**: 472-478.
67. Downing, K.H. and Nogales, E., Tubulin and microtubule structure. *Curr. Opin. Cell. Biol.* 1998, **10**: 16-22.
68. Dubinsky, W.P., Mayorga-Wark, O. and Schultz, S.G., Volume regulatory responses of basolateral membrane vesicles from Necturus enterocytes: role of the cytoskeleton. *Proc. Natl. Acad. Sci. USA* 1999, **96**: 9421-9426.
69. Edmonds, B.T. and Koenig, E., Calcium-dependent volume reduction in regenerating ganglion cell axons *in vitro*. *J. Neurosci. Res.* 1990, **26**: 158-180.
70. Eichinger, L., Koppel, B., Noegel, A.A., Schleicher, M., Schliwa, M., Weijer, K., Witke, W. and Janmey, P.A., Mechanical perturbations elicits a phenotypic difference between Dictyostelium wild-type cells and cytoskeletal mutants. *Biophys. J.* 1996, **70**: 1054-1060.
71. Endow, S.A. and Waligora, K.W., Determinants of kinesin motor polarity. *Science* 1998, **281**: 1200-1202.
72. Erikson, H.P. and Pantaloni, D., The role of subunit entropy in cooperative assembly. Nucleation of microtubules and other two-dimensional polymers. *Biophys. J.* 1981, **34**: 293-309.
73. Felgner, H., Frank, R., Biernat, J., Mandelkow, E.M., Mandelkow, E., Ludin, B., Matus, A. and Schliwa, M., Domains of neuronal microtubule-associated proteins and flexural rigidity of microtubules. *J. Cell Biol.* 1997, **138**: 1067-1075.
74. Ferralli, J., Doll, T. and Matus, A., Sequence analysis of MAP2 function in living cells. *J. Cell Sci.* 1994, **107**: 3115-3125.
75. Fritz, M., Zimmermann, R.M., Barmann, M. and Gaub, H.E., Actin binding to lipid-inserted α-actinin. *Biophys. J.* 1993, **65**: 1878-1885.
76. Fromherz, P., Self-organization of the fluid mosaic of charged channel proteins in membranes. *Proc. Natl. Acad. Sci. USA* 1988, **85**: 6353-6357.
77. Fuchs, E. and Weber, K., Intermediate filaments structure, dynamics, function and disease. *Annu. Rev. Biochem.* 1994, **63**: 345-382.
78. Fuller, N. and Rand, R.P., Water in actin polymerization. *Biophys. J.* 1999, **76**: 3261-3266.
79. Fullerton, G.D., Finnie, M.F., Hunter, K.E., Ord, V.A. and Cameron, I.L., The influence of macromolecular polymerization of spin-lattice relaxation of aqueous solutions. *Mag. Reson. Imag.* 1987, **5**: 353-370.
80. Furukawa, R., Kundra, R. and Fechheimer, M., Formation of liquid crystals from actin filaments. *Biochemistry* 1993, **32**: 12346-123352.
81. Fushimi, K. and Verkman, A.S., Low viscosity in the aqueous domain of cell cytoplasm measured by picosecond polarization microfluorometry. *J. Cell Biol.* 1991, **112**: 719-725.
82. Garcia, A.E. and Hummer, G., Water penetration and escape in proteins. *Proteins* 2000, **38**: 261-272.
83. Garcia-Mata, R., Bebok, Z., Sorscher, E.J. and Sztul, E.S., Characterization and dynamics of aggresome formation by a cytosolic GFP-chimera. *J. Cell Biol.* 1999, **146**: 1239-1254.
84. Gawrisch, K., Barry, J.A., Holte, L.L., Sinnwell, T., Bergelson, L.D. and Ferreti, J.A., Role of interactions at the lipid-water interface for domain formation. *Mol. Membr. Biol.* 1995, **12**: 83-88.
85. Gawrisch, K., Ruston, D., Zimmerberg, J., Parsegian, V.A., Rand, R.P. and Fuller, N., Membrane dipole potentials, hydration forces, and the ordering of water at membrane surfaces. *Biophys. J.* 1992, **61**: 1213-1223.
86. Gershon, N.D., Porter, K.R. and Trus, B.L., The cytoplasmic matrix: its volume and surface area and the diffusion of molecules through it. *Proc. Natl. Acad. Sci. USA* 1985, **82**: 5030-5034.
87. Gicquaud, C. and Wong, P., Mechanisms of interaction between actin and membrane lipids: a pressure-tuning infrared spectroscopy study. *Biochem. J.* 1994, **303**: 769-774.
88. Giorgione, J.R. and Epand, R.M., Role of water in protein kinase C catalysis and its binding to membranes. *Biochemistry* 1997, **36**: 2250-2256.
89. Giorgione, J.R., Huang, Z. and Epand, R.M., Increased activation of protein kinase C with cubic phase lipid compared with liposomes. *Biochemistry* 1998, **37**: 2384-2392.
90. Glogauer, M., Arora, P., Yao, G., Sokholov, I., Ferrier, J. and McCulloch, C.A., Calcium ions and tyrosine phosphorylation interact coordinately with actin to regulate cytoprotective response to stretching. *J. Cell Sci.* 1997, **110**: 11-21.
91. Goldmann, W.H., Galdener, R., Ludwig, M., Xu ,W., Adamson, E.D., Wang, N. and Ezzell, R.M., Differences in elasticity of vinculin-deficient F9 cells measured by magnetometry and atomic force microscopy. *Exp. Cell Res.* 1998, **239**: 235-242.
92. Goni, F.M. and Alonso, A., Structure and functional properties of diacylglycerols in membranes. *Prog. Lipid Res.* 1999, **38**: 1-48.
93. Goode, B.L., Drubin, D.G. and Barnes, G., Functional cooperation between the microtubule and actin cytoskeletons. *Curr. Opin. Cell Biol.* 2000, **12**: 63-71.
94. Goodson, H.V., Valetti, C. and Kreis, T.E., Motors and membrane traffic. *Curr. Opin. Cell Biol.* 1997, **9**: 18-28.
95. Gotz, R., Schulter, E., Shoham, G. and Zimmermann, F.K., A potential role of the cytoskeleton of *Saccharomyces cervisiae* in a functional organization of glycolytic enzymes. *Yeast* 1999, **15**: 1619-1629.
96. Gou, J.P., Gotow, T., Janmey, P.A. and Leterrier, J.F., Regulation of neurofilament interactions *in vitro* by natural and synthetic polypeptides sharing Lys-Ser-Pro sequences with the heavy neurofilament subunit NF-H: Neurofilament crosssbridging by antiparallel sidearm overlapping. *Med. Biol. Eng. Comput.* 1998, **36**:

371-387.
97. Grimm, R., Barmann, M., Hackl, W., Typke, D., Sackmann, E. and Baumeister, W., Energy filtered electron tomography of ice-embedded actin and vesicles. *Biophys. J.* 1997, 72: 482-489.
98. Gyoeva, F.K. and Gelfand, V.I., Coalignment of vimentin intermediate filaments with microtubules depends on kinesin. *Nature* 1991, 353: 445-448.
99. Hamill, D., Davis, J., Drawbridge, J. and Suprenant, K.A., Polysome targeting to microtubules: enrichment of specific mRNAs in a reconstituted microtubule preparation from sea urchin embryos. *J. Cell Biol.* 1994, 127: 973-984.
100. Han, J. and Herzfeld, J., Interpretation of the osmotic behavior of sickle cell hemoglobin solutions: different interactions among monomers and polymers. *Biopolymers* 1998, 45: 299-306.
101. Hancock, W.O. and Howard, J., Processivity of the motor protein kinesin requires two heads. *J. Cell Biol.* 1998, 140:1395-1405.
102. Hannan, A.J., Gunning, P., Jeffery, P.L. and Weinberger, R.P., Structural compartments within neurons: developmentally regulated organization of microfilament isoform mRNA and protein. *Mol. Cell. Neurosci.* 1998, 11: 289-304.
103. Harding, R.J. and Duncan, C.J., Protection against cellular damage in the rat heart by hyperosmotic solutions. *Exp. Mol. Pathol.* 1999, 67: 91-98.
104. Harootunian, A.T., Adams, S.R., Wen, W., Meinkoth, K.J.L., Taylor, S.S. and Tsien, R.Y., Movement of the free catalytic subunit of cAMP-dependent protein kinase into and out of the nucleus can be explained by diffusion. *Mol. Biol. Cell* 1993, 4: 993-1002.
105. Hazlewood, C.F., Rorschach, H.E. and Lin, C., Diffusion of water in tissues and MRI. *Magn. Reson. Med.* 1991, 19: 214-216.
106. Head, M.W. and Goldman, J.E., Small heat shock proteins, the cytoskeleton and inclusion body formation. *Neuropathol. Appl. Neurobiol.* 2000, 26: 304-312.
107. Heald, R., Tournebize, R., Blank, T., Sandaltzopoulos, R., Becker, P., Hyman, A. and Karsenty, E., Self-organization of microtubules into bipolar spindles around artifical chromosomes in *Xenopus* egg extracts. *Nature* 1996, 382: 420-425.
108. Heidemann, S.R., Kaech, S., Buxbaum, R.E. and Matus, A., Direct observations of the mechanical behaviors of the cytoskeleton in living fibroblasts. *J. Cell Biol.* 1999, 145: 109-122.
109. Heise, H., Bayerl, T., Isenberg, G. and Sackmann, E., Human platelet P-235, a talin-like actin binding protein, binds selectively to mixed lipid bilayers. *Biochim. Biophys. Acta* 1991, 1061: 121-131.
110. Henics, T. and Wheatley, D.N., Vacuolar cytoplasmic phase separation in cultured mammalian cells involves the microfilament network and reduces motional properties of intracellular water. *Int. J. Exp. Pathol.* 1997, 78: 345-354.
111. Henningsen, U. and Schliwa, M., Reversal in the direction of movement of a molecular motor. *Nature* 1997, 389: 93-95.
112. Herrmann, H. and Aebi, U., Intermediate filaments and their associates: multi-talented structural elements specifying cytoarchitecture and cytodynamics. *Curr. Opin. Cell Biol.* 2000, 12: 79-90.
113. Herrmann, H., Häner, M., Brettel, M., Ku, N.O. and Aebi, U., Characterization of distinct early assembly units of different intermediate filament proteins. *J. Mol. Biol.* 1999, 286: 1403-1420.
114. Hirai, H., Clustering of delta glutamate receptors is regulated by the actin cytoskeleton in the dendritic spines of cultured rat Purkinje cells. *Eur. J. Neurosci.* 2000, 12: 563-570.
115. Hirokawa, N., Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science* 1998, 279: 519-526.
116. Hitt, A.L., Cross, A.R. and Williams, R.C. Jr., Microtubule solutions display nematic liquid crystalline structure. *J. Biol. Chem.* 1990, 265: 1639-1647.
117. Hoenger, A. and Milligan, R.A., Motor domain of kinesin and ndc interact with microtubule protofilaments with the same binding geometry. *J. Mol. Biol.* 1997, 265: 553-564.
118. Holmes, K.C., Popp, D., Gebhard, W. and Kabsch, W., Atomic model of the actin filament. *Nature* 1990, 347: 44-49.
119. Holowka, D., Sheets, E.D. and Baird, B., Interactions between Fc(epsilon)RI and lipid raft components are regulated by the actin cytoskeleton. *J. Cell Sci.* 2000, 113: 1009-1019.
120. Holy, T.E., Dogterom, M., Yurke, B. and Leibler, S., Assembly and positioning of microtubule asters in microfabricated chambers. *Proc. Natl. Acad. Sci. USA* 1997, 94: 6228-6231.
121. Hoppert, M. and Mayer, F., Principles of macromolecular organization and cell function in bacteria and archaea. *Cell Biochem. Biophys.* 1999, 31: 247-284.
122. Horesh, D., Sapir, T., Francis, F., Wolf, S.G., Caspi, M., Elbaum, M., Chelly, J. and Reiner, O., Doublecortin, a stabilizer of microtubules. *Hum. Mol. Genet.* 1999, 8: 1599-1610.
123. Huang, J.D., Brady, S.T., Richards, B.W., Stenolen, D., Resau, J.H., Copeland, N.G. and Jenkins, N.A., Direct interaction of microtubule- and actin-based motors. *Nature* 1999, 397: 267-270.
124. Hug, T., Koslowsky, T., Ecke, D., Greger, R. and Kunzelmann, K., Actin-dependent activation of ion conductances in bronchial epithelial cells. *Pflugers Arch.* 1995, 429: 682-690.
125. Ingber, D.E., Tensegrity: the architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* 1997, 59: 575-599.
126. Irminger-Finger, I. and Ortega-Perez, R., Analysis of structure and microtubule assembly activity of the *Drosophila* 205K MAP. *Biol. Chem.* 1998, 379: 1381-1386.
127. Isambert, H., Venier, P., Maggs, A.C., Fattoum, A., Kassab, R., Pantaloni, D. and Carlier, M.F., Flexibility of actin filaments derived from thermal fluctuations. Effect of bound nucleotides, phalloidin and muscle regulatory proteins. *J. Biol. Chem.* 1995, 270: 11437-11444.
128. Israelachvili, J. and Wennerstrom, H., Role of hydration and water structure in biological and colloidal interactions. *Nature* 1996, 379: 219-225.
129. Ito, T., Suzuki, A. and Stossel, T.P., Regulation of water flow by actin-binding protein-induced actin gelation. *Biophys. J.* 1992, 61: 1301-1305.
130. Ito, T., Zaner, K.S. and Stossel, T.P., Nonideality of volume flows and phase transitions of F-actin solutions in response to osmotic stress. *Biophys. J.* 1987, 51: 745-753.
131. Janin, J., Wet and dry interfaces: the role of solvent in protein-protein and protein-DNA recognition. *Struct. Fold Des.* 1999, 7: R277-279.
132. Janmey, P.A., Mechanical properties of cytoskeletal polymers. *Curr. Opin. Cell Biol.* 1991, 3: 4-11.
133. Janmey, P.A., The cytoskeleton and cell signaling: component localization and mechanical coupling. *Physiol. Rev.* 1998, 78: 763-781.
134. Janmey, P.A., Euteneuer, U., Traub, P. and Schliwa, M., Viscoelastic properties of vimentin compared with other filamentous biopolymer networks. *J. Cell Biol.* 1991, 113: 155-160.
135. Janmey, P.A., Hvidt, S., Käs J., Lerche, D., Maggs, A., Sackmann, E., Schliwa, M. and Stossel, T.P., The mechanical properties of actin gels. Elastic modulus and filament motions. *J. Biol. Chem.* 1994, 269: 32503-32513.
136. Janmey, P.A., Hvidt, S., Lamb, J. and Stossel, T.P., Resemblance of actin-binding protein/actin gels to crosslinked networks. *Nature* 1994, 345: 89-92.
137. Janmey, P.A., Hvidt, S., Peetermans, J., Lamb, J., Ferry, J.D. and Stossel, T.P., Viscoelasticity of F-actin and F-actin-gelsolin complexes. *Biochemistry* 1988, 27: 8218-8227.
138. Janmey, P.A., Shah, J.V., Janssen, K.P. and Schliwa, M., Viscoelasticity of intermediate filament networks. In: *Subcellular Biochemistry, Intermediate Filaments*, vol. 31, Herrmann, H. and Harris, A. (eds.), Plenum Press, New York, 1998, pp. 381-397.
139. Janmey, P.A., Xian, W. and Flanagan, L.A., Controlling cytoskeleton structure by phosphoinositide-protein interactions: phosphoinositide

binding protein domains and effects of lipid packing. *Chem. Phys. Lipids* 1999, **101**: 93-107.
140.Janson, L.W., Ragsdale, K. and Luby-Phelps, K., Mechanism and size cutoff for steric exclusion from actin-rich cytoplasmic domains. *Biophys. J.* 1996, **71**: 1228-1234.
141.Jansen, R.P., RNA-cytoskeletal associations. *FASEB J.* 1999, **13**: 455-466.
142.Janssen, K.P., Eichinger, L., Janmey, P.A., Noegel, A.A., Schliwa, M., Witke, W. and Schleicher, M., Viscoelastic properties of F-actin solutions in the presence of normal and mutated actin-binding proteins. *Arch. Biochem. Biophys.* 1996, **325**: 183-189.
143.Jibu, M., Hagan, S., Hameroff, S.R., Pribram, K.H. and Yasue, K. Quantum optical coherence in cytoskeletal microtubules: implications for brain functions. *Biosystems* 1994, **32**: 195-209.
144.Johnston, J.A., Ward, C.L. and Kopito, R.R., Aggresomes: a cellular response to misfolded proteins. *J. Cell Biol.* 1998, **143**: 1883-1898.
145.Jones, J.D. and Luby-Phelps, K., Tracer diffusion through F-actin: effect of filament length and cross-linking. *Biophys. J.* 1996, **71**: 2742-2750.
146.Joshi, H.C., Microtubule dynamics in living cells. *Curr. Opin. Cell Biol.* 1998, **10**: 35-44.
147.Kalcheva, N., Rockwood, J.M., Kress, Y., Steiner, A. and Shafit-Zagardo, B., Molecular and functional characteristics of MAP-2a: ability of MAP-2a versus MAP-2b to induce stable microtubules in COS cells. *Cell Motil. Cytoskel.* 1998, **40**: 272-285.
148.Kanai, Y., Chen, J. and Hirokawa, N. Microtubule bundling by tau protein *in vivo*: analysis of functional domains. *EMBO J.* 1992, **11**: 3953-3961.
149.Kao, Y.L., Deavours, B.E., Phelps, K.K., Walker, R.A. and Reddy, A.S., Bundling of microtubules by motor and tail domains of a kinesin-like calmodulin-binding protein from *Arabidopsis*: regulation by $Ca^{2+}$/calmodulin. *Biochem. Biophys. Res. Commun.* 2000, **267**: 201-207.
150.Kapus, A., Szaszi, K., Sun, J., Rizoli, S. and Rotstein, O.D., Cell shrinkage regulates Src kinases and induces tyrosine phosphorylation of cortactin, independent of the osmotic regulation of $Na^+/K^+$ exchangers. *J. Biol. Chem.* 1999, **274**: 8093-8102.
151.Karl, I. and Bereiter-Hahn, J., Cell contraction caused by microtubule disruption is accompagnied by shape changes and an increased elasticity measured by scanning acoustic microscopy. *Cell Biochem. Biophys.* 1998, **29**: 225-241.
152.Käs, J., Strey, H., Tang, J.X., Finger, D., Ezzell, R., Sackmann, E. and Janmey, P.A., F-actin, a model polymer for semi-flexible chains in dilute, semidilute and liquid crystalline solutions. *Biophys. J.* 1996, **70**: 609-625.
153.Kühne, S. and Bryant, R.G., Protein-bound water molecule counting by resolution of $^1H$ spin-lattice relaxation mechanisms. *Biophys. J.* 2000, **78**: 2163-2169.
154.Kim, E. and Reisler, E., Intermolecular dynamics and function in actin filaments. *Biophys. Chem.* 2000, **86**: 191-201.
155.King, S.J. and Schroer, T.A., Dynactin increases the processivity of the cytoplasmic dynein motor. *Nat. Cell Biol.* 2000, **2**: 20-24.
156.Kinosita, K. Jr., Linear and rotary molecular motors. *Adv. Exp. Med Biol.* 1998, **453**: 5-13.
157.Kinosita, K. Jr., Yasuda, R., Noji, H. and Adachi, K., A rotary molecular motor that can work at near 100% efficiency. *Philos. Trans. R. Soc. Lond. B. Biol.* 2000, **355**: 473-489.
158.Knubovets, T., Shinar, H., Eliav, U. and Navon, G., A $^{23}Na$ multiple-quantum-filtered NMR study of the effect of the cytoskeleton conformation on the anisotropic motion of sodium ions in red blood cells. *J. Magn. Reson. B.* 1996, **110**: 16-25.
159.Knull, H. and Minton, A.P., Structure within eucaryotic cytoplasm and its relationship to glycolytic metabolism. *Cell Biochem. Funct.* 1996, **14**: 237-248.
160.Kotani, S., Kawai, G., Yokoyama, S. and Murofushi, H., Interactions between microtubule-associated proteins and microtubules. A proton nuclear magnetic resonance analysis on the binding of synthetic peptide to tubulin. *Biochemistry* 1990, **29**: 10049-10054.
161.Koonce, M.P. and Schliwa, M., Reactivation of organelle movement along the cytoskeletal framework of a giant freshwater ameba. *J. Cell Biol.* 1986, **103**: 605-612.
162.Krendel, M. and Inoue, S., Anaphase spindle dynamics under $D_2O$-enhanced microtubule polymerization. *Biol. Bull.* 1995, **189**: 204-205.
163.Krendel, M., Sgourdas, G. and Bonder, E.M., Disassembly of actin filaments leads to increased rate and frequency of mitochondrial movement along microtubules. *Cell Motil. Cytoskeleton* 1998, **40**: 368-378.
164.Kulp, D.T. and Herzfeld, J., Crowding-induced organization of cytoskeletal elements. III. Spontaneous bundling and sorting of self-assembled filaments with different flexibilities. *Biophys. Chem.* 1995, **57**: 93-102.
165.Kurachi, M., Hoshi, M. and Tashiro, H., Buckling of a single microtubule by optical trapping forces: direct measurement of microtubule rigidity. *Cell Motil. Cytoskel.* 1995, **30**: 221-228.
166.Kusumi, A. and Sako, Y., Cell surface organization by the membrane skeleton. *Curr. Opin. Cell Biol.* 1996, **8**: 566-574.
167.Kusumi, A., Suzuki, K. and Koyasako, K., Mobility and cytoskeletal interactions of cell adhesion receptors. *Curr. Opin. Cell Biol.* 1999, **11**: 582-590.
168.Kuwayama, H., Ecke, M., Gerisch, G. and Van Haastert, P.J., Protection against osmotic stress by cGMP-mediated myosin phosphorylation. *Science* 1996, **271**: 207-209.
169.Lacey, M.L. and Haimo, L.T., Cytoplasmic dynein binds to phospholipid vesicles. *Cell Motil. Cytoskel.* 1994, **28**: 205-212.
170.Lamprecht, J., Schroeter, D. and Paweletz, N., Mitosis arrested by deuterium oxide. Light microscopic, immunofluorescence and ultrastructural characterization. *Eur. J. Cell Biol.* 1990, **51**: 303-312.
171.Lamprecht, J., Schroeter, D. and Paweletz, N., Derangement of microtubule arrays in interphase and mitotic PtK2 cells treated with deuterium oxide (heavy water). *J. Cell Sci.* 1991, **98**: 463-473.
172.Landowne, D., Measuring nerve excitation with polarized light. *Jpn. J. Physiol.* 1993, **43**: 7-11.
173.Landowne, D., Larsen, J.B. and Taylor, K.T. Colchicine alters the nerve birefringence response. *Science* 1983, **220**: 953-954.
174.Lange, K., Microvillar ion channels: cytoskeletal modulation of ion fluxes. *J. Theor. Biol.* 2000, **206**: 561-584.
175.Lee, J.C. and Timasheff, S.N., *In vitro* reconstitution of calf brain microtubules: effects of solution variables. *Biochemistry* 1977, **16**: 1754-1764.
176.Le Gal, F., Gasqui, P. and Renard, J.P., Differential osmotic behavior of mammalian oocytes before and after maturation: a quantitative analysis using goat oocytes as a model. *Cryobiology* 1994, **31**: 154-170.
177.Lehrer, S.S., Golitsina, N.L. and Geeves, M.A., Actin-tropomyosin activation of myosin subfragment 1 ATPase and thin filament cooperativity. The role of tropomyosin flexibility and end-to-end interactions. *Biochemistry* 1997, **36**: 13449-13454.
178.Leterrier, J.F. and Eyer, J., Age-dependent changes in the ultrastructure and in the molecular composition of rat brain microtubules. *J. Neurochem.* 1992, **59**: 1126-1137.
179.Leterrier, J.F., Eyer, J., Weiss, D.G. and Linden, M., *In vitro* studies of the physical interactions between neurofilaments, microtubules and mitochondria isolated from the central nervous system. In: *American Institute of Physics.* Conference Proceedings *The living cell in four dimensions*, Paillotin, G. (ed.), 1991, **226**: 91-105.
180.Leterrier, J.F., Hartwig, J., Käs, J., Vegners, R. and Janmey, P.A., Mechanical effects of neurofilament crossbridges: modulation by phosphorylation, lipids, and interactions with F-actin. *J. Biol. Chem.* 1996, **271**: 15687-15694.
181.Leterrier, J.F., Rusakov, D.A., Nelson, B.D. and Linden, M.,

Interactions between brain mitochondria and cytoskeleton: evidence for specialized outer membrane domains involved in the association of cytokeleton-associated proteins to mitochondria *in situ* and *in vitro*. *Microsc. Res. Techn.* Special Issue on *Mitochondria Dynamics*, Mannella, C.A. (ed.), 1994, **27**: 233-261.

182. Leuchtag, H.R., Indications of the existence of ferroelectric units in excitable-membrane channels. *J. Theor. Biol.* 1987, **127**: 321-340.

183. Liang, P. and MacRae, T.H., Molecular chaperones and the cytoskeleton. *J. Cell Sci.* 1997, **110**: 1431-1440.

184. Liao, G. and Gundersen, G.C., Kinesin is a candidate for cross-bridging microtubules and intermediate filaments. Selective binding of kinesin to detyrosinated tubulin and vimentin. *J. Biol. Chem.* 1998, **273**: 9797-97803.

185. Lillie, M.A. and Gosline, J.M., Swelling and viscoelastic properties of osmotically stressed elastin. *Biopolymers* 1996, **39**: 641-652.

186. Lin, E.C. and Cantiello, H.F., A novel method to study the electrodynamic behavior of actin filaments. Evidence for cable-like properties of actin. *Biophys. J.* 1993, **65**: 1371-1378.

187. Ling, G.N. and Cope, F.W., Potassium ion: Is the bulk of intracellular $K^+$ adsorbed? *Science* 1969, **163**: 1335-1336.

188. Ling, G.N. and Ochsenfeld, M.M., The majority of potassium ions in muscle cells is adsorbed on β- and γ-carboxyl groups of myosin: potassium-ion-adsorbing carboxyl groups on myosin heads engage in crossbridge formation during contraction. *Physiol. Chem. Phys. Med. NMR* 1991, **23**: 133-160.

189. Linshaw, M.A., Fogel, C.A., Downey, G.P., Koo, E.W. and Gollieb, A.L. Role of cytoskeleton in volume regulation of rabbit proximal tubule in dilute medium. *Am. J. Physiol.* 1992, **262**: 144-150.

190. Liou, Y.C., Tocilj, A., Davies, P.L. and Jia, Z., Mimicry of ice structure by surface hydroxyls and water of a β-helix antifreeze protein. *Nature* 2000, **406**: 322-324.

191. Lohman, T.M., Thorn, K. and Vale, R.D., Staying on track: common features of DNA helicases and microtubule motors. *Cell* 1998, **93**: 9-12.

192. Lorenz, M., Poole, K.J., Popp, D., Rosenbaum, G., Holmes, K.C., An atomic model of the unregulated thin filament obtained by X-ray fiber diffraction on oriented actin-tropomyosin gels. *J. Mol. Biol.* 1995, **246**: 108-119.

193. Luby-Phelps, K., Cytoarchitecture and physical properties of the cytoplasm: volume, viscosity, diffusion, intracellular surface area. *Int. Rev. Cytol.* 2000, **192**: 189-221.

194. Ludin, B., Ashbridge, K., Funfschilling, U. and Matus, A., Functional analysis of the MAP2 repeat domain. *J. Cell Sci.* 1996, **109**: 91-99.

195. Luther, P.W., Peng, H.B. and Lin, J.J., Changes in cell shape and actin distribution induced by constant electric fields. *Nature* 1983, **303**: 61-64.

196. Ma, L., Xu, J., Coulombe, P.A. and Wirtz, D., Keratin filament suspensions show unique micromechanical properties. *J. Biol. Chem.* 1999, **274**: 19145-19151.

197. Mackey, A.T. and Gilbert, S.P., Moving microtubule may require two heads: a kinetic investigation of monomeric Ncd. *Biochemistry* 2000, **39**: 1346-1355.

198. Maguire, G., Connaughton, V., Prat, A.G., Jackson, G.R. Jr. and Cantiello, H.F., Actin cytoskeleton regulates ion channel activity in retinal neurons. *Neuroreport* 1998, **9**: 665-670.

199. Mahon, P., Partridge, K., Beattie, J.H., Glover, L.A. and Hesketh, J.E., The 3' untranslated region plays a role in the targeting of metallothionein-I mRNA to the perinuclear cytoplasm and cytoskeletal-bound polysomes. *Biochim. Biophys. Acta* 1997, **1358**: 153-162.

200. Mandelkow, E. and Mandelkow, E.M., Microtubules and microtubule-associated proteins. *Curr. Opin. Cell Biol.* 1995, **7**: 72-81.

201. Margolis, R.L., Job, D., Pabion, M. and Rauch, C.T., Sliding of STOP proteins on microtubules: a model system for diffusion-dependent microtubule motility. *Ann. N.Y. Acad. Sci.* 1986, **466**: 306-321.

202. Marshall, W.F. and Rosenbaum, J., How centrioles work: lessons from green yeast. *Curr. Opin. Cell Biol.* 2000, **12**: 119-125.

203. Martin, M.A., Iyadurai, S.J., Gassman, A., Gindhart Jr, J.G., Hays, T.S. and Saxton, W.M., Cytoplasmic dynein, the dynactin complex and kinesin are interdependent and essential for fast axonal transport. *Mol. Biol. Cell* 1999, **10**: 3717-3728.

204. Marx, A., Pless, J., Mandelkow, E.M. and Mandelkow, E., On the rigidity of the cytoskeleton: are maps crosslinkers or spacers of microtubules? *Cell. Mol. Biol.* 2000, **46**: 949-965.

205. Matsuda, S. and Hirai, H., The clustering of NMDA receptor NR2 subunit is regulated by the interaction between the C-terminal exon cassettes and the cytoskeleton. *Neurosci. Res.* 1999, **34**: 157-163.

206. Matsumoto, G., A proposed membrane model for generation of sodium currents in squid giant axons. *J. Theor. Biol.* 1984, **107**: 649-666.

207. Matsumoto, G., Ichikawa, M., Tasaki, A., Murofushi, H. and Sakai, H. Axonal microtubules necessary for generation of sodium currents in squid giant axons: I. Pharmacological study on sodium current and restoration of sodium current by microtubule proteins and 260 K protein. *J. Membr. Biol.* 1984, **77**: 77-91.

208. Matsumoto, G., Ichikawa, M. and Tasaki, A., Axonal microtubules necessary for generation of sodium currents in squid giant axons: II. Effect of colchicine upon asymmetrical displacement current. *J. Membr. Biol.* 1984, **77**: 93-99.

209. Matthews, J.B., Smith, J.A., Mun, E.C. and Sicklick, J.K., Osmotic regulation of intestinal epithelial $Na^+$-$K^+$-$Cl^-$ cotransport: role of Cl and F-actin. *Am. J. Physiol.* 1998, **274**: 697-706.

210. Maughan, D.W. and Godt, R.E., Equilibrium distribution of ions in muscle fiber. *Biophys. J.* 1989, **56**: 717-722.

211. Mavromatos, N.E., Quantum-mechanical coherence in cell microtubule: a realistic possibility? *Bioelectrochem. Bioenerg.* 1999, **48**: 273-284.

212. McCaig, C.D. and Rajnicek, A.M., Electrical fields, nerve growth and nerve regeneration. *Exp. Physiol.* 1991, **76**: 473-494.

213. Meggs, W.J., Electric fields determine the spatial organization of microtubules and actin filaments. *Med. Hypotheses* 1988, **26**: 165-170.

214. Mehta, A.D., Rock, R.S., Rief, M., Spudich, J.A., Mooseker, M.S. and Cheney, R.E., Myosin-V is a processive actin-based motor. *Nature* 1999, **400**: 590-593.

215. Meier, J., Meunier-Durmort, C., Forest, C., Triller, A. and Vannier, C. Formation of glycine receptor clusters and their accumulation at synapses. *J. Cell Sci.* 2000, **113**: 2783-2795.

216. Melacini, G., Bonvin, A.M., Goodman, M., Boelens, R. and Kaptein, R., Hydration dynamics of the collagene triple helix by NMR. *J. Mol. Biol.* 2000, **300**: 1041-1049.

217. Mentre, P. and Hui Bon Hoa, G., Effects of high hydrostatic pressures on living cells: a consequence of the properties of macromolecules and macromolecule-associated water. *Int. Rev. Cytol.* 2001, **201**: 1-84.

218. Mermall, V., Post, P.L. and Mooseker, M.S., Unconventional myosins in cell movement, membrane traffic, and signal transduction. *Science* 1998, **279**: 527-533.

219. Mickey, B. and Howard, J., Rigidity of microtubules is increased by stabilizing agents. *J. Cell Biol.* 1995, **130**: 909-917.

220. Mills, J.W. and Lubin, M., Effect of adenosine 3'5'-cyclic monophosphate on volume and cytoskeleton of MDCK cells. *Am. J. Physiol.* 1986, **250**: 319-324.

221. Mills, J.W., Schwiebert, E.M. and Stanton, B.A., The cytoskeleton and membrane transport. *Curr. Opin. Nephrol. Hypertens.* 1994, **3**: 529-534.

222. Minaschek, G., Groschel-Stewart, U., Blum, S. and Bereiter-Hahn, J., Microcompartmentation of glycolytic enzymes in cultured cells. *Eur. J. Cell Biol.* 1992, **58**: 418-428.

223. Nedelec, F.J., Surrey, T., Maggs, A.C. and Leibler, S., Self-organization of microtubules and motors. *Nature* 1997, **389**: 305-308.

224.Neely, M.D. and Nicholls, J.G., Electrical activity, growth cone motility and the cytoskeleton. *J. Exp. Biol.* 1995, **198**: 1433-1446.
225.Nicklas, K., Bocker, J., Schlenkrich, M., Brickmann, J. and Bopp, P., Molecular dynamics studies at the interface between a model membrane and an aqueous solution. *Biophys. J.* 1991, **60**: 261-272.
226.Nievers, M.G., Schaapveld, R.Q. and Sonnenberg, A., Biology and function of desmosomes. *Matrix Biol.* 1999, **18**: 5-17.
227.Nogales, E., Whittaker, M., Milligan, R.A. and Downing, K.H., High-resolution model of the microtubule. *Cell* 1999, **96**: 79-88.
228.Noiles, E.E., Thompson, K.A. and Storey, B.T., Water permeability, Lp, of the mouse sperm plasma membrane and its activation energy are strongly dependent on interactions of the plasma membrane with the sperm cytoskeleton. *Cryobiology* 1997, **35**: 79-92.
229.North, A.J., Bardsley, W.G., Hyam, J., Bornslaeger, E.A., Cordingley, H.C., Trinnaman, B., Hatzfeld, M., Green, K.J., Magee, A.I. and Garrod, D.R., Molecular map of the desmosomal plaque. *J. Cell Sci.* 1999, **112**: 4325-4336.
230.Okada, Y. and Hirokawa, N., Mechanisms of the single-headed processivity: diffusional anchoring between the K-loop of kinesin and the C-terminus of tubulin. *Proc. Natl. Acad. Sci. USA* 2000, **97**: 640-645.
231.Oleynikov, Y. and Singer, R.H., RNA localization: different zipcodes, same postman? *Trends Cell Biol.* 1998, **8**: 381-383.
232.Omori, H., Kuroda, M., Naora, H., Takeda, H., Nio, Y., Otani, H. and Tamura, K., Deuterium oxide (heavy water) accelerates actin assembly *in vitro* and changes microfilament distribution in cultured cells. *Eur. J. Cell Biol.* 1997, **74**: 273-280.
233.Onuma, E.K. and Hui, S.W., Electric field-directed cell shape changes, displacement, and cytoskeletal reorganization are calcium dependent. *J. Cell Biol.* 1988, **106**: 2067-2075.
234.Oplatka, A., Critical review of the swinging crossbridging theory and the cardinal active role of water in muscle contraction. *Crit. Rev. Biochem. Mol. Biol.* 1997, **32**: 307-360.
235.Oplatka, A., Are rotors at the heart of all biological motors? *Biochem. Biophys. Res. Commun.* 1998, **246**: 301-306.
236.Orokos, D.D., Bowser, S.S. and Travis, J.L., Reactivation of cell surface transport in *reticulomyxa*. *Cell Motil. Cytoskeleton* 1997, **37**: 139-148.
237.Page, R., Lindberg, U. and Schutt, C.E., Domain motion in actin. *J. Mol. Biol.* 1998, **280**: 463-474.
238.Pagliaro, L., Glycolysis *in vivo*: fluorescence microscopy as a tool for studying enzyme organization in living cells. *Adv. Mol. Cell. Biol.* 1995, **11**: 93-123.
239.Palmer, A., Xu, J., Kuo, S.C. and Wirtz, D., Diffusion wave spectroscopy microrheology of actin filament networks. *Biophys. J.* 1999, **76**: 1063-1071.
240.Panda, D., Chakrabarti, G., Hudson, J., Pigg, K., Miller, H.P., Wilson, L. and Himes R.H., Suppression of microtubule dynamic instability and treadmilling by deuterium oxide. *Biochemistry* 2000, **39**: 5075-5081.
241.Papaseit, C., Pochon, N. and Tabony, J., Microtubule self-organization is gravity-dependent. *Proc. Natl. Acad. Sci. USA* 2000, **97**: 8364-8368.
242.Papaseit, C., Vuillard, L. and Tabony, J., Reaction-diffusion microtubule concentration patterns occur during biological morphogenesis. *Biophys. Chem.* 1999, **79**: 33-39.
243.Parry, D.A.D., Structural features of IF proteins. In: *Guidebook to the Cytoskeletal and Motor Proteins*. Kreis, T. and Vale, R. (eds.), Oxford University Press, UK , 1999, pp. 285-291.
244.Partridge, J., Dennison, P.R., Moore, B.D. and Halling, P.J., Activity and mobility of subtilisin in low water organic media: hydration is more important than solvent dielectric. *Biochim. Biophys. Acta* 1998, **1386**: 79-89.
245.Pedrotti, B., Colombo, R. and Islam, K., Microtubule associated protein MAP1A is an actin-binding and crosslinking protein. *Cell Motil. Cytoskel.* 1994, **29**: 110-116.
246.Pelham, R.J. Jr. and Wang, Y.I., High resolution of mechanical forces exerted by locomoting fibroblasts on the substrate. *Mol. Biol. Cell* 1999, **10**: 935-945.
247.Perides, G., Harter, C. and Traub, P., Electrostatic and hydrophobic interactions of the intermediate filament protein vimentin and its amino terminus with lipid bilayers. *J. Biol. Chem.* 1987, **262**: 13742-13749.
248.Pfaffmann, J.O. and Conrad, M., Adaptative information processing in microtubule networks. *Biosystems* 2000, **55**: 47-57.
249.Pollack, G.H., Blyakhman, F., Shklyar, T., Tourovskaya, A., Tameyasu, T. and Yang, ?., Implications of quantal motor action in biological systems. *Adv. Exp. Med. Biol.* 1998, **453**: 361-369.
250.Popov, S. and Poo, M.M., Diffusional transport of macromolecules in developing nerve processes. *J. Neurosci.* 1992, **12**: 77-85.
251.Prahlad, V., Yoon, M., Moir, R.D., Vale, R.D. and Goldman, R.D., Rapid movements of vimentin on microtubule tracks: kinesin-dependent assembly of intermediate filament networks. *J. Cell Biol.* 1998, **143**: 159-170.
252.Prochniewicz, E., Zhang, Q., Janmey, P.A. and Thomas, D.D., Cooperativity in F-actin: binding of gelsolin at the barbed end affects structure and dynamics of the whole filament. *J. Mol. Biol.* 1996, **260**: 756-766.
253.Provance, D.W. Jr., McDowall, A., Marko, M. and Luby-Phelps, K., Cytoarchitecture of size-excluding compartments in living cells. *J. Cell Sci.* 1993, **106**: 565-577.
254.Puius, Y.A., Mahoney, N.M. and Almo, S.C., The modular structure of actin-regulatory proteins. *Curr. Opin. Cell Biol.* 1998, **10**: 23-34.
255.Punnonen, E.L., Fages, C., Wartiovaara, J. and Rauvala, H., Ultrastructural localization of β-actin and amphoterin mRNA in cultured cells: application of tyramide signal amplification and comparison of detection methods. *J. Histochem. Cytochem.* 1999, **47**: 99-112.
256.Rand, R.P., Fuller, N.L., Bukto, P., Francis, G. and Nicholls, P., Measured changes in protein solvation with substrate binding and turnover. *Biochemistry* 1993, **32**: 5925-5929.
257.Rand, R.P., Parsegian, V.A. and Rau, D.C., Intracellular osmotic action. *Cell Mol. Life Sci.* 2000, **57**: 1018-1032.
258.Richelme, F., Benoliel, A.M. and Bongrand, P., Dynamic studies of cell mechanical and structural reponses to rapid changes of calcium levels. *Cell Motil. Cytoskel.* 2000, **45**: 93-105.
259.Rochlin, M.W., Dailey, M.E. and Bridgman, P.C., Polymerizing microtubules activate site-directed F-actin assembly in nerve growth cones. *Mol. Biol. Cell* 1999, **10**: 2309-2327.
260.Rogers, S.L. and Gelfand, V.I., Membrane trafficking, organelle transport and the cytoskeleton. *Curr. Opin. Cell Biol.* 2000, **12**: 57-62.
261.Rousselet, J., Salome, L. Adjari, A. and Prost, J., Direct motion of Brownial particles induced by a periodic asymmetric potential. *Nature* 1994, **370**: 446-448.
262.Roy, S., Coffee, P., Smith, G., Liem, R.K.H., Brady, S.T. and Black, M.M., Neurofilaments are transported rapidely but intermittently in axons: implications for slow axonal transport. *J. Neurosci.* 2000, **20**: 6849-6861.
263.Ruhrberg, C. and Watt, F., The plakin family: versatile organizers of cytoskeletal architecture. *Curr. Opin. Genet. Dev.* 1997, **7**: 392-397.
264.Rusakov, D.A., Berezovskaya, O.L. and Skibo, G.G., Cytoskeleton-mediated, age-dependent lateral topography of lectin-gold-labelled molecules on the plasma membrane of cultured neurons: a statistical view. *Neuroscience* 1993, **52**: 369-379.
265.Ruthel, G. and Banker, G., Actin-dependent anterograde movement of growth-cone-like structures along growing hippocampal axons: a novel form of axonal transport? *Cell Motil. Cytoskel.* 1998, **40**: 160-173.
266.Sablin, E.P., Kinesins and microtubules: their structure and motor mechanisms. *Curr. Opin. Cell Biol.* 2000, **12**: 35-41.
267.Sako, Y., Nagafuchi, A., Tsukita, S., Takeiuchi, M. and Kusumi, A.,

Cytoplasmic regulation of the movement of E-cadherin on the free cell surface as studied by optical tweezers and single particle tracking: corralling and tethering by the membrane skeleton. *J. Cell Biol.* 1998, **140**: 1227-1240.
268.Sato, M., Schwartz, W.H., Selden, S.C. and Pollard, T.D., Mechanical properties of brain tubulin and microtubules. *J. Cell Biol.* 1988, **106**: 1205-1211.
269.Sattilaro, F., Interaction of microtubule-associated protein 2 with actin filaments. *Biochemistry* 1986, **25**: 2003-2009.
270.Saxton, M.J., Lateral diffusion in an archipelago: Dependence on tracer size. *Biophys. J.* 1993, **64**: 1053-1062.
271.Schmidt, C.E., Horwitz, A.F., Lauffenburger, D.A. and Sheetz, M.P., Integrin-cytoskeletal interactions in migrating fibroblasts are dynamic, asymmetric and regulated. *J. Cell Biol.* 1993, **123**: 977-991.
272.Schutt, C.E. and Lindberg, U., Actin as the generator of tension during muscle contraction. *Proc. Natl. Acad. Sci. USA* 1992, **89**: 319-323.
273.Schwienbacher, C., Magri, E., Trombetta, G. and Grazi, E., Osmotic properties of the calcium-regulated actin filament. *Biochemistry* 1995, **34**: 1090-1095.
274.Seksek, O., Biwersi, J. and Verkman, A.S., Translational diffusion of macromolecule-sized solutes in cytoplasm and nucleus. *J. Cell Biol.* 1997, **138**: 131-142.
275.Shah, J.V., Flanagan, L.A., Janmey, P.A. and Leterrier, J.F., Bidirectional translocation of neurofilaments along microtubules mediated in part by dynein/dynactin. *Mol. Biol. Cell* 2000, **11**: 3495-3508.
276.Sharp, D.J., Brown, H.M., Kwon, M., Rogers, G.C., Holland, G. and Scholey, J.M., Functional coordination of three mitotic motors in *Drosophila* embryos. *Mol. Biol. Cell* 2000, **11**: 241-253.
277.Sharp, D.J., Rogers, G.C. and Scholey, J.M., Roles of motor proteins in building microtubule-based structures: a basic principle of cellular design. *Biochim. Biophys. Acta* 2000, **1496**: 128-141.
278.Shen, M.R., Chou, C.Y., Hsu, K.F., Hsu, K.S. and Wu, M.L., Modulation of volume-sensitive $Cl^-$ channels and cell volume by actin filaments and microtubules in human cervical cancer HT-3 cells. *Acta Physiol. Scand.* 1999, **167**: 215-225.
279.Shimizu, T., Thorn, K.S., Ruby, A. and Vale, R.D., ATPase kinetic characterization and single molecule behavior of mutant human kinesin motors defective in microtubule-based motility. *Biochemistry* 2000, **39**: 5265-5273.
280.Shingyoji, C., Higuchi, H., Yoshimura, M., Katayama, E. and Yanagida, T., Dynein arms are oscillatory force generators. *Nature* 1998, **393**: 711-714.
281.Shoeniger, J.S., Aiken, N., Hsu, E. and Blackband, S.J., Relaxation-time and diffusion NMR microscopy of single neurons. *J. Magn. Reson. B.* 1994, **103**: 261-273.
282.Simson, R., Yang, B., Moore, S.E., Doherty, P., Walsh, F.S. and Jacobson, K.A., Structural mosaicism on the submicron scale in the plasma membrane. *Biophys. J.* 1998, **74**: 297-308.
283.Somers, M. and Engelborghs, Y., Kinetics of the spontaneous organization of microtubules in solution. *Eur. Biophys. J.* 1990, **18**: 239-244.
284.Sotomayor, C.P., Aguilar, L.F., Cuevas, F.J., Helms, M.K. and Jameson, D.M., Modulation of pig kidney $Na^+/K^+$-ATPase activity by cholesterol: role of hydration. *Biochemistry* 2000, **39**: 10928-10935.
285.Suzuki, A., Maeda, T. and Ito, T., Formation of liquid crystalline phase of actin filament solutions and its dependence on filament length as studied by optical birefringence. *Biophys. J.* 1991, **59**: 25-30.
286.Suzuki, M., Shigematsu, J., Fukunishi, Y., Harada, Y., Yanagida, Y. and Kodama, T., Coupling of protein surface hydrophobicity change to ATP hydrolysis by myosin motor domain. *Biophys. J.* 1997, **72**: 18-23.
287.Suzuki, N., Tamura, Y. and Mihashi, K., Compressibility of specific volume of actin decreases upon G to F transformation. *Biochim. Biophys. Acta* 1996, **1292**: 265-272.
288.Suzuki, A., Yamazaki, M. and Ito, T., Osmoelastic coupling in biological structures: formation of parallel bundle of actin filaments i a crystalline-like structure caused by osmotic stress. *Biochemistr* 1989, **28**: 6513-6518.
289.Swaminathan, R., Bicknese, S., Periasamy, N. and Verkman, A.S Cytoplasmic viscosity near the cell plasma membrane: translation diffusion of a small fluorescent solute measured by total intern reflection-fluorescence photobleaching recovery. *Biophys. J.* 1996, 7 1140-1151.
290.Tabony, J., Morphological bifurcations involving reaction-diffusio processes during microtubule formation. *Science* 1994, **264**: 245-24
291.Tabony, J. and Job, D., Gravitational symmetry breaking microtubular dissipative structures. *Proc. Natl. Acad. Sci. USA* 199 **89**: 6948-6952.
292.Tang, J.X., Ito, T., Tao, T., Traub, P. and Janmey, P.A., Opposite effec of electrostatics and steric exclusion on bundle formation by F-act and other filamentous polyelectrolytes. *Biochemistry* 1997, **36**: 1260 12607.
293.Tang, J.X. and Janmey, P.A., The polyelectrolyte nature of F-actin a the mechanism of actin bundle formation. *J. Biol. Chem.* 1996, 27 8556-8563.
294.Tang, J.X., Szymanski, P.T., Janmey, P.A. and Tao, T., Electrostat effects of smooth muscle calponin on actin assembly. *Eur. J. Biochem* 1997, **247**: 432-440.
295.Tasaki, I. and Byrne, P.M., Rapid structural changes in nerve fibe evoked by electric current pulses. *Biochem. Biophys. Res. Commu* 1992, **188**: 559-564.
296.Tasaki, I. and Byrne, P.M., The origin of rapid changes birefringence, light scattering and dye absorbance associated wi excitation of nerve fibers. *Jpn. J. Physiol.* 1993, **43**: 67-75.
297.Terakawa, S. and Nakayama, T., Are axoplasmic microtubul necessary for membrane excitation? *J. Membr. Biol.* 1985, **85**: 65-7
298.Thoumine, O., Cardoso, O. and Meister, J.J., Change in t mechanical properties of fibroblasts during spreading. *Eur. Biophys.* 1999, **28**: 222-234.
299.Togel, M., Wiche, G. and Propst, F., Novel features of the light ch of microtubule-associated protein MAP1B: microtubule stabilizatio self interaction, actin filament binding and regulation by the hea chain. *J. Cell Biol.* 1998, **143**: 695-707.
300.Trombitas, K., Baasten, P., Schreuder, J. and Pollack, G.I Contraction-induced movements of water in single fibres of fi skeletal muscle. *J. Muscle Res. Cell Motil.* 1993, **14**: 573-584.
301.Tuszynski, J.A., Tripisova, B., Sept, D. and Brown, J.A., Select physical issues in the structure and function of microtubules. *J. Stru Biol.* 1997, **118**: 94-106.
302.Umeda, M. and Emoto, K., Membrane phospholipid dynamics duri cytokinesis: regulation of actin filament assembly by redistribution membrane surface phospholipids. *Chem. Phys. Lipids* 1999, **101**: 8 91.
303.Vale, R.D., Coppin, C.M., Malik, F., Kull, F.J. and Milligan, R. Tubulin GTP hydrolysis influences the structure, mechani properties and kinesin-driven transport of microtubles. *J. Biol. Che* 1994, **269**: 23769-23775.
304.Van Oss, C.J., Hydrophobic, hydrophilic and other interactions: epitope-paratope binding. *Mol. Immunol.* 1995, **32**: 199-211.
305.Vassilev, P.M., Dronzine, R.T., Vassileva, M.P. and Georgiev, G. Parallel arrays of microtubules formed in electric and magnetic fiel *Biosci. Rep.* 1982, **2**: 1025-1029.
306.Venier, P., Maggs, A.C., Carlier, M.F. and Pantaloni, D., Analysis microtubule rigidity using hydrodynamic flow and therm fluctuations. *J. Biol. Chem.* 1994, **269**: 13353-13360.
307.Vernos, I. and Karsenty, E., Motors involved in spindle assembly a chromosome segregation. *Curr. Opin. Cell Biol.* 1996, **8**: 4-9.
308.Vikstrom, K.L., Lim, S.S., Goldman, R.D. and Borisy, G.G., Stea state dynamics of IF networks. *J. Cell Biol.* 1992, **118**: 121-129.
309.Vulevic, B. and Correia, J.J., Thermodynamic and structural analy

of microtubule assembly: the role of GTP hydrolysis. *Biophys. J.* 1997, **72**: 1357-1375.
310. Wachsstock, D.H., Schwarz, W.H. and Pollard, T.D., Cross-linker dynamics determine the mechanical properties of actin gels. *Biophys. J.* 1994, **66**: 801-809.
311. Wada, Y., Hamasaki, T. and Satir, P., Evidence for a novel affinity mechanism of motor-assisted transport along microtubules. *Mol. Biol. Cell* 2000, **11**: 161-169.
312. Wagner, O., Zinke, J., Dancker, P., Grill, W. and Bereiter-Hahn, J., Viscoelastic properties of f-actin, microtubules, f-actin/α-actinin, and f-actin/hexokinase determined in microliter volumes with a novel nondestructive method. *Biophys. J.* 1999, **76**: 2784-2796.
313. Walczack, C.E., Microtubule dynamics and tubulin interacting proteins. *Curr. Opin. Cell Biol.* 2000, **12**: 52-56.
314. Wallis, K.T., Azhar, S., Rho, M.B., Lewis, S.A., Cowan, N.J. and Murphy, D.B., The mechanism of equilibrium binding of microtubule-associated protein 2 to microtubules. Binding is a multi-phasic process and exhibits positive cooperativity. *J. Biol. Chem.* 1993, **268**: 15158-15167.
315. Walter, H. and Brooks, D.E., Phase separation in the cytoplasm, due to macromolecular crowding, is the basis for microcompartmentation. *FEBS Lett.* 1995, **361**: 135-139.
316. Wang, L, Ho, C.-L., Sun, D., Liem, R.K.H. and Brown, A., Rapid movement of axonal neurofilaments interrupted by prolonged pauses. *Nat. Cell Biol.* 2000, **2**: 137-141.
317. Wang, N., Mechanical interactions among cytoskeletal filaments. *Hypertension* 1998, **32**: 162-165.
318. Wang, N. and Stamenovic, D., Contribution of intermediate filaments to cell stiffness, stiffening and growth. *Am. J. Physiol. Cell Physiol.* 2000, **279**: 188-194.
319. Wang, Z. and Sheetz, M.P., The C-terminus of tubulin increases cytoplasmic dynein and kinesin processivity. *Biophys. J.* 2000, **78**: 1955-1964.
320. Warner, F.D. and McIlvain. J.H., Kinetic properties of microtubule-activated $^{13}S$ and $^{21}S$ dynein ATPases. Evidence for allosteric behaviour associated with the inner row and outer row dynein arms. *J. Cell Sci.* 1986, **83**: 251-267.
321. Watanabe, T., Ohtsuka, A., Murase, N., Barth, P. and Gersonde, K., NMR studies on water and polymer diffusion in dextran gels. Influence of potassium ions on microstructure formation and gelation mechanism. *Magn. Reson. Med.* 1996, **35**: 697-705.
322. Waterman-Storer, C.M. and Salmon, E.D., Positive feedback interactions between microtubules and actin dynamics during cell motility. *Curr. Opin. Cell Biol.* 1999, **11**: 61-67.
323. Watterson, J.G., The role of water in cell architecture. *Mol. Cell. Biochem.* 1988, **79**: 101-105.
324. Wells, A.L., Lin, A.W., Chen, L.Q., Safer, D., Cain, S.M., Hasson, T., Carragher, B.O., Milligan, R.A. and Sweeney, H.L., Myosin VI is an actin-based motor that moves backwards. *Nature* 1999, **401**: 505-508.
325. Wheal, H.V., Chen, Y., Mitchell, J., Schachner, M., Maerz, W., Wieland, H., Van Rossum, D. and Kirsch, J., Molecular mechanisms that underlie structural and functional changes at the post-synaptic membrane during synaptic plasticity. *Prog. Neurobiol.* 1998, **55**: 611-640.
326. Wiche, G., Role of plectin in cytoskeleton organization and dynamics. *J. Cell Sci.* 1998, **111**: 2477-2486.
327. Wille, H., Mandelkow, E.M., Dingus, J., Vallee, R.B., Binder, L.I. and Mandelkow, E., Domain structure and antiparallel dimers of microtubule-associated protein 2 (MAP2). *J. Struct. Biol.* 1992, **108**: 49-61.
328. Winckler, B., Forscher, P. and Mellman, I., A diffusion barrier maintains distribution of membrane proteins in polarized neurons. *Nature* 1999, **397**: 698-701.
329. Wong, G.C., Tang, J.X., Lin, A., Li, Y., Janmey, P.A. and Safinya, C.R., Hierarchical self-assembly of F-actin and cationic lipid complexes: stacked three-layer tubule networks. *Science* 2000, **288**: 2035-2039.
330. Wriggers, W. and Schulten, K., Stability and dynamics of G-actin: back-door water diffusion and behavior of a subdomain 3/4 loop. *Biophys. J.* 1997, **73**: 624-639.
331. Wriggers, W. and Schulten, K., Nucleotide-dependent movements of the kinesin motor domain predicted by simulated annealing. *Biophys. J.* 1998, **75**: 646-661.
332. Wu, H.W., Kuhn, T. and Moy, V.T., Mechanical properties of L929 cells measured by atomic force microscopy: effects of anticytoskeletal drugs and membrane crosslinking. *Scanning* 1998, **20**: 389-397.
333. Wu, X., Jung, G. and Hammer, J.A. III, Functions of unconventional myosins. *Curr. Opin. Cell Biol.* 2000, **12**: 42-51.
334. Wu, Z., Wong, K., Glogauer, M., Ellen, R.P. and McCulloch, C.A., Regulation of stretch-activated intracellular calcium transients by actin filaments. *Biochem. Biophys. Res. Commun.* 1999, **261**: 419-425.
335. Xian, W., Tang, J.X., Janmey, P.A. and Braunlin, W.H., The polyelectrolyte behavior of actin filaments: a 25 mg NMR study. *Biochemistry* 1999, **38**: 7219-7226.
336. Yamada, S., Wirtz, D. and Kuo, S.C., Mechanics of living cells measured by laser tracking microrheology. *Biophys. J.* 2000, **78**: 1736-1747.
337. Yamamoto, S., Tsutsui, H., Takahashi, M., Ishbashi, Y., Tagawa, H., Imanaka-Yosjida, K., Saeki, Y. and Takeshita, A., Role of microtubules in the viscoelastic properties of isolated cardiac muscle. *J. Mol. Cell Cardiol.* 1998, **30**: 1841-1853.
338. Yamauchi, E., Titani, K. and Taniguchi, H., Specific binding of acid phospholipids to microtubule-associated protein MAP1B regulates its interaction with tubulin. *J. Biol. Chem.* 1997, **272**: 22948-22953.
339. Yamauchi, P.S. and Purich, D.L., Microtubule-associated protein interactions with actin filaments: evidence for differential behavior of neuronal MAP-2 and tau in the presence of phosphatidyl-inositol. *Biochem. Biophys. Res. Commun.* 1993, **190**: 710-715.
340. Yanai, M., Butler, J.P., Suzuki, T., Kanda, A., Kurachi, M., Tashiro, H. and Sasaki, H., Intracellular elasticity and viscosity on the body, leading and trailing regions of locomoting neutrophils. *Am. J. Physiol.* 1999, **277**: 432-440.
341. Yanagida, T., Kitamura, K., Tanaka, H., Iwane, A.H. and Esaki, S., Single molecule analysis of the actomyosin motor. *Curr. Opin. Cell Biol.* 2000, **12**: 20-25.
342. Yang, Y., Bauer, C., Strasser, G., Wollman, R., Julien, J.P. and Fuchs, E., Integrators of the cytoskeleton that stabilize microtubules. *Cell* 1999, **98**: 229-238.
343. Yoon, M., Moir, R.D., Prahlad, V. and Goldman, R.D., Motile properties of vimentin intermediate filament networks in living cells. *J. Cell Biol.* 1998, **143**: 147-157.
344. Young, A., Dictenberg, J.B., Purohit, A., Tuft, R. and Doxey. S.J., Cytoplasmic dynein-mediated assembly of pericentrin and γ tubulin onto centrosomes. *Mol. Biol. Cell* 2000, **11**: 2047-2056.
345. Yu, W., Cook, C., Sauter, C., Kuriyama, R., Kaplan, P.L. and Baas, P.W., Depletion of a microtubule-associated motor protein induces the loss of dendritic identity. *J. Neurosci.* 2000, **20**: 5782-5791.
346. Zambito, A.M. and Wolff, J., Palmytoylation of tubulin. *Biochem. Biophys. Res. Commun.* 1997, **239**: 650-654.
347. Zimmermann, A., Keller, H.U. and Cottier, H., Heavy water ($D_2O$)-induced shape changes, movements and F-actin redistribution in human neutrophil granulocytes. *Eur. J. Cell Biol.* 1988, **47**: 320-326.
348. Zimmerman, S., Zimmerman, A.M., Fullerton, G.D., Luduena, R.F. and Cameron, I.L., Water ordering during the cell cycle: nuclear magnetic resonance studies of the sea-urchin egg. *J. Cell Sci.* 1985, **79**: 247-257.
349. Ziyadeh, F.N., Mills, J.W. and Kleinzeller, A., Hypotonicity and cell volume regulation in shark rectal gland: role of organic osmolytes and F-actin. *Am. J. Physiol.* 1992, **262**: 468-479.