CELL MECHANICS

# Two regimes, maybe three?

We have begun to understand the physical determinants of cell rheology and their relevance to biological functions. Experiments performed on freshly excised cells offer a new perspective in which soft-glass rheology and prestress seem to play central roles.

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The ability of cells to alter their shape in response to mechanical stresses is essential for maintaining life. Certain fundamental functions, such as crawling, spreading and invading, require cells to become highly malleable. However, cells have to maintain their structural integrity under mechanical stress, and in order to do so they must behave like an elastic solid. The apparently ambivalent mechanical behaviour makes the study of cell rheology (the relationship between deformation and applied stress) particularly challenging. On page 636 of this issue, Deng and colleagues managed to reconcile the different mechanical responses of cells by studying, over a wide range of timescales, the microrheology of the cytoskeleton (CSK), the filamentous protein network that constitutes the scaffold of cells, and interpreting the results borrowing concepts from condensed-matter physics[1].

The mechanical responses of cells are determined by the intrinsic mechanical properties of the CSK material, its architecture, and the stress-induced changes in biochemistry that modify the CSK structure. Many *in vitro* experiments on reconstituted actin (the major protein constituent of the CSK) gels suggest that the CSK must behave like a semiflexible polymer. In other words, over short times actin gels are viscoelastic, that is, they exhibit time–frequency dependence of their material moduli, but over long timescales they are elastic and their behaviour is time-independent[2]. Deng and colleagues probe the microrheology of excised smooth muscle cells by monitoring the displacement of ferrimagnetic beads attached to the cell surface and connected to the CSK through receptors that span the cell's membrane. Their results confirm that indeed the cell behaves like an actin gel, but only at small timescales!

A crucial element in this study is that the authors used freshly excised cells, which are one step closer to the cells *in situ*, whereas in previous studies on cultured cells the actin gel-like behaviour was not observed. Deng *et al.* argue that this reflects the difference in the cytoskeletal organization between fresh and cultured smooth muscle cells[1].

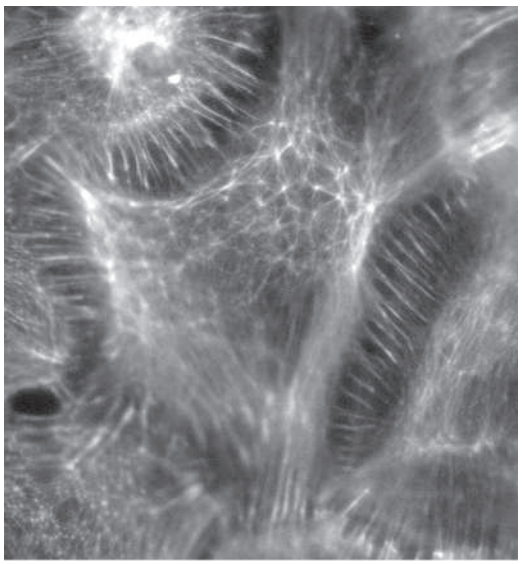

**Figure 1** The cytoskeletal actin network of mouse embryonic fibroblasts. The cells are stained with Alexa488-phalloidin to visualize filamentous actin. Courtesy of Julia Sero and Donald E. Ingber.

A number of microrheological studies on a variety of cell types indicate a similar type of mechanical response. A simple relationship, known as power law, has been found to describe how cells deform with time under a constant or an oscillatory mechanical stress. It suggests that under an applied stress, cells deform continuously and that this process is timescale invariant, that is, it does not depend on loading frequency[3–5]. This behaviour is more similar to soft glasses — fluid-like systems such as slurries or foams — and is well described by the theory of soft-glass rheology (SGR)[3–5]. Soft glasses are characterized by structural disorder; under a load, they undergo structural rearrangements in a never-ending search for order. On the macroscale, this results in a system that slowly deforms over a wide range of timescales. Although the physical basis of SGR in living cells is not yet fully understood, circumstantial evidence justifies its application[5].

The work of Deng and colleagues unifies actin network dynamics and and soft-glass dynamics in cell rheology. They show that the dynamic modulus of the cell scales with the frequency of

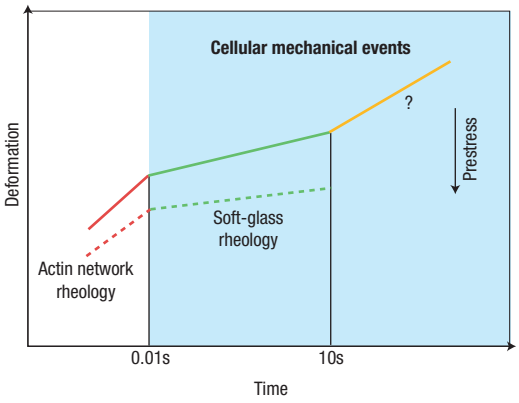


**Figure 2** What is relevant in cell rheology? A log–log plot of the creep response — a time course of cell deformation produced by a constant applied stress — summarizes the current understanding of cell rheology. The creep response is characterized by three regimes: an initial fast creep (~0–0.01 s) governed by the viscoelastic behaviour of the semi-flexible actin network (scales with the power-law exponent $\alpha = 0.75$ indicated by the red segment)[1,2]; a very slow creep (~0.01–10 s) governed by the SGR dynamics ($\alpha \approx 0.05$–$0.35$, indicated by the green segment)[1,3–5], and an intermediate slow creep (above ~10 s, indicated by the yellow segment) governed by mechanisms that are still unknown ($\alpha \approx 0.5$)[6,7]. The slope of each of the segments is indicative of the corresponding $\alpha$. An increase in prestress (direction of the increase indicated by the arrow) causes a decrease in deformation (that is, an increase in stiffness) and a decrease in $\alpha$, as indicated by the dashed lines[1,3,4,9]. Integrated mechanical events of the cell (spreading, crawling, contracting, reorienting, invading and so on) are set within timescales that correspond to the intermediate–slow time regimes.

the applied stress following two distinct regimes: at high frequency (>100 Hz) it is in a fast regime that is consistent with the dynamics of actin networks, in which the modulus scales with frequency with a universal power-law exponent $\alpha = 0.75$; at low frequency (<100 Hz) it is in a slow regime that is consistent with SGR, in which the modulus scales with frequency with a much smaller exponent $\alpha = 0.05$. From a biological point of view, these findings are enlightening. They suggest that the fast dynamics of actin networks is of little relevance because its influence is important only over short timescales (<0.01 s), whereas in cells most integrated mechanical events (spreading, crawling, contracting) are set within much longer timescales.

One lingering question remains though — whether the SGR regime also extends to longer timescales (>100 s). This is important considering the much longer time course of a number of cellular mechanical events. Previous studies performed on cultured cells[6,7] show that under constant stress, the initial slow deformation rate increases sharply after 10–100 s, suggesting that mechanisms other than the SGR might govern cell dynamics at long timescales. A more complete picture of cell dynamics will be achieved when those mechanisms will be identified.

Another issue to consider is that the CSK of living cells is mechanically prestressed[8]. This prestress is generated by molecular motors that generate forces transmitted by the actin network and through adhesion plaques to the extracellular matrix that counterbalances these forces. Microrheological measurements on cells have shown that the power-law exponent $\alpha$ decreases with increasing prestress[9]. Because $\alpha$ is an index of deformability ($\alpha = 0$ corresponds to an elastic solid, whereas $\alpha = 1$ corresponds to a flowing liquid such as water), the fact that $\alpha$ changes with prestress suggests that this regulates the transition between solid-like and fluid-like behaviour in cells. More-recent experiments on actin gels also show a similar dependence of $\alpha$ on prestress[10]. However, this behaviour is interpreted as a direct effect of the materials properties of the actin gel[10], whereas in cells its origin may be related to the architectural organization of the CSK[8]. This is an intriguing question because it implies that dynamic rheological processes within the CSK are regulated by the static mechanical stress it bears, a concept that has not been included in the present rheological models of the cell. A complete understanding of the cell's ability to deform and adapt demands a better understanding of mechanisms by which the prestress influences cell rheology. The results of Deng *et al.* hint at a rheological model where SGR and prestress play central roles.

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