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Integrating cellular dimensions with cell differentiation during early development

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Abstract

Early embryo development is characterized by alteration of cellular dimensions and fating of blastomeres. An emerging concept is that cell size and shape drive cellular differentiation during early embryogenesis in a variety of model organisms. In this review, we summarize recent advances that elucidate the contribution of the physical dimensions of a cell to major embryonic transitions and cell fate specification *in vivo*. We also highlight techniques and newly evolving methods for manipulating the sizes and shapes of cells and whole embryos *in situ* and *ex vivo*. Finally, we provide an outlook for addressing fundamental questions in the field and more broadly uncovering how changes to cell size control decision making in a variety of biological contexts.

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Embryo size, Cell size, Cell shape, Cellular dimensions, Zygotic genome activation, ZGA, Cell differentiation, Cell fate, Cell cycle elongation, DNA:Cytoplasm ratio, Nucleocytoplasmic ratio.

Introduction

A major question in early embryo development is how blastomeres differentiate from a pluripotent state to primary germ layer fates. Previous studies have suggested a link between sizes of blastomeres and early embryonic transitions [1,2]. More recently, it has emerged that the dimensions of a cell—its size and shape—contribute to cell function and fating. Mature oocytes or eggs are among the largest cells in an organism. Following fertilization, blastomeres undergo repeated rounds of divisions in the absence of cell growth driven by maternal factors deposited in the egg (Figure 1a) [3]. This results in an exponential increase in cell number, a massive decrease in cell volume and ultimately an increase in cell types (Figure 1b and c). Reductions of blastomere size during early development have been correlated to the maturation of the cell cycle, cell cycle elongation and titration of inhibitory factors to turn on zygotic transcription [2,4].

After several rounds of embryonic divisions, the embryo transitions from maternal to zygotic control through large-scale activation of the embryonic genome. During this process, hundreds to thousands of genes are expressed [5,6]. The large-scale onset of transcription during zygotic genome activation (ZGA) is required for subsequent establishment of cell fates in the primary germ-layers that become differentiated tissues later in development [5]. In addition, as cells reduce in size, their intercellular contacts increase and they begin to show more variation in shape, which also contributes to early lineage specification [7].

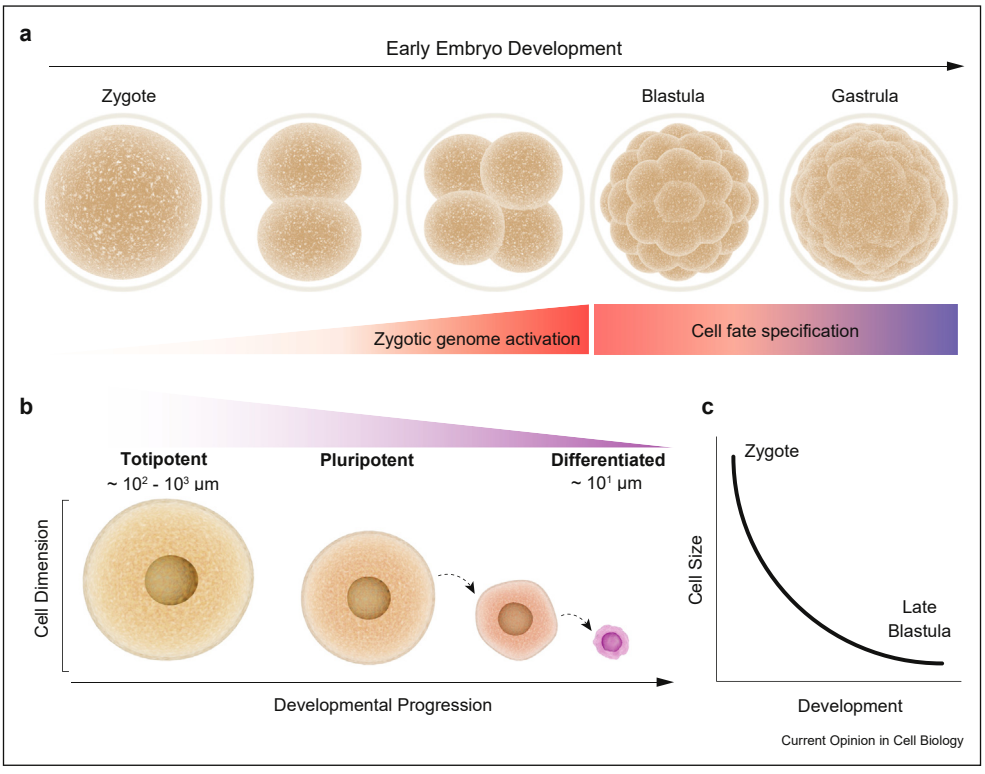
A link between cell size and cell differentiation has been proposed for some organisms [8]. However, how cellular dimensions contribute directly to cell fating and embryonic decision-making in higher organisms is an emerging area of research. Here, we review recent advances in understanding the mechanisms by which blastomere size and shape regulate key events in early embryogenesis. We also highlight the emergence of technologies to manipulate the physical dimensions of cells and embryos.

New concepts

Cell size and spatiotemporal patterning of ZGA

How an embryo regulates the onset of ZGA has been studied extensively in embryos from various model organisms [5,9]. Although ZGA is conserved in all metazoan embryos, its timing varies significantly among different species, ranging from several hours for *Drosophila*, zebrafish and *Xenopus* to 1–2 days for mouse and human [6]. ZGA onset is often coupled to cell cycle elongation and correlated with cells achieving a threshold nucleocytoplasmic (N:C) ratio in embryos from *Drosophila* [10–12], zebrafish [2] and *Xenopus*

Figure 1



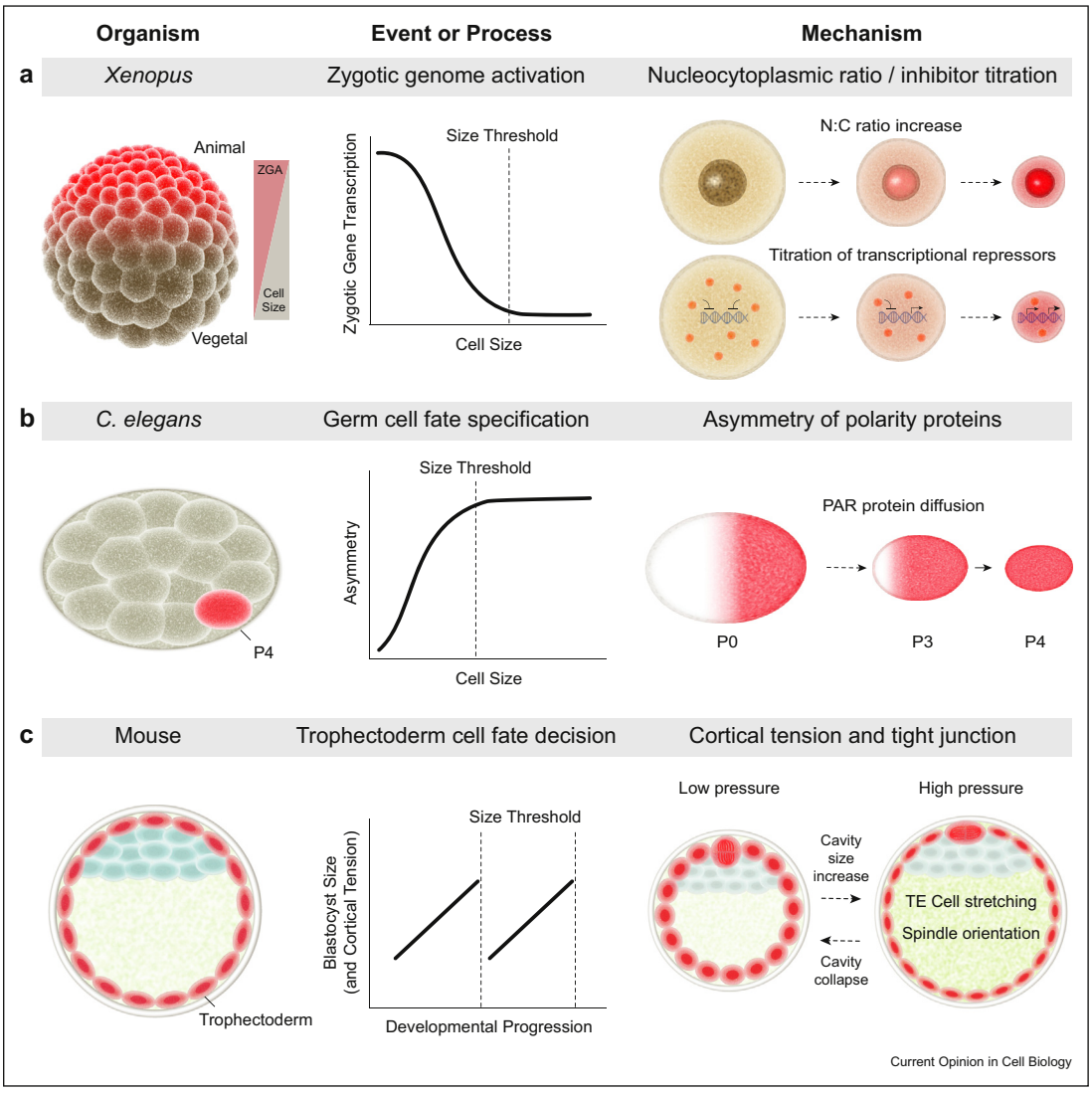
Changes in cell size and cell fate in early embryo development. **(a)** Following fertilization of the egg, blastomeres divide without growing, causing an exponential increase of cell number; volume of the embryo remains relatively constant during cleavage stages. The zygotic genome, which is dormant in early cleavages becomes gradually activated as an embryo reaches certain thresholds. As a result, the embryo initiates a major wave of zygotic gene expression following this large-scale genome activation begins to fate individual cells. **(b)** During early embryo development, the volumes of embryonic cells decrease exponentially (from 2 or more orders of magnitude in the mammalian blastula to up to 5–6 orders of magnitude in vertebrate embryo model systems). The nucleus volume also reduces, although less dramatically than cell volume, and shows a scaling relationship; note that the nucleocytoplasmic (N:C) ratio increases as blastomeres reduce in size. **(c)** Plot of exponential decrease in blastomere size due to division in the absence of cell growth.

[13,14]. In cleavage-stage embryos, blastomeres reduce cytoplasmic volume more drastically than the nucleus volume and thus increase N:C volume ratio and DNA:cytoplasm ratio. Therefore, cell size has been linked to large-scale ZGA onset in various embryonic systems. Previously, the regulatory role of DNA:cytoplasm ratio was evaluated by manipulating DNA ploidy (i.e., diploid vs. haploid embryos). More recently, it has been shown that embryo size can directly control the timing of ZGA [15]. ZGA onset was measured at the single-cell level in whole mount embryos using metabolic labeling of nascent transcription [15,16]. *Xenopus laevis* embryos were particularly effective in this study because a single blastula embryo contains a gradient of cell sizes—smallest at the animal pole and largest at the vegetal pole—and is amenable to physical reduction of embryo size.

ZGA onset is not synchronous but spatially graded in an embryo that contains a blastomere cell size gradient. Large-scale zygotic transcription initiates first in a portion of cells at the animal pole and gradually more cells become activated (Figure 2a) [15]. Quantification of nascent transcription at the single-cell level revealed that blastomeres are largely quiescent until reaching a threshold cell size, but undergo a 20-fold increase in nascent transcriptional output soon after crossing this size threshold. This result is consistent with a model in which a transcription repression is overcome by achieving a sufficiently large single-cell DNA:cytoplasm ratio [13–15], thereby titrating a repressor such as histones [14,17] or inducing cell cycle elongation [4,18] to promote zygotic gene expression. Importantly, cell size is sufficient to regulate the onset of ZGA. By generating miniature embryos the authors achieved a dose-dependent alteration of ZGA timing; approximately one cycle early in half-volume embryos and two cycles early in quarter-volume embryos [15]. These observations provide direct evidence that cell size regulates embryonic genome activation.

Future studies are required to determine whether the cell size-dependent (DNA:cytoplasm ratio dependent) regulation of ZGA onset is a universal feature in

Figure 2



Mechanisms linking cell size and shape to cellular decision-making in early embryogenesis. **(a)** A cell size threshold dictates onset of ZGA (red) during *Xenopus* early embryo development and a cell size gradient shapes the spatially graded pattern of gene expression in single embryos at blastula stage [15]. Proposed mechanisms include increased DNA:cytoplasm or nucleocytoplasmic (N:C) ratio and potential titrations of inhibitory proteins relative to DNA [14,15,63]. For clarity, nucleus is omitted in the bottom panel. **(b)** A cell size threshold switches germ cell division from asymmetric to symmetric in *C. elegans* early embryos via graded distribution of PAR polarity proteins (red) [25]. For clarity, only three generations of germ cells P0, P3, and P4 are shown, and nucleus is omitted. **(c)** Blastocoel cavity size contributes to cell fate specification for the trophectoderm (TE, red) in mouse early embryos [28]. Luminal expansion increases hydraulic pressure and cortical tension in the blastocyst, flattening cells and affecting division pattern and fate in the TE. Embryo size oscillates over development progression (time), expanding with hydraulic pressure until cortical tension reaches a critical threshold and the embryo collapses. Vertical dash lines indicate size thresholds.

embryonic systems. In addition, the finding that ZGA onset occurs first in the animal pole cells—the presumptive ectoderm—and is delayed in the vegetal pole—the presumptive endoderm—raises the question of whether a gradient of cell size contributes to the hierarchical formation of distinct germ layers, as has been observed in mouse [19] and *C. elegans* [20].

Link between cellular dimensions and cell fate specification

Cell size has been implicated in the control of cell fate in several embryonic systems. For example, a cell size threshold in a *Volvox carteri* embryo is correlated with specification of somatic versus germ cells [8]. More recently, it has been appreciated that cell and embryo

sizes can influence cell polarity in the context of germ cells. In many oocytes and early embryos, RNAs and proteins are distributed in a polarized manner and asymmetric cell divisions can generates daughter cells of unequal sizes containing unequal distributions of factors that regulate fate [21,22]. Cell division asymmetry plays a crucial role in cell fate specification [23] and establishing first lineages in early embryos [24]. A recent study suggests that cell size directly controls polarity and division asymmetry in *C. elegans* early embryos (Figure 2b) via size-independent reaction-diffusion gradients [25]. Extent of PAR protein polarization can be cell-size dependent. Once cells become sufficiently small, for example for germ cells at P4, they switch to symmetric divisions and daughters have equal distribution of PAR components. This finding is supported by theory and genetic and physical manipulation of embryo size. Thus, achieving a minimal cell size is a necessary constraint to break cell polarity, and polarization ultimately dictates fate in the *C. elegans* system. Such a mechanism may also contribute to fate decisions of neuroblasts in adult *C. elegans* [26]. However, it is not yet clear whether cell size is linked to germ cell fating for embryonic model systems for vertebrates or mammals.

Mechanical alteration of embryo size influences cell shape, function and fate

Physical forces, such as surface tension and hydraulic pressure, play important roles in shaping early embryogenesis and controlling cell differentiation [27]. A recent study found that fluid filling the blastocoel lumen of mouse embryos generates a hydraulic pressure that stretches and alters the shape of trophectoderm cells on the surface of the embryo (Figure 2c) [28]. As the blastocyst develops, lumenal pressure increases with cavity size, which increases cortical tension, matures cellular junctions and may contribute to proper spindle orientation. The pressure is relieved via breaking of junctions due to high pressure or cells undergoing mitosis. Without this pressure and cell flattening, the embryo loses proper partitioning of trophectoderm cells; they become inappropriately internalized. Interestingly, several recent studies *ex vivo* have identified key mechanical sensors of cell size that regulate stem cell specification [29–31], raising a possibility that such a mechanical relay may also function when embryo size alters.

Cell geometry and cell fate specification

Cell shape influences fate decisions in various embryonic systems. A recent study suggests that cell shape competes with the apical domain to determine the orientation of symmetric and asymmetric divisions, thereby patterning the cells of in the inner cell mass and trophectoderm [32]. Apart from mammalian embryos, cell geometry also controls spatial organization of cell types by determining the selection of asymmetric and symmetric cell division planes in plant early embryos [33]. Mechanistically, microtubules play an important role in sensing the cell geometry in early embryos [34,35]. Aside from embryogenesis, multiple *in vitro* studies also suggest a role for cell shape in regulating cell fate specification. For example, in murine small intestinal organoids, the cellular aspect ratio is found to be a key physical parameter for patterning epithelial cell progeny [36]. Moreover, micropatterning of single cells to manipulate their shapes revealed that cell geometry influences stem cell differentiation by regulating cytoskeletal organization, signaling pathways and gene expression [37–39]. Therefore, cell shape might regulate cell fate decision not only in early embryos but also in later development.

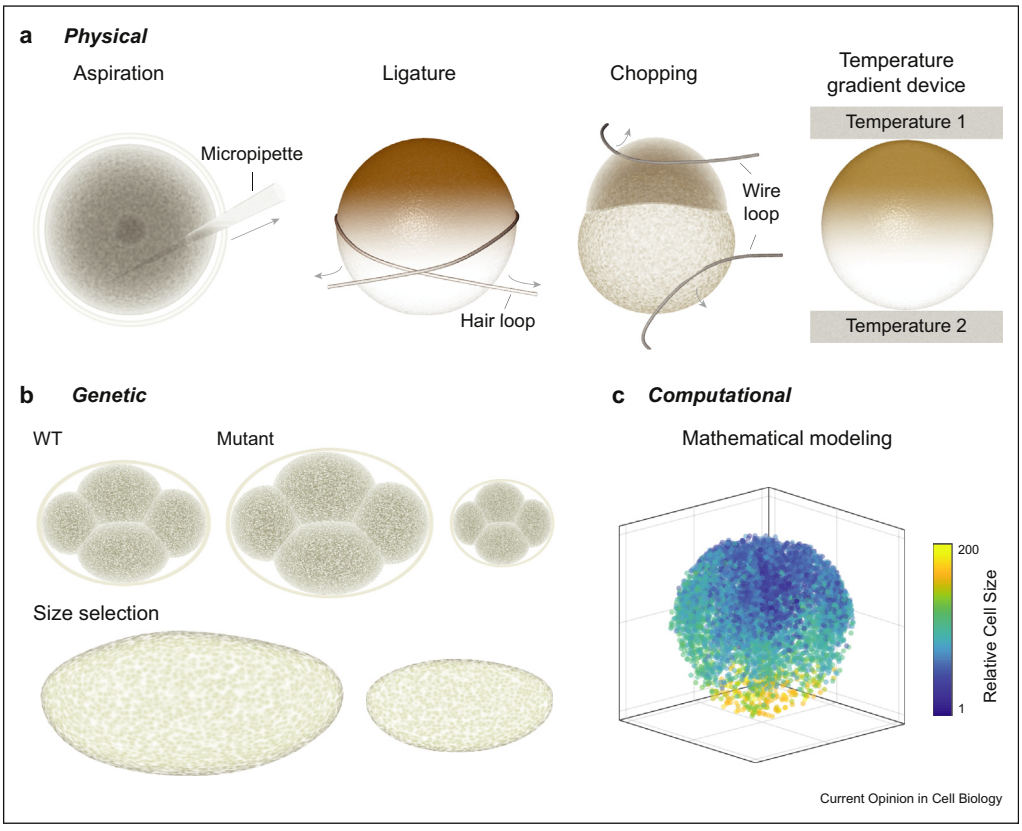
New tools and techniques: manipulating cell and embryo dimensions

Physical manipulation of cell or embryo size

One of the most common methods to alter embryo size is to physically remove part of the cytoplasm from a 1-cell blastomere soon after fertilization. This results in size-reduced embryos containing an increased N:C ratio. For mouse embryos, cytoplasmic removal can be achieved via aspiration using a micropipette (Figure 3a) [40]. Using aspiration to alter embryo size reveals that nuclear size scales with cell size [41], and increasing DNA:cytoplasm ratio induces premature compaction in mouse embryos [42]. Alternatively, embryo size can be manipulated via dissecting or dissociating blastomeres after cleavage has started without altering the DNA:cytoplasm ratio of the cells. For example, eighth-sized embryos from dissociated blastomeres were shown to be instrumental to reveal a role of apical domain in regulating cell patterning [43]. In addition, half-sized embryos showed decreased cavity pressure that affect cell fate of embryonic cells in half blastocysts [28] and changed the ratio of inner cell mass/trophectoderm [44,45]. These studies using size-manipulated embryos lend credence to the notion that the size of embryo or cell can influence cell fate specification in mammalian embryogenesis.

Ligature is also a common strategy for reducing the size of large vertebrate embryos. This can be accomplished using a hair loop and was historically demonstrated in *Xenopus* embryos, and more recently in zebrafish (Figure 3a). Miniature embryos or mini-embryos, generated by ligature at the 1-cell stage, trigger ZGA onset in a size-dependent manner, providing direct evidence that cell size regulates genome activation in early embryogenesis [15]. Separately, partial constriction was used to demonstrate that cell cycle elongation at the MBT is dependent of N:C ratio and not absolute time [1]. Size reduction of zebrafish embryos at later embryonic stages has been useful to uncover new mechanisms of somite scaling [46].

Figure 3



Techniques for manipulating cell or embryo dimensions. **(a)** Physical manipulation of the size of early embryos. Left to right, size reduction of mouse embryos by aspiration of the cytoplasmic material with a micropipette [40–42], generating miniature embryos by ligature of *Xenopus* embryos using a hair loop [15,76], size reduction of zebrafish embryos using a wire loop [46], and manipulating size gradient in *Xenopus* embryos via a temperature gradient device [18]. **(b)** Genetic manipulation of the sizes of eggs and early embryos via knockdown of factors in *C. elegans* [25,48]. Artificial selection can also be used to generate *Drosophila* embryos of altered sizes [50–52]. **(c)** Computational simulation of cell division and blastomere sizes in cleavage stage embryos, for predicting spatial patterns of gene expression [15].

Because the rate of blastomere cell cycle progression is dependent on temperature [47], an embryo temperature controller provides an unique means of altering the gradient of cell sizes present in an blastula embryo [18]. Normally, *Xenopus* embryos contain an animal-to-vegetal blastomere size gradient—the range of cell volumes at ZGA varies more than 100-fold—and this gradient correlates to a spatially graded pattern of ZGA [15]. Alteration of the cell size gradient using a temperature controller to slow division in animal pole and speed it up in vegetal pole can lead to misexpression of mesoderm genes, although the embryo later corrects this following involution [18]. It would be interesting to add a blastomere size gradient to embryos that normally do not have them via an embryo temperature controller. Such a strategy would allow one to characterize the influence of a cell size gradient on the coordinated timing of major early events such as ZGA, cell fate specification and gastrulation.

Genetic manipulation of size in early embryos

For embryos from some invertebrate species, including *C. elegans* and *Drosophila*, it is possible to manipulate egg size through genetic manipulation or *in vitro* selection. An RNAi screen has identified some genes that affect embryo size of *C. elegans* (Figure 3b). These size mutants can be a powerful tool to study how alterations of cell size impact cellular functions in early embryos, from nucleolar size scaling [48,49] to germ cell fate specification [25]. In addition, the dimensions of *Drosophila* eggs can be artificially selected for enlarged or diminished sizes to characterize regulatory mechanisms that control scaling and spatial patterning of gene expression in early development [50–52].

Characterizing the influence of cell size using biomimetic systems and modeling

A multitude of events during early embryogenesis can make it challenging to elucidate the direct roles of cell size and shape on embryo development. A number of *ex vivo* strategies have emerged to create minimal models, including biomimetic cell-like compartments with controllable size and shape [53–55], and computational simulation [15,18,25,28]. For example, microfluidic encapsulation of *Xenopus* egg cytoplasm inside synthetic cell-size compartments, showed that spindle size is directly coupled to blastomere size through cytoplasmic volume [53–55]. In addition, synthetic embryos have been constructed by micropatterning and confining stem cells in two-dimensions (2D) and 3D [56–58].

Mathematical modeling enables control of cell dimensions and reaction-diffusion processes *in silico* (Figure 3c), and it is a powerful tool to characterize various processes controlled by cell size or shape in early embryos, including patterning of cell division [18] and spatiotemporal onset of gene expression in *Xenopus* early embryos [15], hydraulic control of mouse embryo size [28], diffusion of polarity proteins along the membrane in *C. elegans* embryos [25] and cell-shape sensing by microtubules in sea urchin embryos [34]. This expanding toolkit enables important new insights on how cell size and shape regulate cellular decision making, particularly during early embryo development.

Manipulation of cell or embryo shape

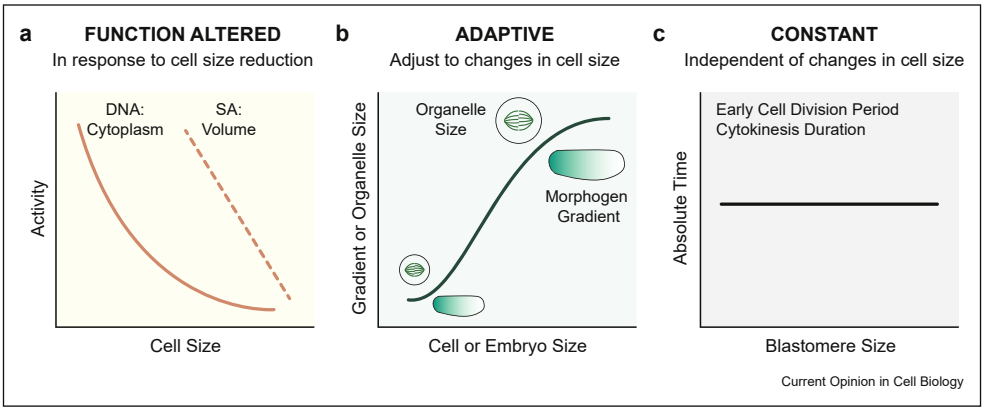
Due to their large size, early embryos are amenable to shape alteration through confinement in microfabricated chambers, such as those made from polydimethylsiloxane (PDMS) patterned by photolithography into predefined shapes [34]. This method unraveled a mechanism by which microtubules sense cell-shape in early sea urchin embryos [34]. Interestingly, the shape of individual mesenchymal stem cells can also be manipulated *in vitro* using a similar strategy and the impact of cell shape on cell differentiation can be studied [37–39]. Such confinement approaches allow alteration of cell size, cell shape or both concurrently, to characterize the downstream effects on cell fate.

Additional emerging concepts related to size regulation in embryonic systems

Thus far, we have discussed how alterations in cell size and shape during early development are leveraged as regulatory mechanisms to trigger downstream responses, including critical events during early embryogenesis. As blastomere cell size reduces, a number of physicochemical parameters alter along with it (Figure 4a). These include the ratio of DNA:cytoplasm volume and plasma membrane surface area:cytoplasm volume (SA:vol). DNA:cytoplasm ratio scales as a function of radius cubed and SA:vol scales with 3/radius. As a result of these altered ratios, in small cells, regulatory components such as histones and replications factors are titrated relative to DNA [14,59], and lipidated proteins and membrane associated proteins partition to the plasma membrane [60].

Some processes adjust to cell size, demonstrating size invariant scaling (Figure 4b), while other processes have constant activity or duration independent of cell size (Figure 4c). Many organelles show size invariant scaling across a wide range of blastomere sizes in early development. These include the spindle [54,55,61,62], nucleus [63,64], centrosome [65] and nucleolus [48,49]. In addition, morphogen gradients often scale with the size of an embryo (highlighted by other excellent

Figure 4



Adaptive and non-adaptive responses to cell size. **(a)** Major embryonic transitions are regulated by reductions in blastomere sizes, via physical parameters linked to cellular dimensions including DNA:cytoplasm ratio and plasma membrane surface area:volume (SA:vol) ratio. **(b)** Numerous processes, including organelles and morphogen gradients, display size invariant scaling; they adapt to alterations of cell size. **(c)** Processes such as early cell division period and cytokinesis duration are constant and independent of cell size in cleavage stages.

reviews [66–69]). Blastomere cell division timing is invariant of cell size, although to date the mechanisms are not understood. The period of cell division in cleavage stage blastomeres is relatively constant and independent of cell size for *Xenopus* [4] and zebrafish [2], and the duration of cytokinesis is independent of blastomere size in *C. elegans* [70].

At the extremes, biochemical or physical processes should in theory become much less functional, such as in very large blastomeres. Instead, biological processes have been adapted to extreme sizes, including trigger waves to overcome simple diffusion limits and enable more rapid signaling in giant cells [71,72], and a nuclear actin meshwork that prevents nuclear condensates from sedimentation in the oocyte germinal vesicle due to gravity [73]. However, not all processes have been adapted to extreme cell sizes. In some cases, the embryo or cell does not compensate for loss of activity, such as the weak spindle assembly checkpoint (SAC) in large blastomeres [74,75].

Outlook and future directions

Recent advances have furthered our understanding of how cell size and shape regulate key events, including zygotic genome activation and cell fate specification during early embryogenesis. However, many fascinating and important questions remain unanswered. For example, the extent to which cellular dimensions directly contribute to cell fate regulation in gastrulation and beyond is largely unknown. Little is understood about the molecular mechanisms by which blastomeres leverage cell size or DNA:cytoplasm ratio to induce large-scale ZGA. Are genome organization and chromatin state tied to cell size, such as through alterations of nucleus size (e.g., by packaging of DNA) or N:C ratio (e.g., by partitioning of regulatory factors between nucleoplasm and cytoplasm)? In addition, are cell size gradients—present in many amphibian embryos—conserved in a variety of other embryonic systems and do they contribute to spatial patterning of ZGA and later embryo development? Furthermore, how does evolution adapt to even larger extremes of size of embryos, such as 4-mm diameter axolotl egg, and what is the minimal embryo size possible to generate a vertebrate embryo? Answering these questions in the context of embryogenesis may provide paradigms to elucidate how dysregulation of cell size and cell fate contribute to tissue pathology and disease progression.

Conflict of interest statement

Nothing declared.

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