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Current Opinion in Cell Biology

Dynamic structure of the cytoplasm

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The cytoplasm is a dense and complex milieu in which a plethora of biochemical reactions occur. Its structure is not understood so far, albeit being central to cellular functioning. In this review, we highlight a novel perspective in which the physical properties of the cytoplasm are regulated in space and time and actively contribute to cellular function. Furthermore, we underscore recent findings that the dynamic formation of local assemblies within the cytoplasm, such as condensates and polysomes, serves as a key regulator of mesoscale cytoplasmic dynamics.

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Current Opinion in Cell Biology 2025, **94**:102507

This review comes from a themed issue on **Cell Architecture (2025)**

Edited by **James Bear** and **Gijsje Koenderink**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.ceb.2025.102507

Introduction

The cytoplasm is the milieu in which cellular processes occur in the interspace between the cell membrane and organelles, including the nucleus. Contrary to a dilute solution where reactants and substrates freely diffuse, the cytoplasmic aqueous phase, called the cytosol, is densely packed with macromolecules, with unique structural properties—such as electrostatic interactions and steric effects—that significantly restrict passive diffusion and distribution of biomolecules and organelles. Translational diffusivity can vary significantly between organisms and cell types, ranging from ~0.1 $\mu m^2/s$ in bacteria [1] to ~0.3 $\mu m^2/s$ in fungi [2,3] and ~0.2–0.6 $\mu m^2/s$ in cancer cells [4–6], as measured using probes (40 nm in diameter) of a size similar to that of ribosomes. Over the past decades, remarkable discoveries have been made about the microscopic organization of the cytoplasm, including the mesoscale dynamics led by macromolecular crowding [7], biomolecular condensation [8], and active dynamics influenced by cytoskeleton [9,10], as well as their physiological roles. The physical properties of the cytosol are evolutionarily optimized and finely regulated, with their modification exerting comprehensive effects on cellular processes [11]. Also, changes in cytoplasmic structure in response to shifts in the physicochemical environment are increasingly recognized as a mechanism for cellular information processing [12].

This brief review will focus on recent discoveries over the past three years regarding the dynamic structural and physicochemical properties of the cytoplasm and their physiological implications, mostly studied in vertebrate, yeast, and bacterial cells. Note that we will not be discussing the role of cytoskeletal elements, to remain general, and which are known to also structure the cytoplasm of animal cells. We regretfully acknowledge the excellent topics and studies could have unintentionally omitted. There are several excellent and comprehensive recent reviews that extensively cover the passive and active rheology of the cytoplasm driven by cytoskeletal structures [13], as well as the physical origins and biochemical roles of its crowded environment [7,14]. Similarly, even if osmotically challenging cells affect their volume and consequently their crowding, we will not be discussing regulatory volume increase or decrease, as we here focus on crowding. Our review highlights recent discoveries regarding changes in the physical properties of the cytosol and the formation of structural assemblies within it rather than the cytoskeleton and membrane-bound organelles. It has been revealed that the physiological functions of the cytoplasm can be dynamically orchestrated through global regulation of cytosolic density and charge, as well as through the reversible formation of condensates and polysomes.

Cytoplasmic density: from the osmolyte scale to the macromolecular scale

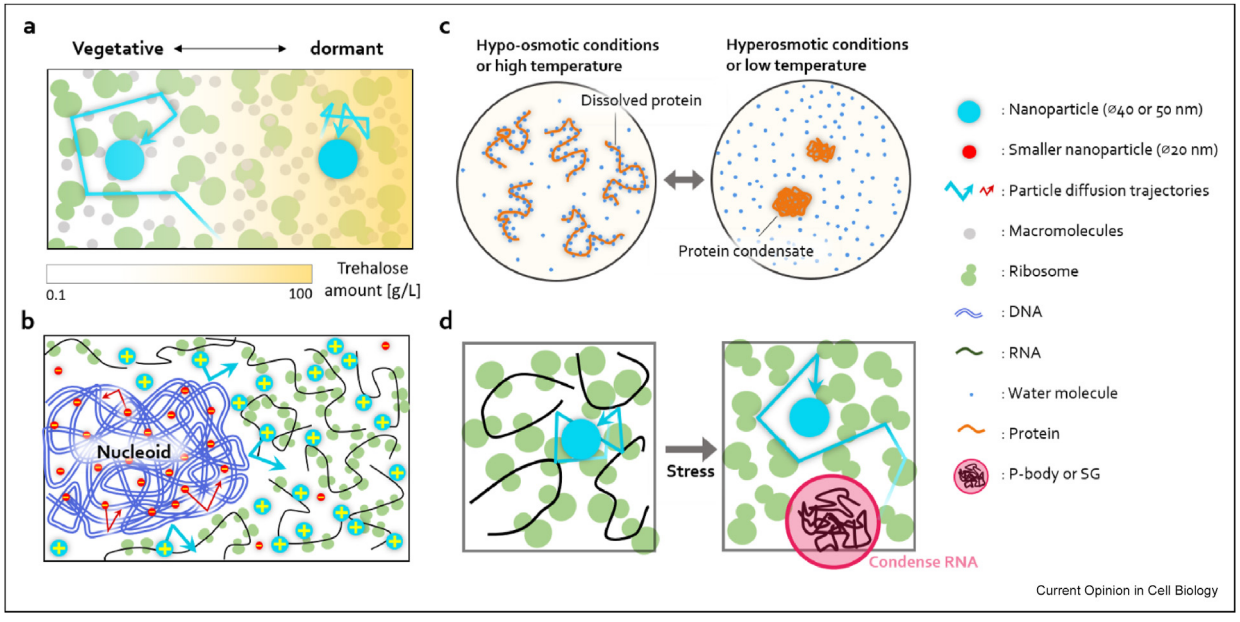
The density of biomolecules within the cytoplasm is a fundamental structural variable, essential for optimizing metabolism and cellular function [15]. The density of small molecules —which function as viscogens and osmolytes— and of macromolecules together regulate cytoplasmic viscosity and excluded volume, collectively governing particle diffusion, which can influence reaction rates. Most of the cytoplasmic volume is occupied by particles of the mesoscale (diameter 10–100 nm),

and the effects of macromolecular crowding dominate mesoscale particle dynamics and assembly. While the behavior of particles much smaller than macromolecules is not affected by the steric effects of crowding [16], the presence of viscogens, such as trehalose and glycogen, can limit the behavior of particles of all ranges of scale [17,18]. By modulating the concentration of crowding agents and viscogen, cells appear to process physiological and stress-responsive processes on a global scale.

Cells can adjust cytoplasmic viscosity to optimize metabolic efficiency and adapt to environmental conditions by modulating the biosynthesis and uptake of small molecules. In response to acute hyperosmotic shock, shrunken *S. cerevisiae* cells rapidly produce glycerol, an osmolyte, through glycolysis-related stress signaling, restoring both original cell volume and protein diffusion levels within minutes. Moreover, the same cells exposed to high temperatures quickly induce the synthesis of glycogen and trehalose, two carbohydrates that increase intracellular viscosity, thereby slowing diffusion-driven reaction rates accelerated by temperature [17]. When the cell enters into dormancy, a reversible state of metabolic stasis under unfavorable cell cycle conditions, the cytosol displays solid or glass-like properties across various organisms [19–21]. Recent studies show that dormant fission yeast spores exhibit a 40 nm particle diffusion coefficient that is 20 times lower than that in nutrient-rich vegetative cells, attributed to the accumulation of trehalose at over 1000-fold higher levels (Figure 1a) [18]**. Interestingly, defects in trehalose degradation inhibited germination, establishing the necessity of cytosolic fluidization in this process.

The density of macromolecules, including proteins and RNA, is a key variable that regulates diffusion dynamics at mesoscopic scales in the cytoplasm by controlling excluded volume. An optimal protein concentration appears necessary for cellular metabolism [22,23] with cytoplasmic mass density tightly regulated within a narrow range [24,25]. In proliferating cells, cytoplasmic mass density shows minimal variation with cell size, suggesting that total osmolytes generally scale with dry mass during cell growth [26,27]. In three human cell lines, direct pharmacological inhibition of protein

Figure 1



Diverse landscapes of cytoplasmic structures. (**a**) The cytoplasm of dormant fission yeast cells is extremely crowded due to macromolecules and a high concentration of trehalose, resulting in diffusion rates for 40 nm particles being 20–40 times slower compared to vegetative cells. Cytoplasmic fluidization via trehalose degradation is essential for dormancy breaking in fission yeast [18]. (**b**) In *E. coli*, particle motion and local concentrations are determined by their size and charge. Small particles (20 nm) are enriched within the nucleoid, whereas larger particles (50 nm) and polysome complexes are preferentially excluded due to the nucleoid's size-selective migration filter. At the same time, the negatively charged polysomes and ribosomes, in contrast with the positively charged nucleoid, result in the localization of particles shifts towards the cellular periphery as the charge becomes less negative. This suggests that particle localization within cells reflects novel properties of fundamental processes [39]. (**c**) Biomolecular condensates buffer cells against osmotic pressure or thermal fluctuations. Dissolved macromolecules, such as proteins, bind one or more layers of water molecules, limiting their mobility in the cytoplasm and restricting their availability for biological processes. However, proteins can condense into membraneless droplets, releasing some bound water and generating free water molecules. Depending on temperature and osmotic pressure, cells adjust the fraction of free and bound water through protein condensation and dissolution [50]. (**d**) Cytoplasmic fluidization requires the disassembly of polysomes and the sequestration of mRNA into P-bodies (process bodies) and SGs (stress granules). This process facilitates the formation of new mesoscale structures, promoting cytoplasmic fluidization [59].

synthesis, degradation, and mTOR activity led to dramatic changes in protein synthesis rate and cellular dry mass, though with surprisingly minor effects on cytoplasmic mass density. *In vitro* experiments with *Xenopus* egg extracts demonstrated that protein synthesis rates are maximized at physiological (1x) cytoplasmic concentrations, while degradation rates increase linearly up to a high concentration (1.8x), suggesting a feedback mechanism that maintains protein concentration homeostasis through balanced synthesis and degradation rates [28]**. Overall, the importance of protein density homeostasis for efficient metabolism and growth has recently been underscored, demonstrating resilience to changes in synthesis and degradation rates.

Excessive cellular growth can lead to cytoplasmic dilution and contribute to aging [29]. In budding yeast, chemical or genetic disruption of cell cycle progression in the G1 phase results in increased cell size. As cell size exceeds approximately twice the normal limit, RNA and protein synthesis rates do not scale accordingly, leading to a substantial decrease in their density. Several studies have shown that DNA copy number becomes rate-limiting in large cells, imposing a universal threshold for the production demands of translation templates proportionate to cell size in mammalian cells and yeasts [30]**. The enlarged cells have activated response pathways to environmental stress, and their proteomes are also remodeled into a phenotype similar to starved cells. The mechanism of this stress pathway is still unclear, but an interesting question is whether the remodeled proteome proceeds in a direction that physically compensates for the diluted cytoplasm. On the other hand, the causality between cytoplasmic mesoscale dilution and stress is still unclear. The abundance of ribosomes, which are the main intracellular mesoscale crowders, is reduced not only by environmental stress [31] but also by treatment with the rapamycin treatment, a ribosome biogenesis inhibitor, fluidizing the cytoplasm with increased mobility of 40-nm passive nanoparticles [4]. In the case of cell enlargement, on the contrary, cytoplasmic decrowding is suggested to induce environmental stress responses. Further investigation will be needed to understand the reciprocity.

Heterogenous structure and dynamics within the cytoplasm: a charge issue?

Most cellular macromolecules carry a net negative charge, with electrostatic repulsion to keep the diffusive encounters strong enough for partner search but weak enough to avoid large-scale clustering [32]. Cells achieve electrical equilibrium in the cytoplasm by regulating the production and transport of counterions and osmolytes across the cell membrane. *Mycobacterium tuberculosis* can adjust the surface charge and composition of its proteome evolutionarily or throughout the cell cycle to adapt to various extreme ecological conditions, including high or low temperatures, acidity, pressure, and radiation [33]. Due to the unique electrostatic environment within the cytoplasm, macromolecules exhibit distinct behaviors and distributions according to their surface charge [34,35]. Interactions between protein partners, including enzyme activities, rely not only on translational diffusion for encounter but also on Brownian surface rotational diffusion to explore binding sites. If translational diffusion is too fast, surface diffusion time is insufficient, while excessively slow lateral diffusion diminishes metabolic efficiency. Beyond density regulation, affinities based on the surface charge of macromolecular components may provide a basis for local search during surface diffusion.

Recently, evidence has emerged that diffusion coefficients within the cytoplasm are heterogeneous in space and time. In the cytoplasm of individual fission yeast cells, the average short-term diffusivity of 40 nm nanoparticles varies over tenfold between cells and over a hundredfold within cells, independent of temperature, cytoskeletal structure, and cell cycle [36]*.Similar findings are reported for mammalian [37] and *E. coli* cells [38]. Gradients in diffusion and density within the cytoplasm appear to influence each other. Bacteria, including *E. coli*, exhibit spatial gradients, with larger macromolecules such as ribosomes and polysomes enriched at the periphery, while the nucleoid—a networked chromatin-like structure in the center—acts as an entropic expeller of large macromolecules (Figure 1b) [39]**. Charged cytoplasmic particles may localize according to charge; highly positively-charged particles tend to cluster around negatively charged ribosomes, restricting their movement toward the nucleoid [40]. Negatively-charged particles, with minimal interaction with other cell components, show prominent clustering with positively-charged entities. Thus, the molecular charge can strongly affect localization and organization within cells relative to other components' charge and distribution [41]. When cells are exposed to energy depletion or excessively acidic environments, some cytoplasmic protein pools acquire a net positive charge when exposed to low pH below their isoelectric point, and the cytoplasm becomes glassy through tangles between macromolecules [42,43]. pH can also modulate the protonation state of histidine residues within the DNA-binding domain of transcription factors, thereby modulating their affinity for specific promoters and controlling gene expression for numerous cellular behaviors [44].

Widespread and versatile condensation

The assembly of membrane-less biomolecular condensates in the cytoplasm bridges nanoscale and mesoscale dimensions, where nanometer-sized molecules organize into higher-order structures with diameters ranging from

tens to thousands of nanometers. Cytoplasmic condensates form in response to biochemical signals or thermodynamic changes, serving numerous physiological and pathological roles [45]. The formation dynamics of these transient and reversible condensates have typically been detected for larger (∼1 μm) structures due to the diffraction limit of optical microscopy. However, a comprehensive understanding of condensates' proteomic composition and their typical size scale has remained elusive. Recently, filtration and size-exclusion experiments on cytoplasmic extracts from *Xenopus* eggs revealed that condensates are predominantly around the 100 nm scale [46]**. As cytoplasm becomes diluted, condensate size decreases, but condensates do not fully dissolve, suggesting they exhibit partially solid-like properties with stable cores, likely formed through specific protein–protein interactions, gelation, or binding with RNA molecules. Proteomics analyses predict that at least 18 % of the cellular proteome—and over half of the cytosolic proteome, excluding membrane-bound organelles (MBP)—could potentially be organized into condensates. This indicates that condensate assembly is strongly influenced by the cytoplasm's physical properties and signaling cues.

Cytoplasmic biomolecular condensates can form not only in response to physiological conditions but also due to changes in temperature [47], osmotic pressure [48], and pH [49] leading to increased local concentrations of specific proteins or altered surface properties of macromolecules. In some cases, the formation of reversible condensates mediates signaling for stress adaptation. Recently, a novel biophysical adaptation mechanism of cells was discovered, wherein macromolecular condensation buffers free water potential in the cytoplasm, enabling rapid water availability under osmotic or temperature stress (Figure 1c) [50]**. Water molecules can form hydration shell around proteins that lowers the entropy of other water molecules surrounding them, reducing the total thermodynamic potential of water [51]. In both yeast and human cells, condensate formation and dissolution either release or sequester free water, effectively buffering the cytoplasm against thermal or osmotic disturbances. Intrinsically disordered proteins were found to play a crucial role in water organization within cells through phase separation. Molecular condensation has also been reported to mediate a heat shock response that is conserved among three morphologically near-identical budding yeast species, which are adapted to different thermal environments and have diverged by up to 100 million years [52]*. These species exhibit a shared stress response, in which homologous proteins lose solubility and, in the case of modified poly(A)-binding protein 1 (Pab1), form condensates slightly above their respective optimal growth temperatures. Pab1 and the orthologs extracted from cells of three thermal conditions are also condensed at slightly higher temperatures than each cell's typical growth temperature in vitro, indicating that Pab1's temperature sensitivity is encoded in its amino-acid sequence. Under conditions where Pab1 failed to condense, the signaling pathway for heat shock adaptation was not activated. These findings show that specific biophysical cellular responses, such as condensation, have been finely tuned across extensive evolutionary timescales, enabling organisms to adapt to their environments.

Cellular metabolism fluidifies the cytoplasm through switching of polysome structures

The cytoplasm can be fluidized through metabolic processes, with the presence and abundance of ATP identified as key determinants of this metabolism-dependent fluidity [19,53]. Since approximately two-thirds of cellular ATP is used in mRNA translation—particularly for aminoacylation of tRNA and GTP regeneration, translation has been pinpointed as a major step sensitive to ATP availability. A recent theoretical study modeled the dynamics of ribosome attachment and detachment on mRNA strands during translation, investigating the effects of these switching dynamics on cytoplasmic fluidity as influenced by ATP availability [54]*. Due to the high copy number and large molecular weight of ribosomes, this ribosomal switching was found to significantly increase the diffusivity of mesoscale tracers within the cytoplasm. This effect appears to arise from repulsive, non-binding interactions proportional to the size of these particles. On the other hand, since ATP—a biological inhibitor of protein aggregation [55]—is depleted during aggregation [56,57], further investigation is required to determine whether metabolism or aggregation primarily drives the mesoscale dynamics influenced by ATP.

Cells become rigid under ATP depletion or environmental stress, but the immediate and constant changes are not always favorable. Recently reported studies of the behavior of crowded and active cytoplasmic condensates in synthetic condensates have shown that excessive crowding accelerates the nucleation process of condensates but greatly impedes their growth by collisions with each other [58]*.Therefore, a precise understanding of the formation of the condensate is required to process the stress responses. Under various stress conditions, yeast cells exhibit a transient increase in intermediate-scale diffusivity within the cytoplasm (Figure 1d) [59]**. Stress-induced inhibition of translation leads to a rapid reduction in the fraction of ribosomes organized into polysomes, with free mRNA subsequently released into the cytoplasm. These released mRNAs condense into processing bodies or stress granules, and inhibition of condensate formation prevents the transient fluidization of the cytoplasm. High concentrations of polysomes or cytoplasmic free mRNA contribute to enhanced elastic confinement of passive rheological probes, whereas mRNA sequestration through condensation alleviates cytoplasmic mesoscale confinement [60].

In human cells, similar changes in diffusion are observed following the blockade of cytoplasmic RNA degradation or condensate formation, suggesting that this response may be conserved across species [59].

Perspectives

The dynamic mechanics of various structures within the cytoplasm discussed in this study highlight the finely regulated spatiotemporal control of cytoplasmic viscosity. However, despite the development of sophisticated methodologies tailored to specific cellular phenomena and scales of interest, broad investigations into variations across cell types and organisms remain limited. This underscores the need for standardized measurements to enable more comprehensive comparisons. Additionally, we emphasize the need for the development of computational models that can more clearly elucidate the various physical causal relationships underlying the experimentally observed dynamic physical properties of the cytoplasm and their subsequent effects. The rapid advancement of desktop computing power, along with the emergence of user-friendly, open-source simulation software such as LAMMPS and GROMACS, has significantly lowered the barrier to entry for researchers.

Conclusion

The cytoplasm is more structured than it appeared, independently of cytoskeletal elements. This structure is dynamic in space and time, and the shape and charge of proteins and protein complexes, together with RNAs, can lead to compartmentalization. At the same time, cells have evolved into elegant ways of dealing with changes in crowding, through the modulation of viscogens or condensates. These mechanisms have perhaps evolved to decrease heterogeneity within the cell. It appears more and more the properties of the cytoplasm are conserved across organisms. This may be so due to evolutionary constraints in how the proteins interact in terms of physics (steric and electrostatic interactions mainly) to keep the cytoplasm fluid enough so that biochemical reactions can take place fast enough.

Author contributions

HK and MD wrote the manuscript.

Declaration of competing interest

None to declare.

Acknowledgment

This work is partly funded by the European Union (ERC, UnderPressure, grant agreement number 101039998). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Council. Neither the European Union nor the granting authority can be held responsible for them.

Data availability

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

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