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Cytoplasm mechanics and cellular organization

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Abstract

As cells organize spatially or divide, they translocate many micron-scale organelles in their cytoplasm. These include endomembrane vesicles, nuclei, microtubule asters, mitotic spindles, or chromosomes. Organelle motion is powered by cytoskeleton forces but is opposed by viscoelastic forces imparted by the surrounding crowded cytoplasm medium. These resistive forces associated to cytoplasm physcial properties remain generally underappreciated, yet reach significant values to slow down organelle motion or even limit their displacement by springing them back towards their original position. The cytoplasm may also be itself organized in time and space, being for example stiffer or more fluid at certain locations or during particular cell cycle phases. Thus, cytoplasm mechanics may be viewed as a labile module that contributes to organize cells. We here review emerging methods, mechanisms, and concepts to study cytoplasm mechanical properties and their function in organelle positioning, cellular organization and division.

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Keywords

Cytoplasm, Cell division, Organelles, Viscoelasticity, Mechanobiology.

Introduction

During cell division, polarization, and migration, cells translocate and position large organelles in their crowded cytoplasm. Cell division, for instance, is accompanied by stereotypical translational and/or rotational movements of the mitotic spindle in bulk cytoplasm, a process key to instruct the size and position of daughter cells [1,2]. Endomembrane networks and vesicles also reorganize through directional movements as cells enter mitosis, and micron-scale chromosomes translocate through the cytoplasm from the spindle centre towards cell poles in anaphase [3]. Egg cell fertilization, karyogamy, or polarized cell migration, are other exemplary processes that involve the motion of large objects like microtubule (MTs) asters and nuclei in the cytoplasm [4,5]. Force exertion by MTs and F-actin cytoskeleton needed to move organelles has been heavily discussed [1,3–6], but resistive forces opposed by the surrounding cytoplasm medium remain much less understood.

However, the cytoplasm is not a simple thin fluid, like water. It is packed with proteins, RNA, ribosomes and polysomes, endomembranes as well as cytoskeletal polymer networks [7]. These endow the cytoplasm with viscosities $\eta \sim 100-1000X$ that of water, close to that of castor oil. The cytoplasm also features solid-like properties, with elasticities of $G \sim 0.1-10$ Pa, similar to a soft hydrogel, and can as such spring objects back to their original position [8–11]. For example, the displacement of an object with a radius R of ~ 5 μm, like a nucleus, on a distance, d, of a few microns in the cytoplasm shall impart a reactive elastic force of $F \sim 6\pi GRd \sim 100$ s of pN. These forces may be partially alleviated by rearrangements of elastic elements in the cytoplasm, but are large enough to quench thermal or active random forces and stabilize organelle position; and also to oppose and limit organelle displacement being largely comparable to forces measured *in vivo* during MT aster, spindle, or chromosome motion [12–14]. The cytoplasm thus poses a fundamental challenge for cellular organization: it has to be stiff/viscous enough to stabilize organelle positions against thermal and metabolically-driven agitation, yet soft and fluid to allow cytoskeletal tracks and motors to transport organelles across cells. These considerations raise fundamental questions on the material properties of the cytoplasm, and on the mechanisms that modulate its viscoelasticity or fluidity in time and space, and on the overall function of cytoplasm mechanics for cellular organization.

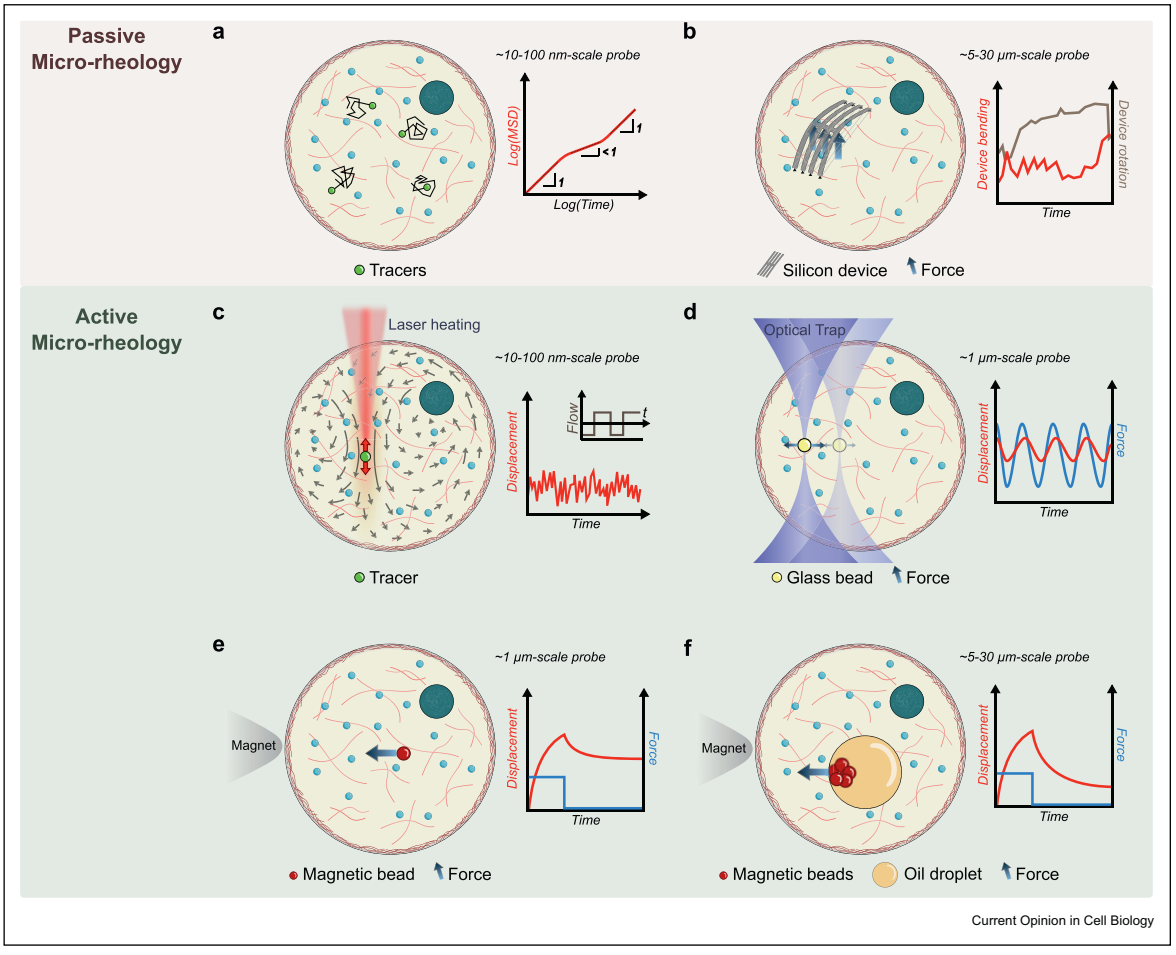
Scale-dependent cytoplasm rheology

The cytoplasm is **viscoelastic**, and its physical properties are studied using the framework of **rheology** (see box). However, as a composite material containing objects ranging from nanometric proteins to micrometric organelles, cytoplasm rheology depends on the scale at which it is being probed [7,15]. Tiny probes navigate within cytoplasm gaps and only feel the resistance of water-like cytosol, while larger objects may bump onto cytoskeletal or membranous networks, feeling enhanced

resistance. Accordingly, many methods were developed to study cytoplasm rheology at different length scales in live cells, which may be split into two categories: passive and active rheology (Figure 1).

Passive rheology uses the tracking of injected or genetically expressed tracers, whose diffusive properties inform on the mechanical properties of the surrounding cytoplasm (Figure 1a). For example, tracers will diffuse slower if the cytoplasm becomes more viscous. Tracers include fluorescently tagged endogenous protein complexes or organelles, injected or engulfed particles, as well as genetically encoded multimeric particles such as GEMs and μNS [15–21]. Passive rheology has been employed to study cytoplasm mechanics at a scale ranging from ∼10 nm to 1 μm, in systems ranging from bacteria and yeasts to stem cells and developing embryos [16,18,22–25]. In many of these systems, tracers exhibit sub-diffusive behaviours: the power dependence of their **mean squared displacement** (see box) with time lag is smaller than 1 [17]. This indicates caging effects, caused by the presence of packed **colloidal suspensions** (see box) in the cytoplasm, such as that formed by ribosome particles [18], and/or reflects the presence of

Figure 1



Methodologies to study scale-dependent cytoplasm material properties. (a) Tracking of diffusing tracers (green dots) in the cytoplasm, allows to compute their mean squared displacement (MSD) as a function of time, and to assay if their motion is sub-diffusive (exponent<1), diffusive (exponent = 1), or super-diffusive (exponent>1). **(b)** The bending and rotations of injected large passive silicon nanodevices allows for mapping the balance between cytoplasmic forces and mechanical properties. Adapted from [28]. **(c)** Generation of cytoplasmic oscillatory flows, through local laser-mediated heating, and concomitant tracking of the resultant tracer's oscillatory displacement allows for extracting cytoplasm rheological properties. Adapted from [34]. **(d)** Optical tweezers microrheology: a micron size dielectric particle is displaced with oscillatory forces created through light-scattering of a laser focused on the particle. Tracking the resultant particle displacement over time allows to compute cytoplasm rheology. **(e)** Magnetic tweezers microrheology: an injected magnetic microbead is displaced with a calibrated magnet tip in the cytoplasm, and its displacement over time is recorded to compute rheological properties. **(f)** Formation of a large magnetic probe by embedding magnetic particles in an oil droplet injected in the cytoplasm. Adapted from [11].

viscoelastic cytoskeletal or membranous networks [20]. These caging or elastic effects become more pronounced for larger tracers, but are alleviated by metabolic or motor-driven activity that agitates and **fluidizes** (see box) the cytoplasm to limit the build-up of gel-like elastic behaviour [16,19,22]. The diffusive tracing of positional fluctuations of larger components above the micron-scale including MT asters, mitotic spindles, nuclei, or injected large micro-fabricated probes, has also been performed, allowing to analyse the balance between active cytoskeletal forces and cytoplasm material properties (Figure 1b) [13,26–28]. A key advantage of passive rheology stands in its relatively simple experimental implementation. Its limitations include the difficulty of extracting exact values of cytoplasm viscoelastic constants [29], and the often-spurious effect of non-specific interactions of probes with cytoplasm elements that can affect the outcome of this method [17,21].

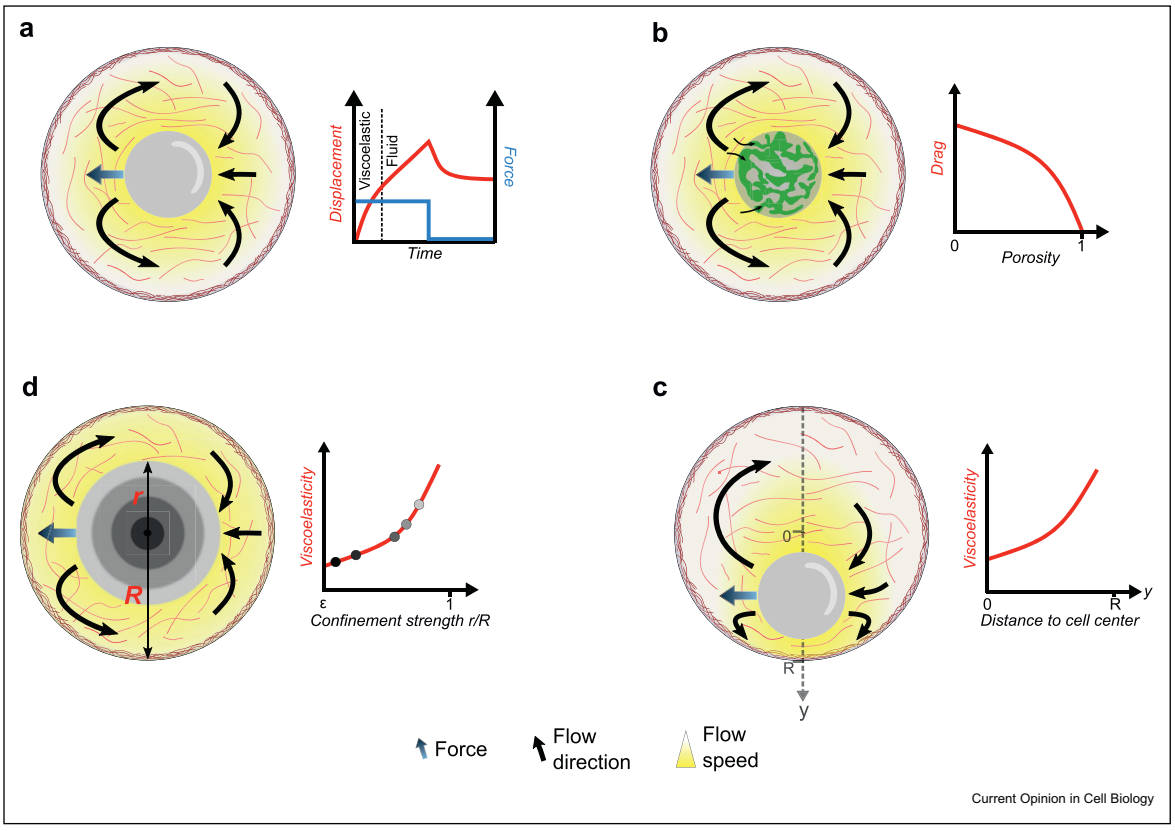
Active rheology differs from passive rheology in that it requires the application of a calibrated external force to the probe. Optical tweezers are a well-spread tool for active cytoplasm rheology. Forces are applied to dielectric (usually glass) particles that range in size from about 100 nm to a few μm, through light scattering of a laser beam focused on the particles. One advent of optical tweezers is to enable the application of periodic forces to probe rheology at multiple frequencies, which informs on time scale-dependent cytoplasm mechanics (Figure 1d). Its limitations stand in the relatively low range of force and displacement amplitudes that can be achieved. Frequency-dependent rheology has been performed with optical tweezers in cytoplasm extracts and *in vivo* in cell types including mouse oocytes, adherent human cells, and stem cells [27,30–33]. These assays showed that the cytoplasm generally behaves more as a viscous fluid when probed at high frequencies (small time scales) and more as a solid-like elastic material at smaller frequencies (longer time scales). Periodic forces have also been applied using light-controlled oscillatory flows evidencing similar frequency dependence of cytoplasm mechanics (Figure 1c) [34]. Magnetic tweezers is another important tool to probe cytoplasm rheology which has a long history, dating from the 1950s [9]. Forces and probe displacements can be much larger than with optical tweezers, and therefore viscoelastic responses are commonly analysed from the shape of the probe displacement-time curve in response to force, and of its recovery curve when force is released (Figure 1e). In agreement with frequency-dependent responses mentioned above, displacement curves usually exhibit a first linear phase reflecting a viscous fluid-like regime at short time scales, that then inflects at longer time scales reflecting solid-like elastic properties of the cytoplasm [9–11]. Magnetic probe size generally ranges from 100s of nm up to a few μm, and was recently expanded to sizes up to 30–50 μm in marine eggs, by embedding magnetic hydrophobic particles in large injected oil droplets (Figure 1f) [11]. Overall, these analyses all underscore size-dependent and time-dependent cytoplasm mechanics, reflecting a hierarchy of pore or mesh sizes, as well as multiple time scales associated with the dynamics and turnover of diverse crowders.

Cytoplasm mechanics and organelle positioning

The function of cytoplasm mechanics has been widely discussed at a small scale relevant to protein diffusion and biochemical reactions [7,15]. However, it may also have fundamental implications at a larger scale, to impact the motion and positioning of many organelles. First, as a viscous fluid, the cytoplasm will influence the speed at which organelles move. For example, micron-scale chromosomes segregate slower when cytoplasm viscosity is increased within mitotic spindles [35]. Viscosity also sets the global speed at which the cytoplasm will flow and drag along organelles, in response to active stresses such as those generated by actomyosin contraction [36]. Second, as an elastic solid, the cytoplasm will participate in the force balance that determines organelle's final position. Consequently, in response to a constant force, such as that generated by a network of cytoskeleton polymers and motors, an organelle will initially move at a constant speed, that will progressively reduce to eventually vanish when the organelle has moved a final distance set by the ratio of the force to cytoplasm stiffness. The cytoplasm may also fluidize in response to force, so that its effective elasticity decays with time, allowing organelles to recover a constant moving speed at longer time scales. For instance, when passive probes with a size typical of a cargo vesicle or a nucleus are moved in the cytoplasm with magnetic tweezers, their displacement exhibit short-term viscoelastic and long-term fluidization signatures [9–11] (Figure 2a). Using magnetic particles that bind spindle poles, Xie et al. displaced entire mitotic spindles in the cytoplasm of live embryos, and obtained similar rheological responses [37]. An implication of such viscoelastic signature is that longer force application will tend to prime the fluid-like response of the cytoplasm, while shorter force applications will favour an elastic response [11,30,37]. Therefore, the cytoplasm may initially resist external forces applied on organelles, but eventually yield by fluidizing to allow their displacement during processes such as endomembrane trafficking, asymmetric division or polarization.

Several physical and biological parameters influence the viscoelastic resistance of the cytoplasm. For instance, if an organelle features many large pores, its **permeability** (see box) will be relatively high, so that the cytoplasm will be able to flow easily through the organelle, alleviating resistive forces (Figure 2b). Nuclei or endomembrane vesicles are typical impermeable organelles. MT asters or spindles, which are built from MT fibres may appear more porous or permeable. However, numerical

Figure 2



Cytoplasm viscoelastic resistance during organelle motion. (a) The cytoplasm is viscoelastic at short time scales and fluidizes at longer times. Hence the displacement of an organelle moved in response to an external force such as that of a cytoskeletal network, follows an initial viscoelastic inflection followed by a linear regime. When force is released, the organelle moves back towards its initial position, reflecting the elastic nature of the cytoplasm. Organelle displacement also creates large-scale cytoplasm recirculation flows whose direction and amplitude depend on cellular boundaries. **(b)** If the organelle is porous or permeable, the cytoplasm will also flow through the organelle, which will alleviate resistive forces exerted by the cytoplasm on the organelle. **(c)** Viscoelastic resistance of the cytoplasm increases nonlinearly with the size ratio of the organelle to that of the cell because of hydrodynamic interactions that couple the organelle with the cell surface. **(d)** Hydrodynamic interactions also render cytoplasm viscoelastic resistance dependent on the initial position of the organelle, with organelles closer to the surface being harder to displace.

studies suggest that MT filaments interact with each other through **hydrodynamic interactions** (see box), which effectively restricts cytoplasm fluid penetration between MTs, thereby reducing the permeability of these assemblies [38]. Asters and spindles also recruit endomembrane, intermediate filament and F-actin networks, which may further reduce their pore size and effective permeability [39,40]. Accordingly, the frictional **drags** (see box) of asters and spindles in the cytoplasm were measured directly *in vivo* to be close to that of impermeable objects [13,37].

Hydrodynamic interactions through the cytoplasm fluid may also couple organelles to the cell surface. The presence of cellular boundaries creates backflows that enhance cytoplasm viscoelastic resistance on moving organelles. This effect will be stronger if the fluid adheres or sticks to the organelle and the cell surface [11], and is also predicted to be larger during translational versus rotational motions [38]. This distinction is attributed to the tangential versus orthogonal movement of the fluid relative to the cell surface [38], and may facilitate mitotic organelle rotations as compared to translations in the cytoplasm [37]. Importantly, this enhancement by cellular boundaries becomes disproportionally more pronounced as organelles increase in size (Figure 2c). It reaches up to ~10−15x when organelles are ~60 % of cell size, and diverges to infinity when their size approaches that of the cell (Figure 2c). This is because the cytoplasm fluid has less and less space to flow around organelles, much like in a piston. This confinement effect was proposed to influence the mobility of many large organelles, including nuclei, MT asters, or mitotic spindles [13,37,38,41]. Similarly, organelles positioned initially closer to the cell periphery may also experience higher cytoplasm resistance (Figure 2d) [11,28]. As a consequence, cell geometry could impact cytoplasm resistance, as an organelle

moving in a tube-like cell such as a columnar epithelial cell, would face large resistance, given the little space left for the fluid to move around it [42]. Interestingly, numerical models indicate that such position-dependent viscoelasticity could in principle induce a directional displacement of large organelles [28], or the local accumulation of smaller particles [43]. Yet, it remains to be tested if position-dependent effects dominate over established gradients of cytoplasm activity that can propel large cargos-like nuclei [27]. In conclusion, the viscoelastic properties of the cytoplasm are predicted to affect the motion and positioning of many organelles, with resistive forces that will depend on several biologically controlled parameters including duration and magnitude of force application, organelle permeability, size, or initial position.

Spatiotemporal regulation of cytoplasm mechanics

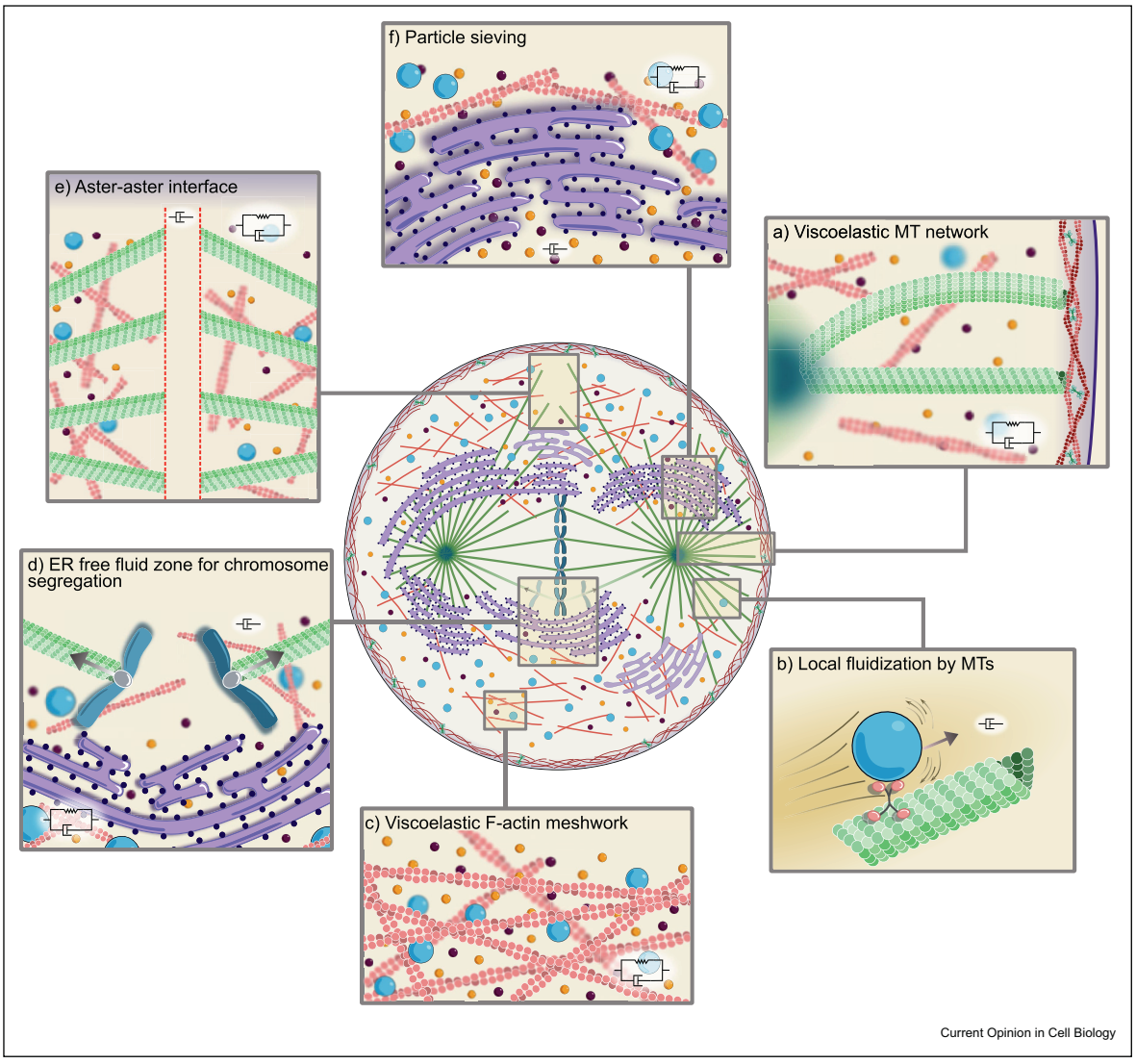
Cytoplasm mechanics can vary in space and time, as cells proceed through cell cycle phases, differentiate or organize spatially. For example, cytoplasm elasticity and viscosity both exhibit a transient peak at the onset of egg fertilization, and decay few minutes after [44]. In adherent human cells and marine zygotes, the cytoplasm was also shown to be stiffer and more solid-like during interphase while rather soft and fluid-like in mitosis [32,44]. Differences in cytoplasm elasticity have been suggested to prime diverse mouse stem cell lineages [33], or mark the entry into dormancy in yeast cells [19,25]. Cytoplasm mechanics can also be highly heterogenous within individual cells, with e.g. stiffer or more fluid zones [45,46] (Figure 3).

Spatiotemporal modulations of cytoplasm mechanics are often caused by variations in cytoskeletal organization or dynamics. MTs, for instance, assemble cell-spanning stable asters in interphase, that become short and dynamic in metaphase [47]. These stable interphase MTs form stiff viscoelastic networks that store and restore elastic energy through filament buckling or bending (Figure 3a) [14,48]. Accordingly, the cytoplasm was measured to be much stiffer within interphase MT asters as compared to outside asters in marine eggs [44]. In contrast, during mitosis, MTs become dynamic and exhibit rapid growth and shrinkage rates and were shown to rather function as fluidizers and softeners of the cytoplasm, in multiple systems (Figure 3b) [11,49–51]. This state-dependent MT contribution to cytoplasm mechanics was proposed to account for temporal variations in cytoplasm rheology between interphase and mitotic human cells [32,44]. Mitotic MTs and associated motors may also structure the cytoplasm and generate local crowded islands that concentrate many mitotic regulators around spindles [52,53].

F-actin is another generic conserved modulator of cytoplasm mechanics (Figure 3c). It forms bulk meshworks that can span the cell interior [54]. These are generally more dilute and softer than those formed at the cell cortex. They behave as tuneable viscoelastic gels with viscosities and elasticities that depend on filament density, crosslinking, or turn-over among other parameters [55]. F-actin was shown to contribute to cytoplasm viscoelasticity, at scales ranging from 100 nm up to tens of μm, in systems including yeast, vertebrate adherent cells, stem cells, and large zygotes [11,18,22,32,33,46]. F-actin meshworks can also fluidize or soften in response to applied forces, much like the cytoplasm does [56]. These bulk F-actin networks evolve during the cell cycle, being generally denser and stiffer in mitosis [32,57]. F-actin may also regionalize cytoplasm mechanics. In adherent cells, the lamella, a zone enriched in cross-linked bulk F-actin, was shown to be stiffer than the rest of the cell interior [45,46]. In marine zygotes, F-actin partitions away from MTs of the mitotic spindle, generating a stiff outer zone that contributes to holding spindles in place, and a soft inner zone plausibly facilitating chromosome segregation within spindles (Figure 3e) [37]. In large eggs, bulk F-actin disassembles locally along a plane defined by sister MT aster interaction, to soften the cytoplasm there and facilitate cleavage furrow ingression (Figure 3e) [58]. The interaction between MTs and F-actin may thus serve as an important module to tune cytoplasm mechanics around cells.

Endomembranes also form dense networks and suspensions that have been proposed in a few instances to influence cytoplasm mechanics [20,46]. Marine eggs are for instance packed with Yolk granules, which can occupy a volume fraction of the cytoplasm from 20 % up to 70 % [59]. These granules form a suspension of large crowding colloids that was suggested to contribute to the large-scale viscosity of the cytoplasm [60]. At high volume fractions such suspension is also predicted to **jam** (see box) and participate in the elastic behaviour of the cytoplasm [61]. The endoplasmic reticulum (ER) is another abundant compartment made of tubules and cisternae, that can span the cell interior [40]. Being much softer than the cytoskeleton, the ER may not contribute to the elasticity of the cytoplasm [62], but has been suggested to act as an important viscous agent that can dampen cytoplasm deformation [63] or slow chromosome segregation (Figure 3d) [35]. The ER may also function as an important sieve to segregate cytoplasm particle suspensions based on size, contributing to regionalize cytoplasm mechanics around structures like the mitotic spindle (Figure 3f) [37,49,52,64]. Overall, cytoplasm mechanics evolve hand in hand with cellular spatial organization. Cytoskeletal and endomembrane networks define regions or time windows with different cytoplasm mechanical properties. These properties in turn feedback on important cellular functions including mitotic spindle positioning [37], chromosome segregation [35], cleavage

Figure 3



Cellular spatial organization and regional cytoplasm mechanics. (a) Microtubule networks exhibit viscoelastic properties and can store elastic energy through filament buckling for instance. **(b)** MT dynamics and transport may serve as local fluidizers of the cytoplasm. **(c)** F-actin meshworks contribute to the viscoelastic properties of the cytoplasm, and can exhibit changes in architecture, density, or turnover that may modulate cytoplasm mechanics in time and space. **(d)** The Endoplasmic reticulum (ER) may enhance cytoplasm viscosity, and is excluded from the inner region of mitotic spindles to facilitate chromosome segregation. **(e)** F-actin is disassembled at the interface between anaphase asters, generating a soft cytoplasm path that may facilitate cleavage furrow ingression. **(f)** The Endoplasmic reticulum (ER) may contribute to soften the interior of the mitotic spindle space by excluding particles larger than its pore size.

furrow ingression [58] or alter the diffusion or advection of smaller components like proteins and macromolecular complexes [49,51,52].

Conclusion

Although organelle positioning and cellular organization have been studied for decades, our appreciation of the regulation and function of the surrounding cytoplasm medium in which reorganization takes place remains fragmented. One important difficulty stands in attributing cytoplasm viscoelastic properties at different scales to a given cellular component or network. As discussed above, cytoskeletal, endomembrane, colloidal suspensions and energy-driven processes are all potentially contributing to determine cytoplasm mechanics, but whether they act as additive layers, or in a more interacting and non-linear manner remains unclear. Therefore, a classical inhibitor or genetic approach may not be sufficient to address the complexity of cytoplasm rheology. Rather, cytoplasm fluid mechanics may be best

studied by combining physical measurements, detailed imaging of cytoplasm organization and dynamics, and modelling. The field is now armed with a plethora of rheological methods to probe cytoplasm mechanics at multiple time and length scales (Figure 1). In addition, emerging methods in electron microscopy now allow to image the whole cell interior at high resolution and in three dimensions [65]. Many lines of models also allow to simulate cytoplasm composition and mechanics [66], accounting for complex hydrodynamic interactions [11,38] or the presence of multiple diverse agents [63,67]. Finally, we propose that a bottom-up approach based on reconstituting cytoplasm complexity, from diverse cytoplasm extracts for instance [31,68], may help dissecting the role of different elements or their potential interactions in determining cytoplasm complex rheology.

In addition to influence organelle positioning, for cell polarity or division positioning, cytoplasm mechanics may have broader implications for morphogenesis. First, cytoplasm viscoelasticity may help cells sense or resist external forces in tissues [15]. Second, global or more local cytoplasm physical properties may contribute to dynamic shape changes by affecting rates or amounts of cell deformations [69], or processes such as cell sorting [33] or cell migration [70]. Finally, cytoplasm mechanics has been proposed to be relevant to fate determination [33,71], suggesting that spatial heterogeneities in cytoplasm properties among cells in tissues could prime local differentiation and impact multi-cellular morphogenesis.

GLOSSARY BOX

Viscoelasticity: The material property of a medium that exhibits both viscous (fluid-like) and elastic (solid-like) deformation when submitted to an applied stress.

Rheology: The study of the deformation or flows of materials in response to applied stress.

Mean Squared Displacement (MSD): Average squared distance of a particle with respect to a reference position over various time lags representing its diffusive properties.

Colloidal suspension: A stable mixture of microscopically dispersed insoluble particles suspended throughout another substance such as a fluid.

Fluidization: Rearrangement of an elastic medium in response to force application leading to a decay in the material elasticity and to flows and dissipation.

Hydrodynamic Interactions: Mutual long-range interactions that couple objects moving through a fluid arising from their respective motion and the resulting flow pattern.

Permeability: A property of porous materials that measures the ability of fluids to flow through them.

Drag: Frictional resistive force experienced by an object moving at a given speed through a viscous fluid.

Jamming: Disordered particle transitions from a flowing state to a solid-like state due to increased density or external forces leading to the loss of mobility and high viscosity.

Author contributions

All authors conceptualized and wrote the manuscript. M.I. A. and J.N. designed figures.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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