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Cytoskeletal Networks and Filament Bundles: Regulation by Proteins and Polycations

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The three-dimensional polymer network formed by the cytoskeleton is the main determinant of cellular mechanics (Elson, 1988; Maniotis *et al.*, 1997) and is required for the cell to resist external forces as well as to generate and transmit the forces used during cell motility (Stossel, 1994). Three types of protein filaments—microtubules, F-actin, and intermediate filaments—form the basis of the cytoskeleton. Certain types of polymers tend to concentrate in separate regions of the cell; typically actin is concentrated at the cell cortex, whereas the microtubules and intermediate filaments are more centrally localized. However, the three types of cytoskeletal filaments can also interpenetrate and form contacts with each other and with specialized structures in cell membranes to provide mechanical continuity throughout the cell. The architecture of these networks depends on local activation of specific regulatory elements, and the variety of structures they form have distinct mechanical characteristics (Satcher and Dewey, 1996). Two distinct types of cytoskeletal assembly are open meshworks of single filaments, and asymmetric assemblies of filament bundles.

The viscoelastic properties of networks formed by F-actin, microtubules, and various intermediate filament types (*e.g.*, vimentin and neurofilaments) differ strongly from each other, as shown in Figure 1. At biologically relevant stresses (*e.g.*, from the 10 dyne/cm^2 of fluid shear stress at the artery wall, to the greater stresses needed for phagocytosis and locomotion) the different types of purified cytoskeletal polymer networks exhibit very different mechanical responses. At a constant weight concentration (2 mg/ml), microtubule networks deform to the largest extent, presumably because there are no bonds to keep the rigid polymers from sliding past each other. In contrast, networks of long actin filaments or of short actin filaments linked to each other by the divalent ABP280 crosslinker initially exhibit very little deformation, but at larger stresses these networks appear to rupture. Vimentin intermediate filaments are more deformable at smaller stresses than F-actin but resist much larger stresses without rupture. This ability to deform without damage, and the downward curvature of the strain/stress plot indicative of strain hardening is also observed in the extracellular fibrin network. The mechanical differences suggest some aspects of the possible biological function of these cytoskeletal elements; they also suggest the molecular basis of the elasticity of such networks, which differs radically from the viscoelastic properties of rubber-like materials (MacKintosh *et al.*, 1995; Kroy and Frey, 1996; Maggs, 1997).

Bundles of filamentous polymers are also a common feature of biological tissues, ranging from partly ordered structures (such as stress fibers), to well-ordered structures (such as sarcomeres and the paracrystalline arrays of actin filaments in microvilli and microtubules in flagella). The formation of such structures *in vivo* is generally thought to be orchestrated by the activity of specific binding proteins (Otto, 1994); but the thermodynamic driving force for the formation of bundles is largely unknown (Grazi, 1994; Tang *et al.*, 1997). Like DNA, all of the cytoskeletal filaments are anionic, with linear charge densities sufficiently high to stabilize electrostatic interactions with polyanions even at physiological ionic strength

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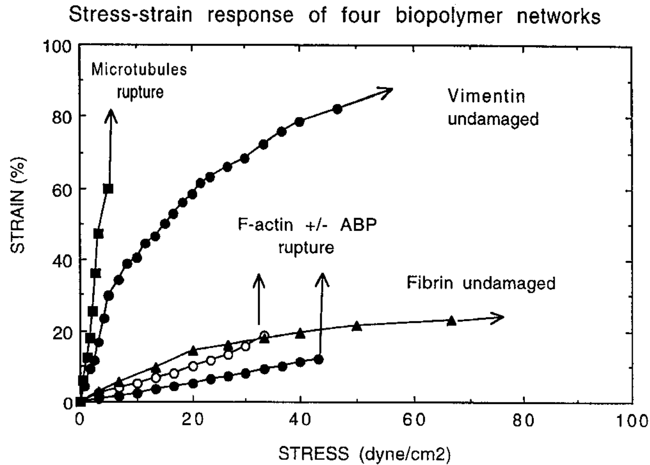

Figure 1. Shear strain of 2 mg/ml samples of polymerized biopolymer networks. The measurements were made 10 s after a range of shear stresses were imposed with a torsion pendulum, as previously described (Janmey, 1991).

(Tang *et al.,* 1996). Theories of polyelectrolytes developed to account for cation-induced condensation of DNA (1) apply equally well to F-actin, microtubules, intermediate filaments, and some filamentous viruses; and these theories provide an explanation for the ability of specific polycationic proteins to be efficient bundling factors for all of these diverse filament types. The effects of metal ions and polyvalent protein ligands on the structure and rheology of cytoskeletal networks likewise provide data relevant to both the biological function of the networks and the molecular structures underlying their mechanical properties.

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Discussion

COULOMBE: I have a question about the behavior of individual filaments in a network of similar filaments. If the filament surface is charged, how do you explain node formation at discrete points along the length of the network?

JANMEY: If the filaments are all anionically charged, you might expect them to be so electrostatically repulsive that they would never come near enough to one another to make nodes. There are two possibilities. One is that some of those nodes are simply kinks in which two filaments, that may be repulsive to each other, are caught in something like a local knot. What we are then looking at is the time it takes for that kind of knot to unravel. A second possibility is that, although the filaments repel each other in a vacuum, when they are in a medium full of counter ions attractive interactions can be created between polymers with like charges. The attraction could be based on the sharing of counterion clouds, or on fluctuations in that cloud. In this way, dipole-dipole interactions may in some cases overcome electrostatic repulsion between the polymers. This is an experimental surprise to us, but it holds up consistently.

FORGACS: If you shear or deform a microfilament network, is it possible to change some rate of polymerization?

JANMEY: I'm not sure that is known for actin. In the case of microtubules, there is evidence that assembly and disassembly rates of tubulin dimers on the microtubule end can be affected by something that looks like a force-generating mechanism. In an actin system it is more likely that you would generate breakage of actin filaments, and accelerate either polymerization or

depolymerization. The short answer to your question is that we do not really know.

FORGACS: You mentioned that microtubule and actin networks do not interact in your assays, whereas intermediate filaments and actin do. Do we know anything about these interactions in the cell?

JANMEY: The filament systems are intimately related to one another—it is very difficult to manipulate one without manipulating the others. The question is whether that kind of connectivity is directly mediated by polymer-polymer contact, or whether it works through specific regulatory proteins that mediate this "talking" of one filament system to another. That is the kind of question we would like to address. In the case of microtubules and actin, it looks as though the interaction must be mediated by some third protein.

FORGACS: If those filaments are all negatively charged, is it possible that some signaling molecules, which are positively charged, can slide or diffuse within those filaments, thereby contributing to their stability?

JANMEY: That is an appealing concept, but I do not know if it has been experimentally tested and verified. There is an interesting split in thinking of the cytoskeleton from a purely mechanical view, which is our lab's prejudice, dissociating it from the connectivity made by a percolated network. That is why the split between making a connective network or a structure rigid enough to resist or support a mechanical stress presented in David Boal's talk is so interesting. We do not know how to separate those two features.

CHEN: Does tagging the actin with a fluorescent group change its stiffness or affect surface interactions with other actin polymers?

JANMEY: That is a really good question. We cannot detect differences in flexibility between a fluorescently tagged actin filament and a non-tagged filament by using techniques such as dynamic light scattering or electron microscopy. Some fluorophores carry their own electrostatic charge. Therefore, we should be able to assemble populations of filaments consisting of the same protein, yet differing by 10%–20% in electrostatic surface charge, due to the fluorophore that we couple to them. If the hypothesis regarding surface charges is correct, then the fluorescently tagged filament types should behave differently. We have yet to test this. In answer to your question, there is no obvious alarm that fluorescent tagging of actin filaments is a problem.

GOLDMAN: Did you mix microtubules and intermediate filaments, especially neurofilaments?

JANMEY: Yes, we have mixed them. One of our problems with intermediate filaments and other filament types is how to take polymerized systems and instill another polymer into them. We start by placing preformed neurofilaments and preformed microtubules next to each other and gently trying to get them to interpenetrate. One of the really interesting findings, which Shah will present, concerns vimentin. We have tried to polymerize vimentin around very low concentrations of labeled actin filaments. The polymerization process breaks the filaments into small pieces. This surprising result might be an interesting mechanical problem. We know it is not simply due to a chemical poison effect, because before the vimentin subunits form a network they don't do anything to destabilize the actin. Only after vimentin begins to polymerize and form a visco-elastic network does it break up the actin filaments. We can polymerize tubulin around actin, but we cannot polymerize vimentin around actin without breaking up the actin. Therefore, it is possible that some of the work of polymerization has the consequence of breaking actin filaments.

TAYLOR: Actin and myosin subfragments have a net negative charge, yet they still interact because of local charge distributions on a protein surface.