The soft framework of the cellular machine

D. A. Weitz*† and P. A. Janmey‡

**Department of Physics and School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138; and ‡Departments of Physiology and Physics, Institute for Medicine and Engineering, University of Pennsylvania, 3340 Smith Walk, Philadelphia, PA 19104*

The mechanical properties of cells are essential in determining a myriad of functions, from mitosis to locomotion. The functional rigidity of a cell is usually thought to result from three interpenetrating networks of filamentous biopolymers: actin microfilaments, microtubules, and intermediate filaments (IF). The mechanical properties of both filamentous actin (F-actin) and microtubule networks have been extensively studied, both directly in cells and in model *in vitro* systems, consisting of reconstituted networks of purified proteins. The assembly and structures of actin and tubulin polymers, coupled to ATP and GTP hydrolysis, respectively, give rise to fascinating dynamics that have attracted experimentalists, theorists, and modelers for decades, trying to understand their properties and functions (1, 2). In addition, the diverse molecular motors that run along these tracks are central to much of cell dynamics and vesicle transport. By contrast, intermediate filaments do not hydrolyze nucleotides, do not exhibit structural polarity, and have no motors that run along them. Also, unlike actin and tubulin, which exist in very similar forms in nearly all eukaryotic cells, IF proteins appeared later in evolution and mutated rapidly to form distinct molecular species in different cell types (3). Some classes of IFs can be genetically ablated in mice without the mice necessarily losing viability and resulting in some cases in a barely discernable phenotype (4). However, the IFs expressed in epithelial cells, keratin IFs (or KIFs), are required for normal epithelial function, and mutations in these proteins can cause devastating human diseases (5–7). In an article in a recent issue of PNAS, Sivaramakrishnan *et al.* (8) reported on the results of a remarkable study of the micromechanical properties of IF networks that should begin to redress our imbalance in understanding of the mechanics of the different biopolymer networks. These authors show that the KIF networks are essential for the mechanical integrity of the cell, and without them, cells such as alveolar epithelial cells would be helpless to withstand the forces they experience as the lung inflates and stretches them.

The work of Sivaramakrishnan *et al.* (8) is remarkable in the means by which the mechanical properties of the IF network are measured. The authors study primary rat alveolar epithelial cells (AEC). They take advantage of the rapid dynamics of F-actin and microtubules compared with KIFs by designing extraction protocols that disassemble and remove these structures while leaving the KIF network intact. Remarkably, the size and shape of the KIF network remains the same as that of the live cell before its membrane and cytoplasm are removed. Sivaramakrishnan *et al.* use a combination of electron microscopy and optical imaging as well as multiparticle tracking microrheology (9, 10) to measure the structure and mechanical properties of the network. They distinguish properties of spatially different regions of the cell and show that the stiffness of the network varies with location. Moreover, they show that it depends inversely on the square of the network mesh size, or the average distance between filament crossings.

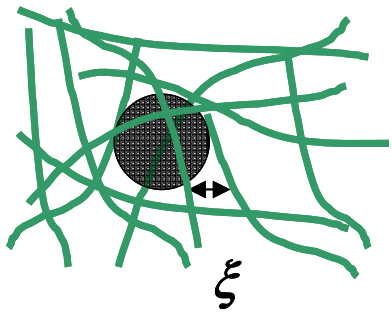

Fig. 1. Schematic view of particle tracking. A probe particle is embedded within the network that constrains it. Its thermal motion reflects fluctuations of the network, providing a measure of the network elasticity. The average mesh size, ξ, is shown by the arrow.

The method that Sivaramakrishnan *et al.* (8) use to measure the mechanical properties is multiparticle tracking microrheology (9, 10). They injected small fluorescent PEG-coated probe particles into cells before extraction, leaving the particles embedded in the KIF network, and measured the thermal motion of these tracer particles by using optical microscopy. The positions of the probe particles are determined to precisions of a few nanometers and are tracked as a function of time. The surrounding network constrains the particle, and its thermal motion provides a measure of the viscoelastic properties of the network, as shown schematically in Fig. 1. There are two distinct portions to this response: The first is the elastic modulus, which measures the recoverable deformation of the network; if a force is applied, the elastic modulus measures the displacement that is reversed when the force is removed. The second is the loss or viscous modulus, which is a measure of how viscous the network is. Sivaramakrishnan *et al.* find that the network is predominantly elastic, albeit with a small but measurable loss modulus. This is most readily seen by the fact that probe particles are constrained to move in a confined volume; they do undergo thermal motion, but they cannot move over very large distances.

Measurements of the mechanical properties of the biopolymer networks that support a cell are exceptionally difficult (1, 2). Ideally, these measurements should be performed on a living cell. Such measurements, however, are particularly difficult to interpret, in part because of the heterogeneity of the cell. Local microrheological measurements are generally not feasible in a living cell because the mechanical motion generated by the motors in the cell confounds any microrheological method, yielding incorrect results. The studies are further complicated by the fact that, in a cell, all three networks, the F-actin, microtubule, and IF networks, are intertwined and are embedded in a crowded, complex environment. Despite these difficulties, there are some valuable attempts to make these measurements. For example, magnetized beads are attached to the surface of the cell by using integrin-specific coatings that bind to the focal adhesions of the cell and couple to the actin, or in some cases the IF networks. By measuring the amount of twisting due to the torque induced by an applied magnetic field, it is possible to determine the viscoelastic response of the cell. However, this method, called magnetic bead twisting cytometry (MTC), does not distinguish contributions of the different networks (11). Other measures entail attaching a cell to the surfaces of two cantilevers, and stretching it (12).

Author contributions: D.A.W. and P.A.J. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 889 in issue 3 of volume 105.

†To whom correspondence should be addressed. E-mail: weitz@seas.harvard.edu.

This method provides a direct measure of the elasticity of the cell, but again cannot distinguish contributions of the different networks within the cell.

The difficulty in measuring the mechanical properties of the individual biopolymer networks in cells has inspired a different approach: Individual proteins are purified, and an *in vitro* network is reconstituted from them, enabling specific networks to be isolated and investigated separately. However, because the networks are formed by random polymerization, their structure and morphology will, at best, only resemble those within the cell. Nevertheless, these measurements offer considerable insight into the mechanical properties of the individual networks and can provide a basis for understanding their behavior. Several studies have also explored the role of different cross-linking proteins on the elasticity of these reconstituted networks and have shown that they have a significant effect, which depends both on the degree of cross-linking and on the specific nature of the cross-linkers (2). Reconstitution of IF networks is particularly problematic because, although the IF proteins themselves are relatively well characterized and feasible to prepare in purified form, much less is known about the way in which IFs are linked to each other by crosslinking proteins or other molecules in the cell (3).

Given the current status of measurements of the elasticity of biopolymer networks, the results of Sivaramakrishnan *et al.* (8) are unprecedented in their precision and in their ability to directly measure mechanical properties of an IF network. This work overcomes the problems associated with reconstituted networks by using a network that is identical to that in the cell. Sivaramakrishnan *et al.* provide a very good measure of the stiffness of the KIF network and obtain quantitative agreement between the measured elasticity and that predicted theoretically. In addition, they show that the elasticity of the KIF network can directly account for the observed displacement of the nucleus in a cell when a modest force is applied to its surface; moreover, the structure of the KIF network is modified upon application of a steady shear stress, with the mesh size decreasing and the elasticity increasing in the periphery of the cell. These results provide strong support for the concept that the KIF network is essential in determining the mechanical properties of the cell, and suggest new experiments to probe the cell's response to a wider range of stresses.

IF networks can be stretched to more than 300% of their resting length.

The measurements of Sivaramakrishnan *et al.* (8) rely explicitly on the thermal motion of the probe particles to make the measurements. As such, the displacements of the network are quite small. Thus, these measurements do not address one key feature of all biopolymer networks but of IF networks in particular. IF networks can be stretched to more than 300% of their resting length (13, 14). By comparison, F-actin and microtubules are much more resistant to deformation and break if stretched by even a few percent. Moreover, the more IF networks stretch, the stiffer they get (15, 16). This nonlinear elastic response, called strain-stiffening, appears very well suited to enable cells to be compliant to small forces but to resist damage when larger forces are applied (17, 18). Clearly an important next step would be to investigate the behavior of the KIF network at larger strains to determine their propensity for strain stiffening. Investigations into the time dependence and relaxation of IF network elasticity are also likely to lead to insights into the functions of these proteins. Moreover, IFs are also involved in a host of cellular functions that are not necessarily related to their mechanical effects (19, 20). The work of Sivaramakrishnan *et al.* represents an important new method for measuring the mechanics of IF networks and sets the standard for measurements of the mechanics of other biopolymer networks. Future studies of IF networks should help elucidate both their mechanical properties and the role of cross-linker proteins and molecular motors in determining their structure and properties.

1. Janmey PA, Weitz DA (2004) *Trends Biochem Sci* 29:364–370.
2. Kasza KE, Rowat AC, Liu J, Angelini TE, Brangwynne CP, Koenderink GH, Weitz DA (2007) *Curr Opin Cell Biol* 19:101–107.
3. Herrmann H, Bär H, Kreplak L, Strelkov SV, Aebi U (2007) *Nat Rev Mol Cell Biol* 8:562–573.
4. Colucci-Guyon E, Portier MM, Dunia I, Paulin D, Pournin S, Babinet C (1994) *Cell* 79:679–694.
5. Fuchs E (1996) *Annu Rev Genet* 30:197–231.
6. Omary MB, Ku NO (1997) *Hepatology* 25:1043–1048.
7. Ku NO, Strnad P, Zhong BH, Tao GZ, Omary MB (2007) *Hepatology* 46:1639–1649.
8. Sivaramakrishnan S, DeGiulio JV, Lorand L, Goldman RD, Ridge KM (2008) *Proc Natl Acad Sci USA* 105:889–894.
9. Mason TG, Ganesan K, van Zanten JH, Wirtz D, Kuo SC (1997) *Phys Rev Lett* 79:3282–3285.
10. Valentine MT, Kaplan PD, Thota D, Crocker JC, Gisler T, Prud'homme RK, Beck M, Weitz DA (2001) *Phys Rev E* 64:61506.
11. Lele TP, Sero JE, Matthews BD, Kumar S, Xia S, Montoya-Zavala M, Polte T, Overby D, Wang N, Ingber DE (2007) *Methods Cell Biol* 83:443–472.
12. Thoumine O, Ott A (1997) *J Cell Sci* 110:2109–2116.
13. Kreplak L, Bär H, Leterrier JF, Herrmann H, Aebi U (2005) *J Mol Biol* 354:569–577.
14. Wagner OI, Rammensee S, Korde N, Wen Q, Leterrier JF, Janmey PA (2007) *Exp Cell Res* 313:2228–2235.
15. Janmey PA, Euteneuer U, Traub P, Schliwa M (1991) *J Cell Biol* 113:155–160.
16. Storm C, Pastore JJ, MacKintosh FC, Lubensky TC, Janmey PA (2005) *Nature* 435:191–194.
17. Gardel ML, Nakamura F, Hartwig J, Crocker JC, Stossel TP, Weitz DA (2006) *Phys Rev Lett* 96:088102.
18. Gardel ML, Shin JH, MacKintosh FC, Mahadevan L, Matsudaira P, Weitz DA (2004) *Science* 304:1301–1305.
19. Helfand BT, Chou YH, Shumaker DK, Goldman RD (2005) *Trends Cell Biol* 15:568–570.
20. Ku NO, Omary MB (2006) *J Cell Biol* 174:115–125.