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REVIEW

# Recent advances in understanding nuclear size and shape

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**ABSTRACT**

Size and shape are important aspects of nuclear structure. While normal cells maintain nuclear size within a defined range, altered nuclear size and shape are associated with a variety of diseases. It is unknown if altered nuclear morphology contributes to pathology, and answering this question requires a better understanding of the mechanisms that control nuclear size and shape. In this review, we discuss recent advances in our understanding of the mechanisms that regulate nuclear morphology, focusing on nucleocytoplasmic transport, nuclear lamins, the endoplasmic reticulum, the cell cycle, and potential links between nuclear size and size regulation of other organelles. We then discuss the functional significance of nuclear morphology in the context of early embryonic development. Looking toward the future, we review new experimental approaches that promise to provide new insights into mechanisms of nuclear size control, in particular microfluidic-based technologies, and discuss how altered nuclear morphology might impact chromatin organization and physiology of diseased cells.

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## Introduction

Size and shape are distinctive aspects of nuclear structure. Within a given cell type, nuclear size is generally maintained within a defined range. Changes in stereotyped nuclear morphologies are associated with a wide range of disease states. In each of these instances, it is unclear if altered nuclear size or shape contributes to the pathology or is a secondary effect of disease. In order to answer these questions, we require a better understanding of the basic cell biological mechanisms that contribute to the maintenance of normal nuclear size and shape. In this review, we discuss recent studies that have provided new insights into mechanisms of nuclear size regulation. While the primary focus of this review is nuclear size, we also include some discussion of factors that affect nuclear shape, as altered nuclear shape may reflect changes in nuclear size. In particular, it has been proposed that changes in nuclear size may manifest as altered nuclear shape, so as to preserve a constant ratio of nuclear-to-cytoplamic (N/C) volume that is important for proper cell function.[1,2] We end the review with some thoughts on the potential functional significance of nuclear morphology in normal development and disease.

### *Nucleocytoplasmic transport and nuclear morphology*

Proteins moving between the nucleoplasm and cytoplasm pass through the nuclear pore complex (NPC), which is important for selective nucleocytoplasmic transport and nuclear permeability. The NPC is composed of ~30 nucleoporins (Nups) present in multiple copies, with Nups typically organized into sub-complexes.[3-5] The cylindrical NPC core spans the nuclear envelope (NE) through a pore formed by the fusion of the inner nuclear membrane (INM) and outer nuclear membrane (ONM).[6,7] Nucleocytoplasmic transport involves nuclear transport factors, including importins that bind cargos that contain a nuclear localization signal (NLS) and exportins that bind cargos with a nuclear export signal (NES). Certain Nups influence nuclear morphology. For example, Nup1 and Nup60 contain amphipathic helices that impart curvature to the INM, and overexpression of Nup1/Nup60 amphipathic helices in yeast led to deformation of the NE.[8]

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Overexpression of Nup53 in yeast caused formation of intranuclear double membrane lamellae that lined the INM.[9] In *Xenopus*, depletion of Nup188 increased nuclear size through increased import of INM proteins,[10] and *Arabidopsis thaliana* deficient for Nup136 exhibited spherical rather than ellipsoid nuclei.[11]

Changes in NPC composition can impact nucleocytoplasmic transport and nuclear size. NPC differences in macronuclei (MAC) and micronuclei (MIC) of the ciliated protozoan *Tetrahymena thermophila* determine the differential nuclear import of MAC-specific or MIC-specific linker histones. MAC and MIC are 2 morphologically and functionally distinct nuclei within the same cell. MIC is smaller, transcriptionally inert, and contains a diploid genome originating from the zygote. MAC, on the other hand, is much larger and generated by programmed DNA rearrangements and amplifications. Loss of MAC- or MIC-specific linker histones leads to nuclear enlargement of the MAC or MIC, respectively, demonstrating that reduced chromatin compaction increases nuclear size in a nucleus-specific manner.[12] Four Nup98 homologs showed differential localization in MAC and MIC nuclei, with 2 being specifically targeted to the MIC and 2 to the MAC. MacNup98A and MacNup98B possess typical FG-repeats, specifically repeats of the amino acid sequence GLFG, which interact with nuclear transport factors. In place of these GLFG repeats, MicNup98A and MicNup98B contain atypical importin docking repeats consisting of the amino acid sequence NIFN or SIFN. Nucleoporin domain swapping experiments were performed to test the model that GLFG repeats block import of MIC cargos while NIFN repeats block import of MAC cargos. When the N-terminal GLFG repeats of MacNup98A were fused to the C-terminal domain of MicNup98A (BigMic), the chimera localized to the MIC nucleus, reduced micronuclear linker histone import, and increased MIC size. Conversely, a fusion protein consisting of the C-terminus of MacNup98A joined to the N-terminal NIFN repeat domain of MicNup98A (BigMac) was targeted to the MAC nucleus, macronuclear linker histone import was reduced, and MAC size increased. Collectively, these data suggest that GLFG/NIFN repeats help to prevent misdirected protein transport to a given nucleus, thereby impacting nuclear size in *T. thermophila*.[13] Interesting questions that arise from these studies are if linker histones are the only importin cargos necessary to induce these nuclear size differences, and whether linker histones impact nuclear size by altering chromatin structure or changing gene expression.

NPC number and density could theoretically impact nuclear size as well. However, comparing different cell types and organisms revealed an inverse relationship between NPC density and nuclear volume,[14] with NPC number being controlled independently of nuclear volume and surface area.[15] Studies in *Xenopus* showed that increased nuclear expansion rates could be uncoupled from increased NPC numbers.[10] Furthermore, NPC assembly and nuclear expansion are independently regulated in mammalian tissue culture cells, as blocking interphasic NPC assembly in HeLa cells did not alter nuclear expansion or size.[16,17] On the other hand, mutations that cause NPC clustering and/or mislocalization frequently give rise to altered nuclear morphology.[18-21] Taken together, NPC composition seems to contribute more to the regulation of nuclear size than NPC number or density.

## Nuclear lamins and nuclear morphology

Structural elements of the NE play important roles in defining nuclear size. The nuclear lamina, a meshwork of lamin intermediate filaments that lines the INM, is one of the major structures implicated in the regulation of nuclear morphology in metazoans.[22-26] The nuclear lamina is important for chromatin organization, DNA metabolism, and providing mechanical strength to the nucleus. Four major lamin isoforms constitute the nuclear lamina in vertebrate cells – lamins A and C (alternatively spliced products of the LMNA gene), lamin B1 (encoded by LMNB1), and lamin B2 (encoded by LMNB2). Lamins B3 and C2 are found in germline cells and are products of alternative splicing of the LMNB2 and LMNA genes, respectively.[27]

General lamin structure includes an N-terminal globular head domain, a central $\alpha$ helical rod domain, and an immunoglobulin like C-terminal domain with NLS.[28,29] Lamin monomers interact through their central coiled coil $\alpha$ helical domains to form 50 nm long dimers,[30,31] which in turn interact in a head-to-tail manner to form higher order lamin polymer structures.[32-36] Although the nuclear lamina is apposed to the INM, ~10% of A-type lamins localize to the nuclear interior and interact with the chromatin and

nucleoplasmic proteins, such as pRB and PCNA, rather than assembling into large polymers.[37-40] In addition, interphase phosphorylation of lamin A induces its redistribution from the NE to nucleoplasm,[41,42] and how a nucleoplasmic pool of lamins might influence nuclear morphology is an open question. Lamins also play a role in resisting dynein-mediated clustering of NPCs via the dynein adapter BICD2,[43] thus lamins may indirectly contribute to proper nuclear morphology by preventing NPC aggregation that has been linked to aberrant nuclear membrane structures.[18]

Lamin expression and nuclear size are often correlated. Changes in lamin isoform expression during frog, chicken, and mouse development coincide with reductions in nuclear size.[44-46] During granulopoiesis, lamin A/C expression is downregulated in neutrophils, leading to more deformable nuclei that facilitate cell passage through narrow constrictions.[47] Nuclear volume also influences cell migration efficiency,[48] and expression of certain lamin mutants or altering lamin expression levels affect nuclear deformability and cell migration.[49-52] Knocking down lamin B1 in HeLa cells led to an increase in lamina meshwork size, formation of NE blebs enriched in lamin A/C, and increased nucleoplasmic lamin A mobility.[53] Reduced lamin B1 levels are frequently associated with altered nuclear and cell shape and increased cellular senescence.[54-60] Abnormal lamin localization and expression correlate with aberrant nuclear size in various disease states, notably cancer.[61] For example, lamin B1 expression is elevated in prostate cancer, and lamin B1 expression levels directly correlate with tumor stage in hepatocellular carcinoma.[62,63] Reduced lamin A/C levels are detected in small-cell lung carcinoma (SCLC) relative to non-SCLC, which might contribute to the differences in nuclear morphology between these 2 cancers.[64,65] LAP2$\beta$, a lamina associated protein that connects the lamina and chromatin, is highly expressed in colorectal adenocarcinoma and SCLC, potentially contributing to nuclear enlargement in these cancers.[66]

Nucleocytoplasmic transport and lamin import regulate nuclear size in *Xenopus*. The eggs, cells, and nuclei of *X. laevis* are larger than those of *X. tropicalis*, and nuclear import rates differ in egg extracts from these 2 species, with *X. tropicalis* nuclei exhibiting slower import rates than *X. laevis* nuclei. The levels of 2 nuclear transport factors, importin $\alpha$ and NTF2, differ between the 2 extracts, and altering the levels of these 2 proteins was almost sufficient to account for the differences in nuclear size and import between these 2 species.[67] Lamin B3, the major lamin type found in the egg, was found to be one of the imported cargos accounting for these differences in nuclear size, which is consistent with the observation that depletion of lamin B3 from egg extract blocks nuclear growth.[67-69] Ectopic addition of lamin B3 to *Xenopus* egg extract increased the rate of nuclear growth,[67] with the lamin immunoglobulin fold being required for post-mitotic lamina assembly and NE growth.[23] It has recently been shown that nuclear size is sensitive to the total lamin concentration in *Xenopus* egg and embryo extracts, with low and high concentrations increasing and decreasing nuclear size, respectively. Recombinant lamin B1, B2, B3, and A similarly affected nuclear size when tested individually or in combination. Altering lamin levels *in vivo*, both in *Xenopus* embryos and mammalian tissue culture cells, also influenced nuclear size in a concentration-dependent manner.[22]

Developmental nuclear scaling in *Xenopus* embryos also depends on nuclear import capacity and lamins. Early *Xenopus* embryonic development is a robust system to study cellular scaling mechanisms in the absence of DNA ploidy changes because cell division is rapid with no overall change in the size of the embryo itself. The 1.2 mm fertilized egg undergoes 12 rapid synchronous cell divisions (each approximately 30 min) to produce several thousand 50 $\mu$m cells at the midblastula transition (MBT).[70] Nuclear size decreases throughout early embryonic development in both *X. laevis* and *X. tropicalis*.[67,71,72] Nuclear size reductions prior to the MBT correlate with reduced bulk import rates and levels of cytoplasmic importin $\alpha$. Ectopic importin $\alpha$ expression was sufficient to increase nuclear size in pre-MBT embryos, while expression of both importin $\alpha$ and lamin B3 was required to increase nuclear size in later developmental stages.[67,71] Experiments in *C. elegans* similarly demonstrated the involvement of nuclear transport and lamins in nuclear size regulation.[73,74]

In pre-MBT *Xenopus* embryos, nuclei expand throughout interphase, while at the MBT and beyond nuclei reach a steady-state size that is smaller than nuclei in pre-MBT embryos. A 3-fold decrease in NE surface area occurs between the MBT and gastrulation (stages 10.5–12).[67,71] When large nuclei assembled in

*Xenopus* egg extract were incubated in gastrula stage embryo extract, they became smaller. This nuclear shrinkage was dependent on conventional protein kinase C (cPKC) activity and correlated with removal of lamins from the NE. Based on these data, it was proposed that nuclear recruitment of cPKC leads to interphase phosphorylation of lamins that alters their residence time at the NE and contributes to reductions in nuclear size. This activity might account for the post-MBT developmental scaling of nuclear size in *Xenopus* embryos, as cPKC activity and nuclear localization increase in embryos after the MBT.[75,76]

### The endoplasmic reticulum and nuclear morphology

The endoplasmic reticulum (ER) plays an important role in NE formation and regulating nuclear size and shape. The ER is an interconnected lipid bilayer membrane network, consisting of ER tubules and sheets, that is continuous with the NE. Post-mitotic NE reformation is initiated by the targeting of ER tubules to chromatin, through the action of ER-resident DNA binding proteins. This is followed by flattening of ER membranes around the developing nucleus,[77] with final sealing of the NE accomplished by ESCRT-III.[78,79] In mammalian tissue culture cells, the INM proteins MAN1, Lap2$\beta$, and lamin B receptor contribute to NE formation by anchoring ER membranes at the chromatin surface, promoting membrane spreading onto the chromatin.[80] Subsequent expansion of the NE requires an intact ER network, as detachment of ER membranes from the NE by shear mechanical stress hampered nuclear growth in *Xenopus* extract, and withdrawing mechanical stress resulted in recovery of NE growth.[77] Impairing the function of the AAA-ATPase p97, generally required for ER network maintenance, also inhibited NE expansion.[81] Conversely, selective macroautophagy of ER and nuclear membranes has recently been observed in *S. cerevisiae*. Atg39, a perinuclear-localized autophagy receptor, regulates autophagic sequestration of NE. Under nitrogen starvation conditions, cells lacking Atg39 exhibited lobulated and distorted nuclei with a concomitant loss of viability.[82]

Structural proteins of the ER network contribute to NE morphology by affecting the proportion of ER tubules to ER sheets. Reticulon (Rtn) proteins are responsible for shaping the ER tubules and stabilizing membranes with high curvature by inserting a hydrophobic wedge into lipid bilayers.[83-85] In tissue culture cells, Rtn overexpression increased ER tubulation and reduced NE surface area, while Rtn depletion by siRNA knockdown reduced ER tubulation and increased nuclear size (Fig. 1A).[86] NE formation was inhibited in *Xenopus* egg extract supplemented with a neutralizing Rtn4 antibody,[77] and ectopic Rtn4 expression in early *Xenopus* embryos led to altered nuclear size.[71] Furthermore, ectopic addition of recombinant Rtn4b to *Xenopus* egg extract decreased the rate of nuclear expansion, leading to an ~2.4-fold reduction in nuclear cross-sectional area (Fig. 1B). These observations suggest a tug-of-war relationship between membranes of the ER and NE.[80,86,87] Other ER structural proteins like Climp63 and atlastins, which set ER sheet width and generate 3-way junctions, respectively, might also impact nuclear morphology. For example, Climp63 levels might affect the relative amounts of ER sheets versus tubules,[88] and atlastins might control expansion of the NE by regulating the extent of ER tubule branching.[89,90] Also of potential relevance are members of the conserved Lunapark protein family that reside at ER 3-way junctions in yeast and mammalian cells, reducing ER 3-way junction dynamics and preventing ER tubule fusion.[91] Membrane biogenesis can also impact nuclear morphology. Lipin is a phosphatidate phosphatase important in glycerolipid biosynthesis, and inactivation of lipin or genes that regulate lipin leads to disorganization of peripheral ER structures with concomitant defects in NE morphology in *C. elegans* and yeast.[2,92-94]

The interplay between the ER and nuclear lamina may be important in determining steady state nuclear size. During interphase, nuclei expand and import nuclear lamins into the forming NE, strengthening the lamina meshwork. The increased mechanical force exerted by the expanding lamina might resist the ability of the ER network to extract membrane from the NE, so that optimal lamin import promotes NE growth.[22] As already discussed, lamins are phosphorylated during interphase,[42,95-97] and increased phosphorylation of lamins by cPKC may result in increased loss of lamins from the NE and/or altered nuclear lamina dynamics. This phenomenon might be compensated for by retraction of NE membrane back into the ER, resulting in decreased NE surface area and constant nuclear lamina density, potentially

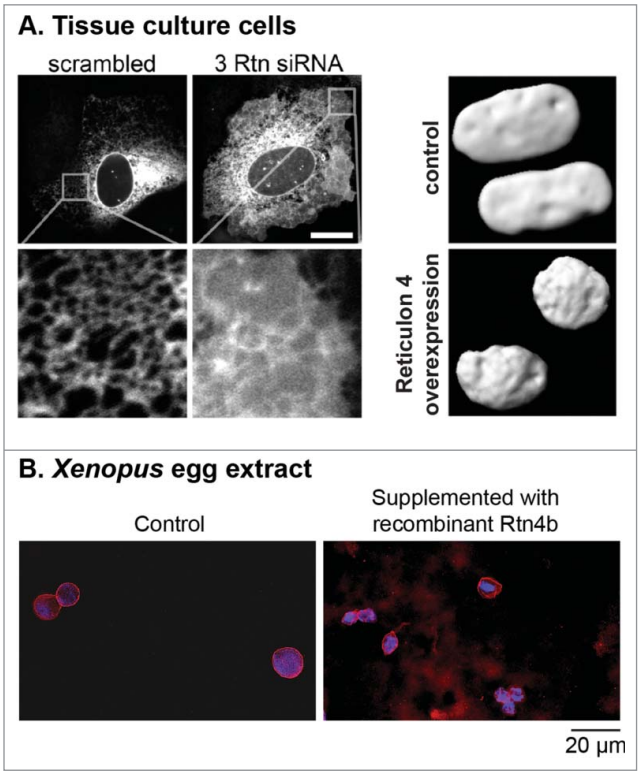


**Figure 1.** Reticulon expression levels affect ER structure and nuclear size. (A) In the left panel, the ER is visualized in U2OS cells with a Sec61-GFP construct. Knockdown of Rtn1, Rtn3, and Rtn4 by siRNA (labeled 3 Rtn siRNA) leads to less ER tubulation and more ER sheets, with a concomitant increased rate of post-mitotic nuclear formation. The scale bar is 20 $\mu$m. In the right panel, nuclei in U2OS cells are visualized with GFP-NLS at 160 minutes after nuclear formation. In cells overexpressing V4-Rtn4, nuclei are smaller due to slower nuclear expansion. Images used with permission from.[86] (B) Nuclei were assembled in *Xenopus laevis* egg extract for 45 min. The extract was then supplemented with 67 nM recombinant purified Rtn4b protein and incubated for another 45 min. Nuclei were fixed, spun onto coverslips, and stained with mAb414 to visualize the NPC and NE (red) and Hoechst to visualize the DNA (blue). Nuclear cross-sectional areas were quantified. Exogenous addition of Rtn4b led to an ~2.4-fold reduction in nuclear cross-sectional area (our unpublished data).

contributing to the steady-state regulation of nuclear size.[75]

Interestingly, Rtn levels are sometimes altered in different cancers, potentially contributing to cancer-associated abnormalities in nuclear morphology. Rtn4a overexpression has been observed in malignant brain tumor cells, and sufficiently high Rtn expression levels could have a dominant negative effect leading to increased nuclear size, as observed in *Xenopus*.[71] Downregulation of Rtn4 Interacting Protein 1 (Rtn4IP1) has been observed in thyroid cancers,[98,99] potentially decreasing the efficiency with which Rtn4 shapes tubulated ER and leading to an increase in nuclear size.[100] As another example, Rtn1 is upregulated in malignant pancreatic carcinoma, diffusely infiltrating gliomas, and neuroendocrine tumors,[98,101-103] again possibly influencing ER and nuclear morphology in these cancers.

## Nuclear and organelle scaling relative to cell size

Cell growth and division influence the sizes of intracellular organelles, but mechanisms responsible for regulating how organelle size scales relative to cell size are largely unknown.[104] It is well established that nuclear size varies as a function of cell size and that the N/C volume ratio is a tightly regulated cellular feature. Varying the sizes of *S. cerevisiae* cells by mutation or differing growth conditions demonstrated that large cells possess large nuclei and smaller cells exhibit smaller nuclei, maintaining a constant N/C volume ratio.[105] Similar studies were performed in fission yeast *S. pombe*, where a constant N/C volume ratio was maintained over a 35-fold range of cell sizes. A 16-fold increase in DNA amount did not change nuclear size, demonstrating that ploidy has little effect on nuclear size in this system.[106] Interestingly, nucleolar size scaled proportionately with nuclear and cell sizes in these yeast studies as well. Nucleolar size also exhibited a positive correlation with cell/nuclear size in early *C. elegans* embryos. Surprisingly, when embryo size was altered by various RNAi treatments, nucleolar size showed an inverse scaling relationship, in which large nucleoli assembled in small cells/nuclei and vice versa. The model proposed to explain this observation is that oocytes are maternally loaded with a fixed number, rather than a fixed concentration, of nucleolar components. As a result, the concentration of nucleolar proteins is higher in RNAi-treated embryos with small cells/nuclei, thus giving rise to larger nucleoli.[107] Nucleolar assembly that depends on an intracellular phase transition could explain this behavior, and such concentration-dependent phase transitions might be a useful paradigm for understanding size scaling of other intracellular structures.[108]

Limiting component models have been invoked to explain size scaling of a variety of different organelles and intracellular structures.[109] As these models may be relevant to nuclear size scaling, it is worth briefly touching on what is known about size scaling of other

organelles. Centrosome size scales linearly with cell size in early embryonic stages of *C. elegans* development. A limiting component hypothesis has been suggested in which centrosome size is determined by cytoplasmic volume, which dictates the total amount of centrosomal components available for assembly.[110] The size of mitochondrial networks directly correlates with cell size in *S. cerevisiae*, with aging mother cells showing a continual reduction in the mitochondria-to-cell size ratio over successive generations.[111] How mitotic spindle size might influence nuclear size, and vice versa, is a particularly intriguing question. As discussed later in this review, microfluidic encapsulation of mitotic *X. laevis* egg extracts demonstrated that changes in cytoplasmic volume are sufficient to drive spindle length scaling that occurs during early *X. laevis* development.[112-114] These data suggest that spindle scaling might be explained by limiting amounts of cytoplasmic components, acting in concert with other mechanisms that affect the activity of microtubule regulatory factors.[115-117] For example, in early stages of *Xenopus* development, the kinesin-13 microtubule depolymerase kif2a is inhibited by importin $\alpha$, but becomes activated later in development when the cytoplasmic importin $\alpha$ concentration decreases through redistribution to a membrane pool, thus giving rise to smaller spindles.[118] As nuclear transport has been clearly implicated in nuclear size control, this function for importin $\alpha$ provides a potential link between size scaling of the spindle and nucleus.

## Nuclear size in embryonic development

For a given cell type, nuclear size is generally maintained within a defined range, and the size of the nucleus tends to scale as a function of cell size.[119] How nuclear size might impact cell function and physiology is an important question. As already described, *Xenopus* development offers a useful animal model to investigate scaling relationships. During early *X. laevis* embryogenesis, nuclear volume decreases on average ~3-fold up to the MBT, while cytoplasmic volume shows a much more dramatic ~70-fold reduction in volume. As a consequence, the average N/C volume ratio increases abruptly prior to the MBT (Fig. 2). The MBT is associated with a dramatic increase in zygotic transcription and acquisition of slower, asynchronous cell cycles. Identifying the mechanisms that contribute to proper MBT timing has been an area of active research for decades. Experimentally increasing nuclear volume in embryos by microinjecting different nuclear scaling factors, including import proteins, lamins, and reticulons, increased the N/C volume ratio in pre-MBT embryos and led to premature activation of zygotic gene transcription and early onset of longer cell cycles. Conversely, decreasing the N/C volume ratio delayed zygotic transcription and resulted in additional rapid cell divisions.[71] Similarly, in early *C. elegans* embryonic development, cell cycle duration is correlated with the N/C volume ratio.[120] These data show that nuclear size and the N/C ratio can impact timing of the MBT, providing insight into the physiological significance of the relationship between cell and nuclear size. These findings are potentially relevant to cancer where deviations from normal N/C volume ratios are frequently observed.[100]

Another factor that contributes to proper MBT timing in the *Xenopus* embryo is the ratio of DNA to cytoplasm.[121,122] By varying the DNA content or cytoplasmic volume of early *X. laevis* embryos, it was shown that increasing the ratio of DNA to cytoplasm resulted in an earlier MBT.[122,123] The proposed mechanism is that the egg is loaded with a fixed amount of DNA binding proteins that serve to inhibit the MBT. As development proceeds, cell number and total DNA amount increase exponentially in the embryo. Once a threshold amount of DNA is reached, there are insufficient numbers of MBT inhibitory molecules to sufficiently bind all DNA, thus leading to induction of the MBT.[122,124] Several potential limiting DNA binding factors have been identified: histones,[125] phosphatase PP2A,[126] and DNA replication initiation factors.[127] Another protein that acts though a limiting titration mechanism is RAD18, a ubiquitin ligase responsible for monoubiquitination of PCNA. It functions to silence the DNA damage checkpoint in *Xenopus* embryos prior to the MBT.[128] Importantly, none of these factors appear to fully account for the proper regulation of MBT timing, so it seems likely that redundant mechanisms are involved, with both DNA amount and nuclear volume contributing. We propose that nuclear volume may be relevant to the DNA titration model, by

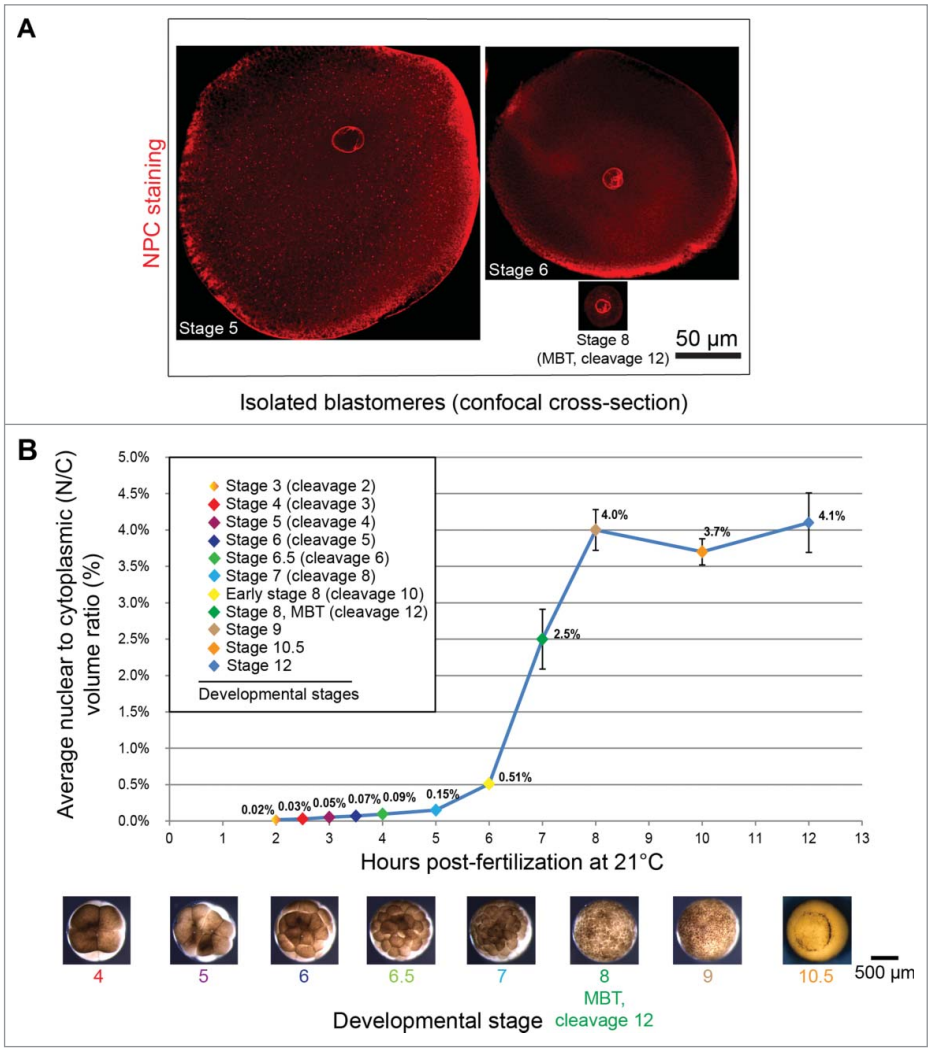


**Figure 2.** Nuclear scaling in early *Xenopus* embryos. (A) Isolated blastomeres from different stage *X. laevis* embryos were stained with mAb414 antibody against the NPC and imaged by confocal microscopy. (B) Average nuclear and cell volumes were quantified for the indicated stages of development, and used to obtain average nuclear-to-cytoplasmic (N/C) volume ratios. Error bars are SE. Images used with permission from[71].

regulating intranuclear concentrations of the limiting DNA binding factors.[71]

Just as nuclear size affects MBT cell cycle length, cell cycle progression also impacts nuclear morphology. Identified in *Xenopus*, Dppa2 and REEP3/4 are required to remove microtubules and ER membrane, respectively, from chromatin at the end of mitosis to ensure a nucleus of the proper size and shape forms in the subsequent interphase.[129,130] In *C. elegans*, partial inactivation of polo-like kinase PLK-1 leads to defects in NE breakdown giving rise to 2 nuclei that fail to merge into one.[131] In yeast, formation of NE flares adjacent to the nucleolus in response to excess membrane production or mitotic arrest is dependent on polo kinase Cdc5.[132,133] A question for future research is precisely how kinases with well-established roles in mitosis, including cyclin-dependent kinases, polo kinases, and PKC, contribute to the maintenance of proper nuclear morphology.

Given the potential impact of the cell cycle on nuclear morphology, it is worth considering the interplay between size scaling of mitotic structures and the nucleus. Scaling of mitotic spindle length as a function of cell size during developmental progression is conserved across metazoans,[134] as has already been discussed in the context of early *Xenopus* embryogenesis. Comparing nearly 100 natural isolates of *C. elegans* showed that selection for cell size, that impacts spindle size, can account for differences in spindle size that span over 100 million years of evolution.[135]

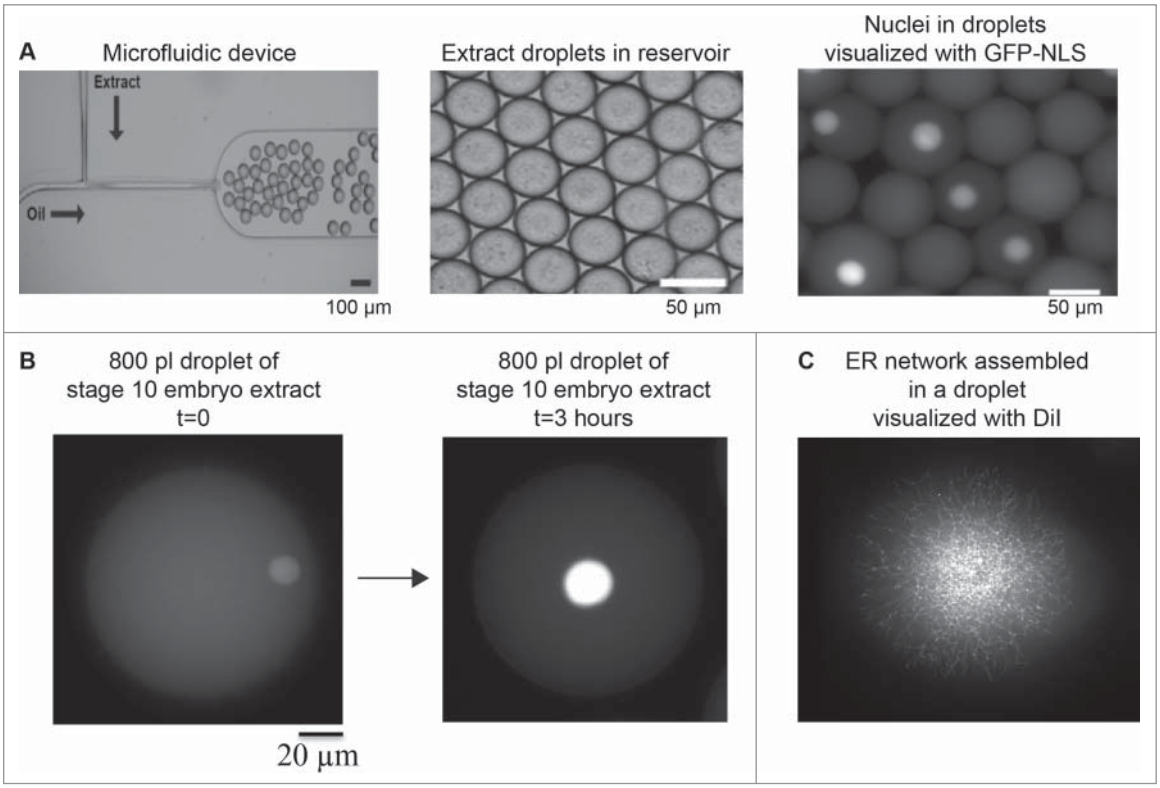


**Figure 3.** Microfluidic encapsulation technology to study organelle size scaling. (A) A standard microfluidic T-junction device is shown. At the junction where oil/surfactant and *X. laevis* egg extract mix, droplets are generated. Droplet size can be tuned by altering device geometry and flow rates. Image courtesy of John Oakey and Jay Gatlin. (B) Large 800 pl droplets containing stage 10 *X. laevis* embryonic cytoplasm and endogenous nuclei are shown. Nuclei are visualized by import of GFP-NLS. Over the course of ~3 hours at room temperature, nuclear size expands (our unpublished data). (C) Partially fractionated *X. laevis* egg extract was encapsulated in a droplet, and ER network formation was visualized with DiI (our unpublished data).

Furthermore, during early *C. elegans* development the physical length of condensed mitotic chromosomes scales with cell and nuclear size, such that a smaller nucleus contains shorter chromosomes as measured just prior to NE breakdown when chromosome condensation is nearly complete.[74] Similar evolutionary pressures may act to control the size of the nucleus as a function of cell and embryo size. As already discussed, multiple mechanisms likely contribute to the proper regulation of nuclear size. Regardless of mechanism, nuclear size normally scales with cell size, suggesting that correct nuclear scaling is important for nuclear, cell, and organismal function, as is particularly evidenced in the case of early embryonic development.

### *New technologies to study nuclear size regulation*

During early *Xenopus* development, reductions in nuclear size might be regulated by changes in the composition of the embryonic cytoplasm and/or reductions in cytoplasmic volume as cells become smaller. We have already reviewed data showing how developmentally regulated changes in cytoplasmic composition contribute to nuclear scaling, including roles for nuclear import and cPKC activity. More recently, microfluidic-based technologies enable the testing of how cytoplasmic volume directly impacts nuclear size (Fig. 3). Encapsulating *X. laevis* egg or embryo extracts in droplets of tunable and defined size is becoming a popular approach to investigate mechanisms of organelle scaling. The open nature of the extract system allows for precise manipulation of cytoplasmic composition, for instance through the addition or depletion of specific proteins, while microfluidic droplet generating devices allow for exquisite control of cytoplasmic volume and droplet shape. This approach facilitates the study of how organelle size is regulated by varying cytoplasmic volumes, limiting components in fixed cytoplasmic volumes, and sensing of droplet shape and boundaries.[136]

Microfluidic techniques have provided insight into scaling of the mitotic spindle, where droplet encapsulation of *X. laevis* egg cytoplasm demonstrated that small spindles form in small droplets and larger spindles form in larger droplets. Interestingly, spindle length was more sensitive to cytoplasmic volume than droplet shape, arguing against a boundary-sensing model of spindle length regulation.[112,113] As cytoplasmic volume regulates spindle length scaling, these data support a limiting component model of spindle size regulation. A variant of this approach, in which *X. laevis* egg extract was pumped into microfluidic channels of varying dimensions, demonstrated that the rate of nuclear growth correlated with the volume of accessible cytoplasm. Additionally, when *X. laevis* egg extract was treated with a dynein inhibitor prior to encapsulation in the microchannels, nuclear expansion was greatly inhibited, implicating a microtubule- and dynein-based mechanism of nuclear size regulation.[137]

Other new technologies allow for measuring the entire transcriptomes of individual cells, by coupling microfluidic encapsulation of individual cells with next generation sequencing.[138] For example, whereas only 5 mouse retinal cell types were previously known, this so-called Drop-seq method identified 39 distinct cell types based on individual cell profiles.[139] We envision coupling these approaches with encapsulation of *Xenopus* extracts, in order to test how cytoplasmic volume impacts the onset of zygotic transcription associated with the MBT.[71]

## Does nuclear morphology affect chromatin organization?

The genome is highly compacted in the eukaryotic nucleus and each chromosome generally occupies a preferred but not fixed position. Chromosomes near the interior of the nucleus tend to be gene-dense while genes at the periphery are usually poorly expressed and associated with the nuclear lamina.[140,141] How nuclear size and morphology affect chromosomal positioning and gene expression is an important unanswered question. Additionally, it has been showed that there is a correlation between nuclear volume and genome size in animals and plants.[142,143] Here we discuss some aspects of chromatin organization that may be influenced by nuclear size (Fig. 4).

Topologically associated domains (TADs) in metazoan genomes are chromosomal regions that tend to be in close proximity to one another. Within a single TAD there are interactions and chromatin loops

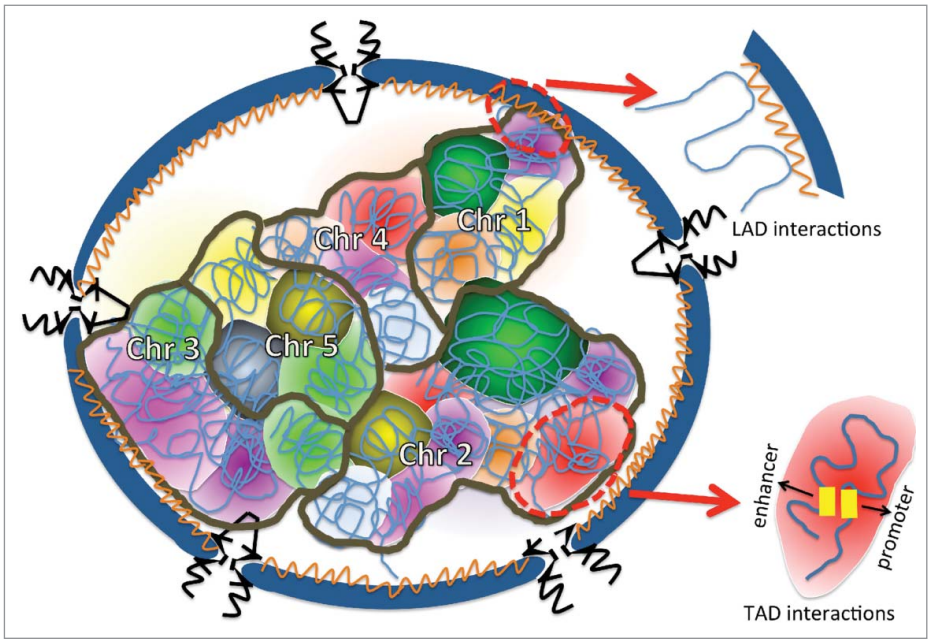


**Figure 4.** Chromosomes are spatially organized within the nucleus. The NE is blue, and the nuclear lamina is the orange structure lining the nucleoplasmic face of the NE. NPCs are black and inserted into the NE. Each chromosome is outlined in brown and is composed of multiple different TADs that are depicted with different colors. An example of the type of chromatin interaction occurring within a TAD is shown within the red oval. LAD interactions of chromatin with the nuclear lamina are also depicted. It is easy to imagine how a change in nuclear volume and/or shape might impact this chromosomal organization.

between *cis*-regulatory regions, such as enhancers, promoters, and insulators, which regulate gene expression. Neighboring TADs are isolated from one another by boundary elements, including the insulator binding protein CTCF, housekeeping genes, and short interspersed element retrotransposons.[144,145] The importance of CTCF for chromosome organization has been demonstrated by changing the orientation or position of CTCF binding sites, which leads to altered chromatin looping and 3D chromosome architecture.[146,147] While some aspects of TAD organization are conserved among different cell types,[144] changes in TAD interactions are associated with cell differentiation and response to environmental signals,[144,148-151] demonstrating plasticity in this level of chromatin positioning. How nuclear volume and shape might influence TAD organization is an open question.

Lamina-associated domains (LADs) are transcriptionally repressed chromatin domains localized at the NE. LADs are generally enriched in repressive histone modifications,[152] and repositioning of active genes to the lamina can result in their repression. For instance, in human embryonic stem cells and derived embryoid bodies, active circadian rhythm genes are silenced when moved into LADs by PARP1 and its co-factor CTCF.[153] The nuclear lamina provides an interaction platform among LADs, and lamins regulate this chromatin organization through diverse interactions. For example, *Drosophila* B-type lamins interact with the actin nucleation protein Wash to maintain proper LAD and chromosome organization.[154] Deletion of B-type lamins in mouse embryonic stem cells reduced interactions between LADs and the nuclear lamina,[155] and cortical neurons in lamin B1 deficient mice exhibit misshapen nuclei with nuclear blebs.[156] As discussed earlier in this review, lamins and components of the nuclear lamina affect nuclear size, which we speculate could influence LAD organization.

Besides the lamina, other nuclear components can affect chromosome structure and gene expression. In yeast, some nuclear transport factors and Nups bind to transcriptionally active genes.[157] Loss of *Drosophila* Nup62 or Nup93 alters chromatin attachments to the NPC,[158] and depletion of *Xenopus* Nup188 increases nuclear size, potentially impacting chromatin localization.[10] Centromeric regions of human chromosomes also adopt defined positions within the 3-dimensional space of the nucleus that are likely important for nuclear function and may be influenced by altered nuclear size in cancer.[159]

Novel methods are being developed to investigate chromatin and chromosome structure. We anticipate these approaches will be useful in ascertaining how altered nuclear morphology impacts global chromatin organization. One of the most widely used methods to detect higher-order chromatin structure is the chromosome conformation capture (3C) family of techniques.[146,153,160] Chromatin immunoprecipitation sequencing (ChIP-seq), micrococcal nuclease sequencing (MNase-seq), and chromatin immunoprecipitation exonuclease (ChIP-exo) methodologies provide information over the ~1–150 bp length scale, allowing for analysis of nucleosome fiber folding [149,161-164]. While these types of approaches provide information on populations of cells, single-cell approaches have now become feasible. A modified DamID (DNA adenine methyltransferase identification) method enables genome-wide mapping in single human cells,[144,147,165,166] and CRISPR-based approaches have been used to image individual chromosomal loci in live cells and in vitro extracts.[167,168] To identify molecular mechanisms that contribute to proper chromatin organization, HIPMap (high-throughput imaging position mapping) was coupled with siRNA screening to identify human genome positioning factors, including chromatin remodelers, histone modifiers, and NE and NPC proteins.[169] As we gain a better understanding of how proper chromosome positioning is established, it becomes possible to test how nuclear size and shape impact these pathways.

## Nuclear morphology and disease

Aberrant nuclear morphology is associated with many diseases, most notably cancer in which altered nuclear size and shape are used by pathologists to assess the degree of malignancy.[61,100,170] Chromosomal gains and losses, amplification or deletion of smaller genomic fragments, and changes in higher-order chromatin structure are all associated with cancer.[171,172] Cancer-associated changes in nuclear morphology may disrupt normal chromatin positioning, gene

expression, and DNA damage pathways, potentially contributing to disease progression. In a recent study, it was shown that loss of the developmentally regulated GATA6 transcription factor in ovarian cancer resulted in deformation of the NE, cytokinesis failure, and aneuploidy.[173] Molecular mechanisms that may contribute to altered nuclear size and shape in cancer have been touched on throughout this review and comprehensively reviewed elsewhere.[1,61,87,100], [170,174-176] An important question for future research is if correcting the altered nuclear morphology of cancer cells might mitigate disease.

Mutations in nuclear proteins contribute to other diseases. In mammalian cells, loss or mutation of NE structural proteins, such as emerin [177] and lamin A/C,[178] cause altered nuclear morphology associated with diseases such as muscular dystrophy,[179] premature aging,[180] laminopathies,[181] and cancer.[182] For example, a heterozygous mutation in exon 11 of LMNA results in Hutchinson-Gilford progeria syndrome, a rare premature aging disease associated with alterations in nuclear shape and structure.[183] Amyotrophic lateral sclerosis and frontotemporal dementia result from hexanucleotide repeat expansions in the *C9orf72* gene, which leads to an inhibition of nuclear protein import and abnormal nuclear architecture.[184,185] Torsin proteins are ER membrane embedded AAA+ ATPases. While most torsins are found in the peripheral ER, torsinA (encoded by *TOR1A*) is also localized in the INM where it affects nuclear morphology. An in-frame deletion in the *TOR1A* gene that removes a single glutamic acid residue (ΔE-torsinA) causes the neurodevelopmental disorder DYT1 dystonia and results in redistribution of torsinA from the ER to the NE.[186-189] In both torsinA null and homozygous ΔE-torsinA knockin mice, the neuronal NE exhibited ultrastructural abnormalities, including INM-derived vesicles within the NE lumen[190]. Other data indicate that torsinA affects connections between the INM and ONM.[191] Mutating *OOC-5*, the *C. elegans* homolog of torsinA, led to abnormal germ cell and intestinal nuclear morphologies, with vesicle-like blebs present in the perinuclear space and protrusion of ONM into the cytoplasm.[192]

Nuclear architecture can also be targeted by pathogens. Human immunodeficiency virus type 1 (HIV-1) associates with particular Nups in order to integrate into transcriptionally active host genes that are located within 1 $\mu$m from the nuclear periphery, while avoiding heterochromatin regions in LADs,[193] suggesting that host nuclear topology is an essential determinant of the HIV-1 life cycle. SINC is a type III secreted protein of *Chlamydia psittaci*. When Hela cells are infected by *C. psittaci*, SINC incorporates into the NE of the host cell nucleus and interacts with Nups, ELYS, lamin B1, and emerin, leading to alterations in nuclear shape and function in both infected cells and neighboring uninfected cells.[194] Many diseases are associated with altered nuclear morphology, so identifying approaches to correct these morphological defects may lead to novel therapeutics.

## Concluding remarks

Throughout this review, we have touched on some important outstanding questions in the field of nuclear morphology regulation. A variety of new experimental methodologies promise to provide new insights into the mechanisms that control nuclear size and shape. With a better handle on mechanism, it should become possible to directly address the functional significance of nuclear morphology, both in normal cells and in disease. How does nuclear morphology affect nuclear function and the morphology of other intracellular structures? How does nuclear volume impact chromatin positioning and gene expression? Does nuclear morphology represent a novel target for cancer therapeutics? Answers to these questions and others are hopefully forthcoming, as exciting work remains to elucidate the regulation of nuclear size and shape.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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