Dynamic organization of the cytoplasm

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Abstract
The cytoplasm is a dense and complex milieu in which a plethora of biochemical reactions occurs. Its structure is not resolved so far, albeit being central to cellular functioning. In this review, we will discuss recent insights on the organization of the cytoplasm which seems to be dynamic and dependent on how its molecular components interact to create local arrangements or condensates, without the need for cytoskeletal elements.

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 is densely packed with macromolecules, with unique structural properties—such as electrostatic interactions and steric effect—that significantly restrict passive diffusion and distribution of biomolecules and organelles. Over the past decades, remarkable discoveries have been made about the microscopic organization of the cytoplasm, including the mesoscale dynamics led by macromolecular crowding [1], biomolecular condensation [2], and active dynamics influenced by cytoskeleton [3,4], as well as their physiological roles. The physical properties of the cytoplasm are evolutionarily optimized and finely regulated, with their modification exerting comprehensive effects on cellular processes [5]. Also, changes in cytoplasmic organization in response to shifts in the physicochemical environment are increasingly recognized as a mechanism for cellular information processing [6].

This brief review will focus on recent discoveries investigating the dynamic structural and physicochemical properties of the cytoplasm and their physiological implications, mostly studied in animal cells, yeasts, 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 we could have unintentionally omitted. In this review, we address recent findings on the composition and concentration of cytoplasmic contents, micro-compartmentalization, and highlight the novel discovery that the collapse of polysome, structure formed when several ribosomes transverse the same mRNA molecule— can contribute to cytoplasmic fluidization.

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 [7]. The density of small molecules —which function as viscogens and osmolytes— and of macromolecules regulates cytoplasmic viscosity and excluded volume, collectively governing particle diffusion, which can influence reaction rates. The movement of particles can be distinguished by their scale within the cytoplasm. 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

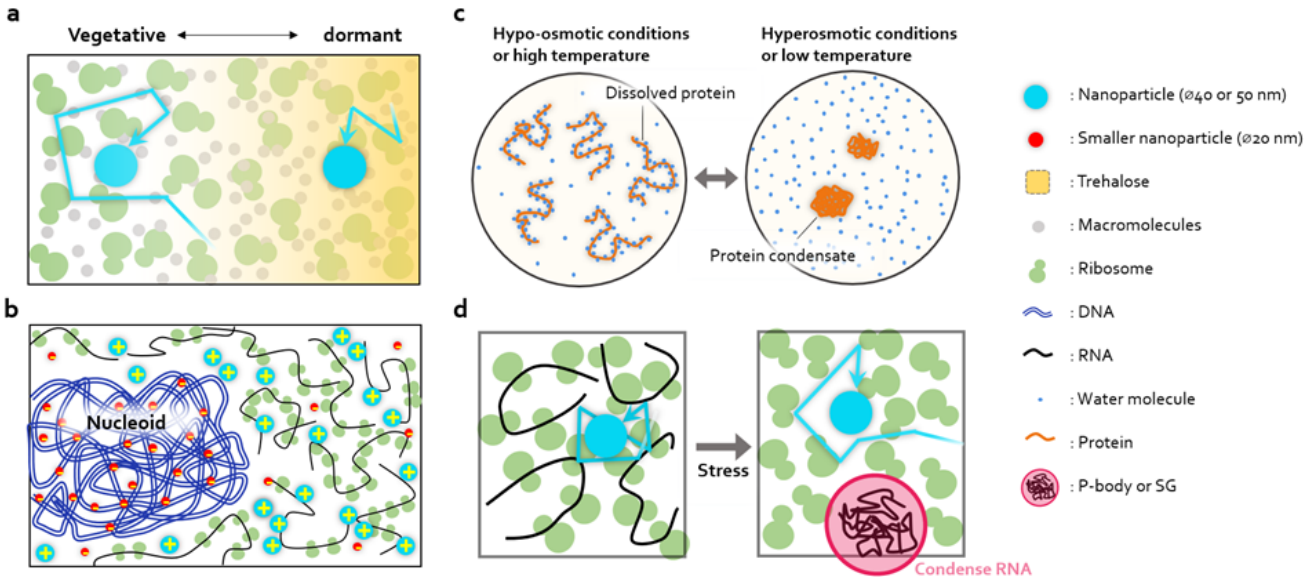

Figure 1. Diverse landscapes of cytoplasmic structures. (a) The cytoplasm of dormant fission yeast cells is extremely crowded due to macromolecules and a high concentration of trehalose, resulting in diffusion rates for 40 nm particles being 20–40 times slower compared to vegetative cells. Cytoplasmic fluidization via trehalose degradation is essential for dormancy breaking in fission yeast [12]. (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 [26]. (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 [41]. (d) Cytoplasmic fluidization can occur through the disassembly of polysomes and the sequestration of mRNA into P-bodies (process bodies) and SGs (stress granules) [50].

effects of crowding, the presence of viscogens such as proline, glucose, and trehalose can limit the behavior of particles of all ranges of scale. By modulating the concentration of crowding agents and viscogens, 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 [8]. When the cell enters into dormancy, a reversible state of metabolic stasis under unfavorable cell cycle conditions, the cytoplasm displays solid or glass-like properties across various organisms [9–11]. Recent studies show that dormant fission yeast spores exhibit a 40nm particle diffusion coefficient that is 20 times lower than that in nutrient-rich vegetative cells, attributed to the accumulation of trehalose at over 1,000-fold higher levels (Figure 1. a) [12]**. Interestingly, defects in trehalose degradation inhibited germination, establishing the necessity of cytoplasmic 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 [13,14] with cytoplasmic mass density tightly regulated within a narrow range [15,16]. In proliferating cells, cytoplasmic mass density shows minimal variation with cell size, suggesting that total osmolytes generally scale with dry mass during cell growth [17,18]. In three human cell lines, direct pharmacological inhibition of protein 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 [19]**. 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[20]. 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 homeostatic size, 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 proportional to cell size in mammalian cells and yeasts [21]**. 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 [22] but also by treatment with the rapamycin treatment, a ribosome biogenesis inhibitor, fluidizing the cytoplasm with increased mobility of 40-nm passive nanoparticles [23]. 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 this reciprocity.

Heterogenous organization 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[24]. Cells achieve electrical equilibrium in the cytoplasm by regulating the production and transport of counterions 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 [25]. Due to the unique electrostatic environment within the cytoplasm, macromolecules exhibit distinct behaviors and distributions according to their surface charge [26,27]. Interactions between protein partners, including enzyme activities, rely not only on translational diffusion for encounter but also on 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 [28]*. Similar findings are reported for mammalian [29] and *E. coli* cells [30]. 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 1. b) [26]**. Charged cytoplasmic particles may localize according to charge; highly positively-charged particles tend to cluster around negatively charged ribosomes, restricting their movement towards the nucleoid [31]. 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 [32]. 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 [33,34]. 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 [35].

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 [36]. 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 [37]**. As cytoplasm becomes diluted, condensate size decreases but 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 [38]**,** osmotic pressure [39]**,** and pH [40] 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 1. c) [41]**. Water molecules can form hydration shell around proteins that lowers the entropy of water molecules surrounding them, reducing the total thermodynamic potential of water [42]. In both yeast and

human cells, condensates 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 [43]*. 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

Cells can fluidize their cytoplasm through metabolic processes, with the presence and abundance of ATP identified as key determinants of this metabolism-dependent fluidity [9,44]. Since approximately two-thirds of cellular ATP is used in 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 [45]*. 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 [46]—is depleted during aggregation [47,48], further investigation is required to determine whether metabolism or aggregation primarily drives the mesoscale dynamics influenced by ATP.

Cells become less fluid 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 [49]*.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 1. d) [50]**. 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 [51]. 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 [50].

Conclusion

The cytoplasm is more organized than it appeared, independently of cytoskeletal elements. This organization 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 that the properties of the cytoplasm are conserved across organisms. This may be so due to evolutionary constraints in how 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.

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Author Contributions

HK and MD wrote the manuscript.

Competing interests

None to declare.

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Brief descriptions for the selected references

Special interest (·): *

outstanding interest (··) : **

**[12] High concentrations of trehalose, a glucose-derived viscogen that increases cytoplasmic viscosity in dormant fission yeast cells, are degraded through the PKA1-Ntp1 pathway upon exit from dormancy, fluidizing the cytoplasm. Changes in cell volume, protein synthesis, and cytoskeletal dynamics during germination do not significantly affect this fluidization, emphasizing the role of trehalose in altering cytoplasmic viscosity.

**[19] Using *Xenopus* egg extracts, the rate of protein synthesis and degradation is demonstrated to depend on cytosol concentration. The cytosol, maintained at an optimal concentration under physiological conditions, acts as a negative feedback homeostatic system by modulating viscosity in proportion to its concentration.

**[21] During cell growth, cytoplasm expands relative to the constant genome content, diluting the genome concentration. Overly enlarged cells show altered cellular composition and exhibit senescent-like phenotypes. Genome dilution induces a starvation-like growth state and proteome remodeling, which is observed in both yeast and mammalian cells.

*[28] Experimental and theoretical measurements demonstrate cytosolic heterogeneity at the scale of large protein complexes. This heterogeneity arises independently of the cytoskeleton, cell cycle phase, and temperature, though it increases under hyperosmotic shock. Its source remains unclear.

*[26] The mesoscale dynamics of bacterial cytoplasm, characterized by polydispersed macromolecules with varying sizes and charges, are analyzed experimentally and simulationally. Particle localization and heterogeneity appear to result from cytoplasmic polydispersity, nucleolar structures, geometric constraints, and forces such as entropic and electrostatic interactions with biomolecules like ribosomes and DNA.

**[37] It is revealed that at least 18% of the proteome in *Xenopus* egg extracts is organized into mesoscale biomolecular condensates (~100 nm in size) stabilized by RNA or gelation using quantitative proteomics, filtration, size exclusion, and dilution experiments.

**[41] Macromolecules can modulate water potential by restricting free water within their hydration layers. In concentrated cytosolic environments, temperature changes significantly affect water potential, counteracted by opposing osmotic adjustments. It is demonstrated that biomolecular condensates of intrinsically disordered proteins act as a rapid compensatory mechanism, buffering water potential by capturing or releasing free water during thermal or osmotic fluctuations.

*[43] Cellular and molecular responses to temperature are measured in three budding yeast species that diverged approximately 100 million years ago and adapted to different thermal environments. The biomolecular condensation response to heat shock is conserved across individual proteins but tailored to the thermal niches of each species.

*[45] Frequent ribosome turnover, representing a significant portion of the cellular proteome, appears to enhance the mobility of other macromolecules within the bacterial cytoplasm.

Evidence from agent-based simulations suggests that protein translation is a key energy-dependent process underlying metabolism-driven cytoplasmic fluidization.

*[49] A synthetic phase separation system, synDrops, is developed to investigate how the cellular environment influences condensate formation. Together with simulations, synDrops shows that macromolecular crowding promotes condensate nucleation but inhibits droplet growth through coalescence. These findings suggest that mesoscale molecular assembly is favored by the combined effects of crowding and active processes in the cytoplasm.

*[50] Various stresses are proposed to induce transient cytoplasmic fluidization, which requires polysome disintegration. Stress responses involve mRNA condensation into stress granules or P-bodies, and polysome collapse promotes condensate growth by fluidizing the cytoplasm.