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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 modulated 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 occurrence 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
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 heterogeneous 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 adapting 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 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 IF building block is made of antiparallel homo- or heterodimers, 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 resistance 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 their 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 labeling polymer (MTs et MFs) to the organization of polymers into networks and their anchorage to various membraneous compartments (46,53,59,112,200,254,313). A second group of cytoskeleton-associated proteins is constituted 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 reflect 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 interphase, divided cells, a MT network seeded in one or several MT organisation centers (MTOC: nuclear membrane and specialized domains underneath the plasmalemma in plants and 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 (148,202). MT-associated proteins allow the anchorage of many organelles to MTs in a static (structural) or 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 (ameboid 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-solution 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 interdependence 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 intimitiy 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 immobile 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 and the phosphorylation of actin-associated proteins (43,150,168,220). In addition, some types of cells exhibit a strong resistance 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 direct contribution of the cytoskeleton to regulation of cell volume and exchanges of water, metabolites and ions with the extracellular medium led to the hypothesis that the whole actin network acts as an osmotic sensor, alone or in cooperation with MTs (43,68,257,278).

The molecular basis of such a property of cytoskeletal polymers is not well known. Indirect evidence was obtained in model systems, suggesting that polymerization mechanisms of macromolecules and their organization into networks may be directly related to their osmotic properties (57,100,158,185,273), which raises the possibility that interactions between water molecules and protein domains of the cytoskeleton subunits are key factors in the properties of cytoskeleton toward the behavior of water compartment in the cytoplasm. The changes of actin hydration during the transition of G-actin to F-actin may be part of the major involvement of actin in osmotic properties of cells (78,130,287). However, other mechanisms of interaction of cytoskeletal polymers with water are likely required for their contribution to the osmotic balance of cells. A more effective component of this function of cytoskeleton in water control could be the hydration level of polymers and of crossbridges between polymers in cytoskeletal networks of cells, as reflected by the changes in compressibility associated with polymerization and network formation, consecutive to the modification of the area of solvent exclusion of polymers (257,312). The shrinkage of F-actin under osmotic pressure (273), the bundling of F-actin induced by osmotic stress (288), and the inhibition of osmotically driven water flow by actin filaments, regulated by the crossbridging protein ABP (129,130), suggest strongly that the immobilization of cytoskeletal polymers into crossbridged networks affects directly the mobility of water in cells. The control of osmotic pressure through the water release or adsorption by the cytoskeleton may depend primarily on the physical organization of cytoskeletal polymers together into dense and loose networks, as suggested by the regulation of cell volume by the reversible polymerization of F-actin (349).

Another element of the cell volume regulation by the cytoskeleton results from the direct control of ion channels by actin filaments and proteins (3,124,209,221,257,278). The involvement of actin networks in the regulation of stretch-activated channels (90,133,354) further showed that the dynamic organization of F-actin underneath the plasma membrane is a major effecter of the exchange of ions between the cell and the extracellular medium. In this perspective, the hypothesis was recently raised that the regulation of cell volume occurs through the modulation of the life time of crossbridges between filaments, affecting
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 D₂O versus H₂O 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 accompanied 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 accompanied 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 D₂O 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 (D₂O) on polymerization and stability of MTs and actin filaments in vitro and in living cells. D₂O 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, D₂O 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 D₂O in cells occurs likely through polymerization-independent mechanisms involving D₂O-enhanced stabilization of polymers by associated proteins. Second, these findings suggest that the differences in the physical properties of H₂O and D₂O molecules have direct consequences on the association between tubulin or actin monomers into polymers. A hypothesis on the molecular mechanisms of action of D₂O 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 bonds 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 D₂O allowed a denser gel than that obtained in H₂O 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 that 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 control their diffusion in the cytoplasm, thus providing a high 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 a 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 resist 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 coordinate-mechanical actions. In all steps of these molecular crosstalks from which arise life, a local and long range order is generated which is imposed to the water-based medium c
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 primarily 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-Pi and ATP/ADP-Pi) suggests 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 resistance 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 resistant 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, resistant, stable, they are the bones of the cytoskeleton, withstanding strong stresses.

In contrast to F-actin, IFs are less elastic at low strain, but exhibit a strain hardening under increasing strains, which is consistent with their presumed role in stabilizing the cell integrity against strong deformations. This property is shared by all types of the large IF family tested (134,138,180,196) with a particularly high resistance to large strains specific for the unusual NF polymers which are unique in having numerous interfilament contacts through the lateral projections of their HMW subunits that induce the formation of semi parallel alignments of these flexible IFs similar to that of NF bundles in neurons (180). Although several IF-associated proteins have been identified which were shown to mediate interactions between IFs and other cytoplasmic structures (112), no studies similar to those on F-actin have been performed yet on their effect on IF viscoelasticity, with the exception of preliminary evidences that small heat shock proteins interacting with type III IFs in cells may strongly interfere with IF gelation *in vitro* (63).

Nevertheless, the comparison between the physical strength of gels made of NFs and vimentin or desmin IFs suggests that the presence of NF sidearms is a major determinant of the mechanical properties of NFs in axons, and raises the possibility that similar effects may be achieved by IF-associated proteins in other cell types.

- **Viscoelasticity of MTs**: → lonely, stiff, however often bundled. Are they the brain of the cytoskeleton, organizing without direct involvement in action? Their MAPs and motors do the work.

Rheological studies of MTs are rare, and suggest that they exhibit little viscoelasticity under small strains which does not increase with larger strains that induce further the breaking of the sample (39,132,134,138,268,312). These data are consistent with the fact that MTs are stiff polymers not interacting with each others, and raise in addition the hypothesis that their associated proteins (MAPs) do not mediate significant interactions between MTs. However, studies of viscosity changes in high concentrations of MT purified from brain with saturated amounts of MAPs showed that the weak viscosity of MT suspensions is affected by nucleotides, and by cytoplasmic organelles such as mitochondria which bind to MTs via MAPs sidearms (179). Although preliminary, these indications support the hypothesis that weak interactions between MAPs-bearing MTs may occur, which remain to be analyzed in further work, such as with non-destructive methods recently devised (239,312). No evidence of neuronal MAPs-mediated crossbridging of MTs have been obtained *in vitro*, although...
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 cytoskeletal 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 lambrush-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 tensivegrity hypothesis, in which mechanotransduction is thought to occur through a hard-wired cytoskeleton (125). However, a direct test of the tensivegrity 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 organization 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 tensivegrity 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 salatory motion of axonal NFs in association with MTs suggest the alternate contribution of retrograde and anterograde molecular motors (262,275,316). Salatory motor 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 addressed with in vitro models in which both the presence of motors and the geometry of the limiting compartment determine the morphology of the cytoskeleton arrays (107,120,223). The self-organization of cytoskeletal polymers in the presence of a limited number of associated proteins may thus be a major principle of the pattern formation of cytoskeleton arrangements in living cells involved in morphogenesis or differentiated cell specialization. Furthermore, evidence that gravity-dependent reaction-diffusion limited MT patterns occur in 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 mechanical ordering within the MT structure through interactions of tubulin monomers with the water molecules of their hollow core (143,211) bring support to a theory of information processing in MT structure (248). The intriguing recent finding that a motion of gold-coupled antibodies on the MT surface occurs in the absence of direct binding to the motor kinesin or dynein but as a consequence of these motor-dependent motions of MTs, adds experimental support for such hypotheses, since these data suggest that coherent conformational changes of tubulin drive this lateral diffusion of bound proteins, a process which could be also at the origin of the diffusion-dependent sliding of the STOP protein on MTs (201,311).

From these attractive hypotheses, the possibility is likely that the physicochemical properties of cytoskeletal polymers determine several levels of molecular ordering of the cytoplasm, from the controlled diffusion of ions and soluble proteins resulting from their organization as an anionic cytoplasmic scaffold, to sophisticated topography of integration of signals inside their three-dimensional structures. Moreover, may also involve the strong cooperativity between monomers, inducing conformational transitions during the polymerization mechanism, or inside polymers following the binding of associated proteins (72,252). Similarly, the binding of associated proteins to the activity of cytoskeleton-associated motors exhibit cooperativity, which may result in a discontinuous (patch) distribution of associated proteins along the polymer (45,101,197,314,320) or induce long range conformation changes of the polymer subunits such as in actomyosin, a change in the twist of F-actin is thought to produce the contraction force (177,272).

**Molecular motors**

Molecules that move on their own, provided some sort of fuel: a great discovery of the last 20 years. But the rail seems to do as much work as the engine. How water molecule 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 the 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 motion 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 quanta 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 channeling 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 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 weakly associated. The bidimensional mobility of transmembrane proteins in lipid bilayers is directly or indirectly affected by the membrane skeleton and the cytoskeleton, as a result from the strength of interactions between proteins of the two structures (28,139,166,264,267,271,282,328). The physiological consequences of this topographic constraint are numerous: the tension-induced reinforcement of integrin-based adhesion sites is the result of clustering of integrin molecules through the recruitment of integrin-binding proteins in the immediate submembranous cytoskeleton domain (59,133), and the cytoskeleton-dependent clustering of many plasma membrane proteins such as N-CAM, neuroreceptors, Na/KATPase and other ion channels is a key event in their physiological function at specific locations on the cell surface (28,60,114,167,205,215,325,328). Similar conclusions were reached when studying intracellular membranous compartments such as tubulovesicular reticulum membranes or mitochondria in which clusters of membrane proteins are induced by interactions with the cytoskeleton (6,181).

These functional relationships between cytoskeletal elements and membranes might involve particular interactions of the cytoskeletal polymers with water molecules at the vicinity of lipid bilayers, where lipid-bound water molecules contribute to dipole potentials in membranes (33,85). Model systems suggested that the interaction between proteins and lipid membranes affects both the organization of lipid molecules and the activity of the protein, and that the hydration water plays a key role in these interactions (85,87,284). Detailed indications for such rearrangements were obtained from studies of the binding of the protein kinase C (PKC) to membranes, which occurs through a higher hydration of the enzyme (88). The binding to membranes promotes the activity of PKC in direct correlation with the phase of the lipids (89). Thus, the activation of PKC in cell membranes by its activator, diacylglycerol could be the consequence of the induction by diacylglycerol of the transition of the bilayer to hexagonal cubic phases (92). Similar mechanisms might be at the heart of interactions between cytoskeletal proteins and membranes. The binding of actin to lipid bilayers through tight interaction with few lipid molecules induces conformational change of the protein (87,329). The insertion of tubulin into biological membranes involves postaductional modifications of the tubulin polypeptide (acetylation, palmytoylation) and the interaction of the modified tubulin subunit with transmembrane proteins (8,346). The binding of the vimentin IF subunit to lipid bilayers involves both electrostatic interactions of the
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 privileged 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 occurrence 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 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 D₂O-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 kinesin-mediated bidirectional motility of NF fragments along MTs (275), are preserved for days (even months for NF properties) in buffers containing 50-80% D₂O. Thus, it seems that D₂O allows in vitro what H₂O 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 D₂O 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 of the variable region of the last 10-18 amino acids (6)), supports further the possibility that this domain (and the hydration state) plays a crucial role in the dialogue between MTs and their numerous binding partners.

The supramolecular organization of subunits in cytoskeletal polymers determines the emergence of new phenomena, which are most clearly represented by the polyelectrolyte nature of all polymers and the self-organization of concentrated suspensions following distinct processes such as liquid crystal arrays, motor-crossovering protein-dependent bundles, asters and geodes and dissipative energy structures. The probability for spontaneous organization of cytoskeletal polymers in liquid crystal arrays in living cells is high: In cytoplasmic subdomains such as axons and dendrites of neurons, the concentration of MTs is close to 200 μM (estimated from their density in electron microscopic sections compared that of sedimented MTs); F-actin is concentrated in growing cones of neurons, in the front lamella of moving cells or muscle cells (were F-actin concentration is about 10 times the concentration of 60 μM that is required for the nematode phase shift). These properties of cytoskeletal structures have profound consequences on the structure of water and solutes in the cytoplasm. Whether a hydration layer of polymers is stable, metastable or highly labile organization of water molecules on the surface of the polymer remains to be established. Nevertheless, evidence for the direct consequences of crossbridging between polymers on the diffusional characteristics of water is emerging. If transport to the real situation of the cytoskeleton in cells, these indications suggest that all cellular events involving changes in the stability, the stiffness and the relative organization of cytoskeletal elements through associated and crossbridged proteins, likely result in strong modifications of the local diffusion of water and solutes. The numerous observations of the contribution of crossbridging and polymerization of the cytoskeleton to the osmotic properties of living cells are in direct support of this hypothesis. The importance of the polyelectrolyte function of cytoskeletal polymers in the spatial organization together and the association of many cellular proteins, including non-cytoskeleton molecules, the cytoskeleton scaffold, has been reviewed recently (13). Moreover, another consequence of this property to consider with respect to the presence of a cloud of counterions surrounding the polymer structure, is the channelling of intra-transport along the cytoskeleton (44,186), possibly in relation with membrane potentials and electric fields. With new developments of this approach in the next years, one can expect that the studies of functional interactions between...
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 might 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 might 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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