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Mechanochemical control systems regulating animal cell size

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Cell size regulation arises from physical manifestations of cell proliferation and metabolic pathways. On one hand, coordination between these systems yields a constant cell size over generations to maintain cell size homeostasis. However, active regulation of cell size is crucial to physiology and to establish broad variation of cell sizes within an individual organism, and is accomplished via physical and biochemical pathways modulated by myriad intrinsic and extrinsic cues. In this review, we explore recent data elucidating the mechanobiological regulation of the volume of animal cells and its coordination with metabolic and proliferative pathways.

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Introduction

Cellular size control plays a critical role in cellular function, tissue organization, and disease progression. Historically, histology has provided extensive characterization of size variations between terminally differentiated cells and size misregulation in disease progression. Recently, it has become evident that changes in cell volume impact cell mechanics, organelle and nuclear size, and differentiation [1]. Changes in cell size can occur in response to environmental or intrinsic signaling cues; these changes can be seen during processes such as cell adhesion [2], spreading [3], tissue deformation [4], migration [5], apoptosis [6], and mitosis [7]. Therefore, understanding the mechanisms of cell size regulation is essential for advancing our knowledge of cellular physiology and developing new therapeutic strategies.

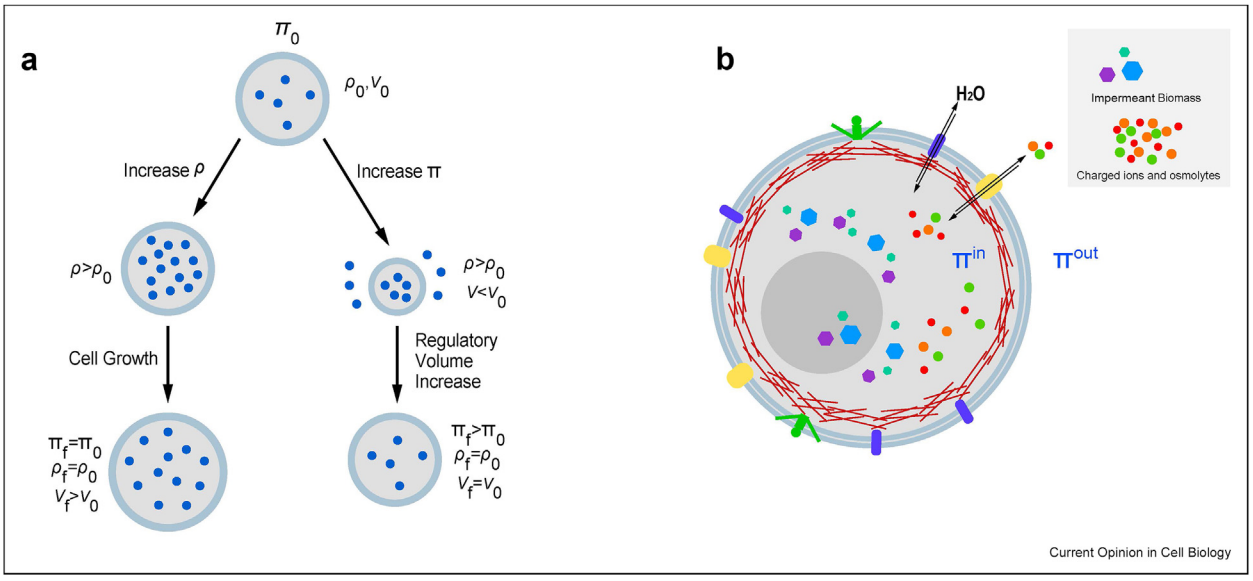
Cell size, or volume, regulation sits at the nexus of core mechanochemical systems, leading to challenges in characterizing the underlying systems contributing to the regulation of cell size. Coordination of proliferative (cell cycle) and metabolic (biosynthesis) cellular systems clearly lead to changes in cell size, and significant progress has been made to understand biochemical pathways and regulators (e.g. checkpoints) of cell cycle and size regulation [8,9]. However, cell volume is a physical metric, and is regulated by the physical biology of the cell interior and surface. Consequently, cell volume is governed by mechanochemical feedbacks that act across vast time scales, from rapid timescales (seconds) of force-sensitive ion channels and water exchange, to biochemical and transcriptional pathways that operate over slower timescales (minutes to hours) [10]. Foundational knowledge of mechanochemical cell size regulation has been explored extensively in single cell models [5,6]. Here, we explore recent work to establish a similar framework for understanding volume regulation in animal cells.

Physical basis of volume regulation

The mass of a cell is approximately 70 % water, with the remaining 30 % consisting of “dry mass” made up of organic matter (e.g. proteins, lipids, nucleic acids) and inorganic ions [11,12]. Much of this dry mass is both impermeant and negatively charged. Thus, although the solid, nonaqueous components are a small fraction of the cell volume, they are the primary driver of water exchange across the membrane to modulate osmotic stress. Because the flow of water is passive, changes in osmolyte concentration can lead to rapid changes in cell volume.

The forces driving fluid exchange are dominated by osmotic pressure differences between the cell interior and exterior. The classic pump-leak model originally described 60 years ago details a framework by which mechanical and osmotic force balance across the plasma membrane is established to regulate cell volume homeostasis [13]. This model proposes that the balance of osmolytes and ions within the cell regulates water

Figure 1



a) Illustration of processes that regulate cell volume, V, due to changes in impermeant biomass, ρ, and external osmotic pressure, Π. Consider initial conditions of all three parameters to be V_0, ρ_0, Π_0, (top). Metabolism drives transient increases in ρ that, through cell volume increase reestablishes the dry mass homeostasis such that $\rho_f = \rho_0$. Similarly, when the external osmotic pressure increases ($\Pi > \Pi_0$), the process of volume regulatory increase reestablishes both the volume and dry mass density such that the final values are the same as those initially ($V_f = V_0$; $\rho_f = \rho_0$). **b**) Cell volume is determined by water fluxes as needed to maintain the force balance across the cell membrane, and includes contributions from cytoplasmic osmotic pressure (Π_{in}), extracellular osmotic pressure (Π_{out}) and cortical elasticity (red lines). Contributions to cytoplasmic osmotic stress arise not only from impermeant biomass (e.g. DNA, proteins, nucleotides, amino acids), but also from inorganic ions and osmolytes.

flux (Figure 1), maintaining cellular homeostasis. A modernized pump-leak model, incorporating updated estimates of cellular components, demonstrates that empirical scaling laws for cell and nuclear size with biomass can be understood to arise from the interplay of proteins and small osmolytes ([3,14]).

This framework lends insight into mechanisms underlying cell volume homeostasis in response to hyper or hypoosmotic stress, known as regulatory volume increase (RVI) and decrease, respectively (Figure 1) [15]. For example, in the case of hyperosmotic stress, transient osmotic swelling drives channel-mediated efflux of K^+ and Cl^- to reduce cell volume [15]. The ability to adapt to volatile osmotic environments to maintain physiological function is crucial to both the function and survival of the cell. Active regulation of osmolytes via channels therefore serves to enforce dry mass homeostasis under evolving conditions such as osmotic shock and biosynthesis.

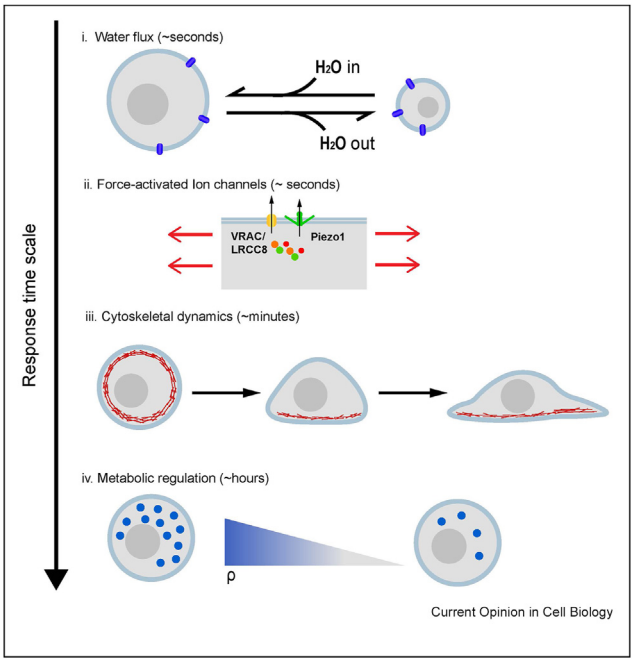
Cell volume regulation by mechanoosmotic coupling

Recent work has motivated the modification of the pump-leak model to include mechanosensitive processes [3,16]. For instance, mechanosensitive stretch-activated channels, such as Piezo1, offer a direct mechanism for coordinating membrane stresses with ion channel activity to regulate cell volume [17]. Further, the cytoskeleton plays crucial roles in mechanotransduction pathways that can alter cell volume. The actin cortex is coupled to the plasma membrane and membrane proteins, including ion channels [18]. Venkova et al. found that while the direct contributions of cortical actin stiffness to cell volume are minimal (<10 %), actin mechanobiology may serve to regulate ion channel activity [3]. Recent work has demonstrated that the actin cytoskeleton plays a central role in regulating osmolyte transport via volume-regulating ion channels (e.g. VRACs [19]) and the leucine transporter LAT1 [20]. Further, this cell surface mechanotransduction can directly trigger osmotic stress-mediated activation of metabolic pathways such as AKT/ERK and mTOR signaling [19,21].

A mechanoosmotic pump-leak model is sufficient to capture more complex time-dependent changes that occur during cell morphological processes [3] (Figure 2). For instance, the transient volume decrease that occurs during cell spreading depends on the spreading rate and arises from the dynamic regulation of cortical tension [3]. Moreover, spatial regulation of cortical mechanics during cell migration results in polarized NHE1 and SWELL1 at the leading and trailing edges, respectively, which drives cell locomotion through local volume regulation [5].

The impact of the mechanoosmotic coupling on other aspects of the physicochemical cell state has yet to be

Figure 2



Cell volume changes happen across diverse time scales. At the shortest time scales (~seconds), changes in osmotic pressure gradients can drive movement of water in or out of the cell through aquaporin channels (i), and myriad ion channels (e.g. VRAC, LRCC8, Piezo1) regulate osmolyte transport (ii). Force regulation can couple membrane tension to ion channel activity and endow rapid force response. Cytoskeletal dynamics mediate mechanotransduction signaling at second to minute time scales. For instance, cell spreading decreases cell volume (iii). At longer time scales, metabolic regulation drives changes in the amount of impermeable biomass within the cell, leading to variable dry mass density ρ (iv).

fully explored within this emerging picture. For example, many dynamic cellular processes, such as membrane potential, are set by the intracellular and extracellular ionic compositions (reviewed in Ref. [16]). Recent work outlines a mechanism by which physical crowding at high cell number densities modulates the mass density setpoint of epithelial cells, resulting in membrane hyperpolarization [22]. Membrane potential may therefore function as a dynamic readout of cell size and physical state.

Cell volume regulation through osmotic pressure modulation coupling to cell mechanoresponsive systems has recently been shown to play a deeper role in many areas of cellular function outside of simple osmotic regulation and homeostasis, and further investigation into the role of cell volume dynamics during diverse cellular processes may yield new insights.

Coordination of cell cycle and metabolism in volume control

Proliferative cells maintain a balance between biosynthesis and cell cycle to maintain a consistent size across generations and cell populations. Cell size control has been an area of active study for many years, revealing molecular mechanistic pathways [23,24]. Here we focus on recent developments in monitoring the dynamics of cell volume and dry mass density over the cell cycle, which have significantly advanced our understanding of the physical basis of size regulation during proliferation [10,25].

Models of cell size regulation during the cell cycle typically assert various physical requirements (e.g. mass added, "time spent", minimal size) at various stages [26]. A leading model is that during the cell cycle, the G1/S transition requires satisfying a minimal cell volume checkpoint [27–29]. In this 'sizer'-like model, the duration of G1 is modulated to account for varying initial birth size or growth rates until a constant size checkpoint is achieved. Recent developments in the evolutionary analysis of cell cycle control networks reveal a tendency to drive the emergence of cell size set points [30].

Recent work has demonstrated that alternative mechanisms can yield a consistent cell size at the G1-S transition without the need for an explicit sizer [31]. For instance, biochemical systems regulating cell cycle progression tune cell size throughout the cell cycle [32]. A "thermostat" model of cell size regulation suggests that cells can finely tune their size homeostasis through a variety of modular feedback mechanisms that regulate the cell size setpoint and its variability [32]. An understanding of the mechanochemical feedback that allows for robust homeostasis of cell size across generations, yet also allows for variable cell size, remains to be developed. For instance, recent advances now enable direct measurement of cytoplasmic dry mass density through the cell cycle [33]. While the dry mass density is different for cell types, for any given cell type it stays constant throughout the cell cycle [33]. These studies suggest that the dry mass density set point and homeostasis in a cell may be closely linked to size regulation.

Further insights into cell size regulation have come from studies that make strong perturbations to the cell cycle. This work has revealed how cell growth can continue even in cell cycle-arrested cells, elucidating the origins of cell senescence and aging [34,35]. A recent study showed that cell size itself regulates the proteome, likely through scaling of the ratio of DNA to cytoplasm [36]. This finding underscores the importance of cell size control in preventing the generation of overly large cells which may trend towards senescence and malignancy.

Volume reduction during contact inhibition of proliferation

Cell proliferation can also be modulated by environmental cues. A classic example is contact inhibition of proliferation (CIP), where cell proliferation arrests

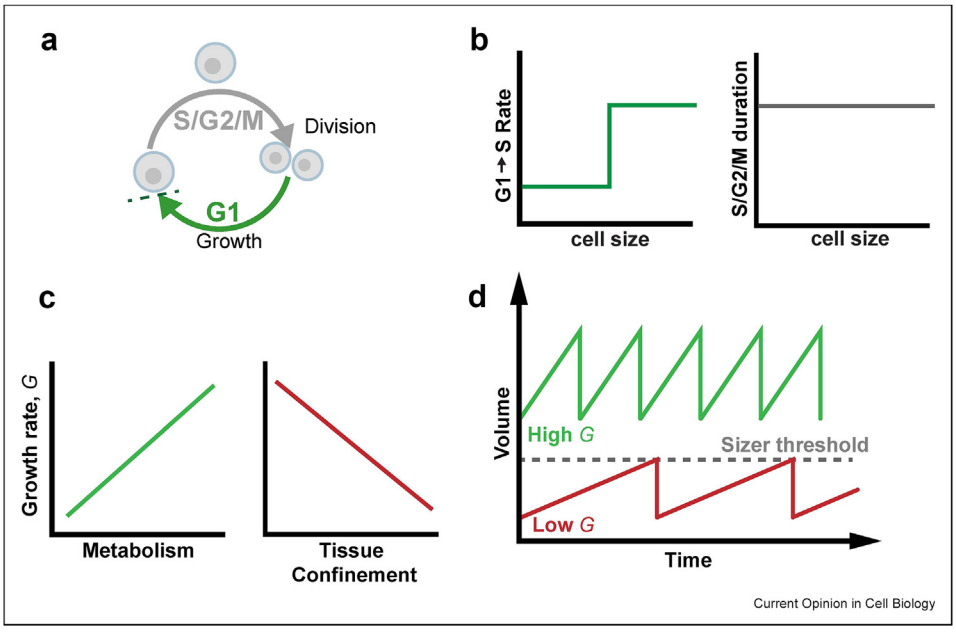
due to changes in intracellular signals at high cell densities. For epithelial tissue, the uniform shape and size of quiescent cells in epithelial tissue are central in maintaining physiological function and barrier integrity [37,38]. Cell size variation has long been observed as a hallmark of cancers and other disorders, histology routinely assesses for large variance of epithelial cell size and shape to gauge disease progression [39,40].

We recently showed that the volume of cell cycle arrested epithelial cells is ∼2.5-fold smaller than those in sparse, proliferative cultures [29]. This reduction in mean cell volume occurs over 1−2 cell cycles in which cells divide without growing, resulting in volume-reducing divisions [29]. Further, we found that a G1 sizer model with variable cell growth rate (i.e. rate of change of cell volume) was sufficient to describe these data (Figure 3). In epithelial tissue, the cell growth rate is restricted by the amount of free space available throughout tissue growth dynamics, reflecting confinement imposed by tissue-scale growth dynamics. In sparse cultures with low confinement and maximized cell growth rates, cell cycle progression is dominated by time spent in S/G2/M, and size regulation is consistent with a characteristic time between divisions (e.g. a "timer" model). On the other hand, the reduced growth rate at high confinement drove the system to a "sizer" regime, dominated by a prolonged G1 phase needed to achieve the minimal size to proceed through G1-S [29].

One intriguing question for future research is understanding how epithelial mechanics enforce these cell growth dynamics through the regulation of apicobasal height and/or extrusion [41]. For instance, recent work found that RVI in response to hyperosmotic stress is suppressed in confluent epithelia [42]. This suggests the possibility that changes in cell size could arise both from changes in metabolic pathways and/or the biophysical regulation of volume regulation.

Another interesting line of inquiry will be exploring the consequences of physiological growth modulation. For instance, recent findings show that changes in ERK activity due to local cell crowding prior to cell division promote p27 expression and suppress cyclin D1 in daughter cells, leading to cell cycle arrest and quiescence, even if cells are no longer crowded [43]. This suggests that the maternal cytoplasm can significantly influence daughter cell physiology. One possibility is that changes in growth rate alter the physicochemical state of the cytoplasm, resulting in modifications to the expression or activity of regulators of biochemical pathways.

Figure 3



a) Schematic of cell cycle, with a focus on variable growth in G1. **b**) In a simple G1 sizer model as described previously [29], the G1-to-S transition rate is sharply cell size dependent whereas the duration of S/G2/M is size independent. (**C**) The growth rate, *G*, or rate of change of cell size, is modulated by many intrinsic and extrinsic factors. For instance, increased metabolism will yield a higher growth rate. Within epithelial tissue, the local confinement suppresses growth rate. (**d**) Representative cell volume as a function of time in a simple sizer model from for two growth rates (G = 1 and G = 0.05) yield a transition from regimes dominated by G2/S/M duration (e.g. "timer" model) at high growth rates to those dominated by size thresholds (e.g. a "sizer" model) at low growth rates.

Towards mechanochemical control systems framework of cell size regulation

Within any given terminally differentiated cell type, cell size control systems robustly maintain cell size across generations and populations. In contrast, in early development, cell sizes change smoothly over relatively short times and coincide with fate determination. Such cell size control is essential for morphogenesis and organ development [44]. While transcriptional regulation of cell cycle and metabolic pathways play critical roles, it is also plausible that mechanobiological pathways contribute to cell volume regulation in the early embryo. Understanding the nature of the mechanochemical systems that variably confer robustness while also allowing for smooth transitions in cell volume will provide insight into the nature of mechanochemical control systems regulating cell size.

Some recent work has pointed to the idea that cell volume regulation may additionally be modulated by environmental cues. For example, cyanobacterium changes size based on a circadian clock [45], and kidney epithelial cells decrease in size through cilia-mediated flow sensing [46]. Further, cells actively regulate their concentration of organic osmolytes in response to extrinsic osmotic stresses [47,48]. Couplings between osmolyte production and cell adhesion mechanobiology have recently been shown [49]. Given its central role in cell identity, exploring the mechanochemical system's regulation of cell size will be exceptional to explore in future work.

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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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* of special interest
** of outstanding interest

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The authors find that that sub-populations of proteins scale differently in relation to cell size, with some proteins such as nuclear factors like Rb, histones, nucleosome sub-scaling; and cathepsins, and lysosomal peroxisome proteins super-scaling compared to cell volume. Additionally, they show that populations of primary fibroblasts which have been selected for larger cell size become senescent at an earlier passage than smaller sized cells. They ultimately show that changes in proteome content is most strongly dependent on changes in DNA-to-cytoplasmic ratio, as larger but polyploid cells do not exhibit similar proteomic changes. This demonstrates that the size of a cell can lead to differential regulation of the proteome.

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