Turn Up the Volume: Uncovering Nucleus Size Control Mechanisms

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Reporting in *Developmental Cell*, Hara and Merten (2015) apply the use of microfabrication and in vitro analysis in cell-free extracts to the old problem of nuclear size control. The authors make insights into the regulation of nuclear growth that potentially explain the widely reported correlation between nucleus size and cell size.

An intriguing observation in biology is that the sizes of organelles are not static; they adapt to variation in cell size and shape. But how is this coupling achieved? The prototypical example of organelle scaling is the nucleus, a structure whose size is tightly linked to cellular dimensions (Jorgensen et al., 2007; Levy and Heald, 2010; Neumann and Nurse, 2007). This scaling phenomenon is broadly conserved and has been reported for many single-cell and multicellular eukaryotes, including humans. Currently, the question of intracellular size regulation has received renewed interest because loss of the homeostatic coupling between nucleus and cell volume—termed nucleocytoplasmic ratio—is diagnostic of the progression of various cancers (Zink et al., 2004). Although many factors likely play a role in determining nucleus assembly, only a subset have been implicated in controlling nucleus growth or scaling. Interestingly, a number of studies have proposed a direct role for cell size, through cytoplasmic volume, in regulating nucleus expansion (Jorgensen et al., 2007; Neumann and Nurse, 2007). But why is cytoplasmic volume so important for nucleus growth (Goehring and Hyman, 2012)? One idea is that the building blocks of the nucleus or regulators of nuclear assembly are present in limited quantities, creating an intrinsic feedback mechanism between cell size and nucleus growth. For example, in a study by Levy and Heald (2010), the authors demonstrated that the levels of nuclear import and the concentration of lamin B determined nucleus size in *Xenopus* cytoplasmic extracts and during early embryo development, a period in which cell size is rapidly reduced due to cell division in the absence of growth. Although observations of nucleus scaling with cell size in early embryogenesis have been informative (Hara and Kimura, 2009; Levy and Heald, 2010), to truly decipher the impact of spatial constraints and limiting volumes on nucleus size, it is necessary to move away from the embryo and into a system in which boundary conditions can easily and directly be controlled.

In a study published in this issue of *Developmental Cell*, Hara and Merten (2015) develop and apply an in vitro reconstitution approach—combining cell-free cytoplasmic extracts and confinement—to uncover relationships between the cytoplasmic space surrounding the nucleus and nuclear growth (Figure 1A). The power of their experimental system is that individual parameters, such as compartment size and membrane concentration, can be studied in isolation and without adverse effects on the cell or embryo. By varying the number of sperm nuclei added to extracts in a test tube, the authors found that nucleus size was reduced as nucleus concentration increased (Figure 1B). This result suggested that competition for a limiting amount of cytoplasmic volume restrains nuclear growth. To explicitly test this hypothesis, the authors confined preassembled nuclei in cytoplasm-filled channels and measured nucleus size as a function of channel dimensions (Figure 1C). They discovered that nucleus growth is maximal in unconfined cytoplasm and decreases as channel dimensions are reduced. To determine whether physical confinement was responsible for the decrease in nucleus size, the authors varied channel aspect ratio while keeping cross-sectional area constant. Intriguingly, the short axis of the channel had no impact on nucleus size, suggesting that nucleus growth is regulated by the volume of cytoplasm in the immediate vicinity of the nucleus and not by mechanical constraints. Furthermore, by providing growing nuclei with additional cytoplasm, the authors demonstrated that cytoplasmic volume is sufficient to increase nucleus growth. Similar scaling relationships have been reported for the mitotic spindle (Good et al., 2013) and centrosome (Decker et al., 2011), suggesting that cytoplasmic volume may be a universal regulator of organelle growth (Goehring and Hyman, 2012).

Hara and Merten (2015) also identified molecular factors, including the amount of membrane and the levels of the motor protein dynein, that contribute to the rate of nucleus expansion. Inhibition of microtubule assembly or dynein activity restricted the growth of nuclei both in test tubes and while encapsulated in microfabricated channels. The authors postulate that centrosomal asters, composed of microtubules and dynein, are necessary to transport membrane to the growing nucleus. Addition of a purified membrane fraction was sufficient to increase the size of confined nuclei, shifting the nucleus scaling curve to the left (Figure 1D). Importantly, this result was dependent on microtubules and dynein because their inhibition blocked the effects of membrane supplementation. These results suggest that membranes and membrane transport are factors that can become limiting due to

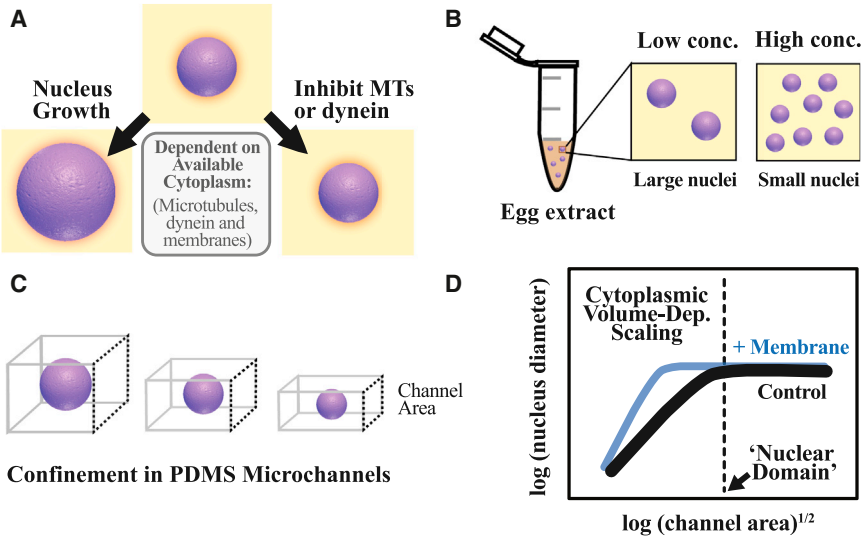

Figure 1. Investigating Nucleus Growth In Vitro

(A) Cartoon model of nucleus expansion in cell-free cytoplasmic extracts from the eggs of *Xenopus laevis*. Growth is dependent on the amount of locally accessible cytoplasm. Key molecules include microtubules (MTs), the motor protein dynein, and the amount of membranes. Other studies have found that the levels of nuclear import and lamin B also regulate nucleus size (Levy and Heald, 2010). Inhibition of microtubule polymerization or dynein motor activity blocks nucleus growth. (B) In unconfined cytoplasm (test tube), nucleus expansion is restricted by the number of nuclei, suggesting that the availability of cytoplasm dictates growth. (C) In microchannels with varying cross-sectional areas, nucleus expansion is dependent on channel dimensions. The concentration of nuclei loaded into devices was held constant (150/μl). (D) Nucleus size scaling trends: If "nuclear domain" is larger than the volume of the sphere whose diameter is 170 μm, nucleus expansion plateaus. If dimensions are reduced below this nuclear domain, nucleus expansion is restricted by cytoplasmic volume. The addition of membranes counteracts the scaling effect of reduced cytoplasmic volume, likely because the amount of available membrane is volume-limited.

cytoplasmic volume, potentially explaining the link between nucleus size and cell size.

This work from Hara and Merten (2015) is one of a number of recent studies that utilize microfabrication and confinement to investigate how cellular dimensions influence intracellular function. For example, centrosomal aster growth and positioning has been studied in microfabricated wells, and microfluidic droplets have been invaluable in the reconstitution of complex cell-cycle processes, including spindle assembly (Good et al., 2013) and actomyosin ring organization and contraction (Miyazaki et al., 2015). These cell-like systems allow precise control over compartment dimensions and, when coupled to quantitative imaging, provide tremendous insights on subcellular assembly and scaling. We are in an exciting time in which technology has made it possible to characterize biochemical reactions in a cell-like context. It is now conceivable to reconstitute complex cell-biological processes in vitro without losing the geometric constraints previously present only in intact cells.

Findings from this work represent an important step in our understanding of the causal link between cell and nucleus size. However, because microchannels can only approximate the effects of a limiting volume surrounding the nucleus, future studies should include reconstitution of nucleus assembly inside of cytoplasm-filled microfluidic droplets. Additionally, to determine whether cytoplasmic volume has an impact on nucleus scaling in *Xenopus* early embryogenesis, cytoplasm extracts from various stages of development should be encapsulated. Future work is also required to determine the downstream phenotypic effects of altering nucleus size. For example, do changes in nucleus size directly alter transcription or the spatial organization of the genome? Given that an altered nucleocytoplasmic ratio is linked to cellular senescence and cancer, in vitro studies like this one by Hara and Merten (2015) may provide paradigms for understanding size control relevant to both healthy and diseased cells.

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