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Bringing balance to the (osmotic) force

1. **Balance of osmotic pressures determines the nuclear-to-cytoplasmic volume ratio of the cell**
   Authors: Dan Deviri and Samuel Safran
   PNAS, 119(21) e2118301119 (2022)

2. **Control of nuclear size by osmotic forces in *Schizosaccharomyces pombe***
   Authors: Joël Lemière, Paula Real-Calderon, Liam J. Holt, Thomas G. Fai and Fred Chang
   bioRxiv, doi: https://doi.org/10.1101/2021.12.05.471221

Recommended with a Commentary by Ajay Gopinathan, University of California, Merced, CA 95340.

Since its first observation a long time ago (at least more than a century), a fully satisfactory explanation for a particular phenomenon in cells has eluded the community. This phenomenon is the remarkable robustness of the ratio of the volumes of the nucleus to the cytoplasm (N/C ratio) of cells, which is maintained constant for almost any given cell type under a wide variety of conditions including different cell growth stages, osmotic environments, and mutations [1] . To put this question in context, the nucleus is the defining feature of the cells of all eukaryotic species which range from yeast to humans. The nucleus houses the cell's genetic material and acts as the master regulator of an enormous number of highly complex cellular processes depending on gene regulation. While we have learned a lot about the intricacies of these processes, it is amazing that the regulation of something as basic as nuclear size evades a full explanation. It is perhaps additionally aggravating that the question is so easy to state and the phenomenon apparently so universal. Two recent papers have addressed this question independently and come to the same conclusion in

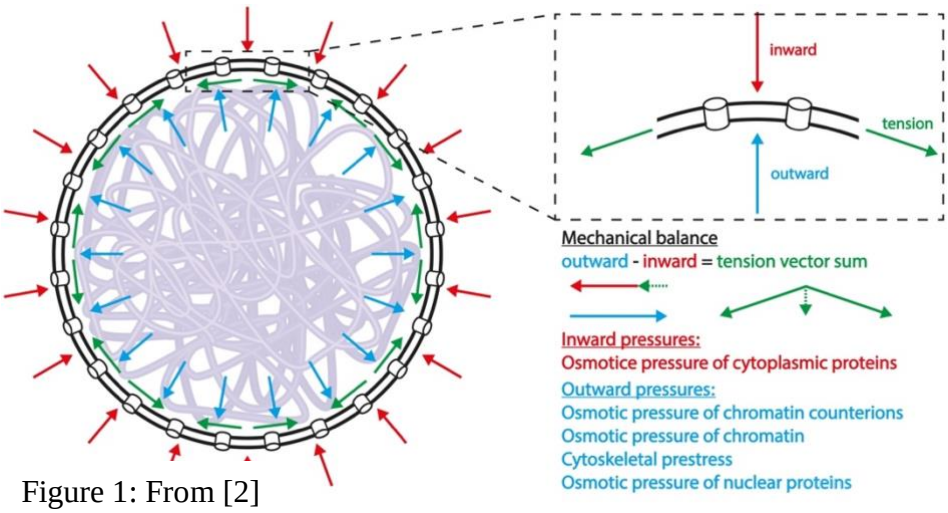

Figure 1: From [2]

serendipitously complementary ways. The main common finding from both papers is that the nuclear volume does not depend on the amount of genetic material or some complex regulation mechanism, but that purely physical forces (specifically osmotic forces) are sufficient to set nuclear size and explain the N/C ratio robustness.

In the first recommended paper, Deviri and Safran [2] do a systematic analysis of the important physical forces involved and come up with bounds and estimates for their magnitudes. To understand the forces involved, we need to look a bit more closely at the nucleus which contains the genetic material in the form of chromatin (which forms chromosomes and is composed of DNA condensed with histones and other accessory proteins) within a nuclear envelope (see fig.1). The double lipid membrane nuclear envelope (NE) keeps the interior of the nucleus distinct from the contents of the rest of the cell and provides mechanical and biochemical protection. The NE therefore defines the nuclear boundary and its volume. Transport of material between the nucleus and cytoplasm (medium outside the nucleus) occurs through the nuclear pore complexes which allows the free diffusion of small molecules and ions across the NE while strictly regulating the transport of larger macromolecules and assemblies (>~4 nm) via an energy consuming process.
The size of the nucleus is assumed to be set by the mechanical balance of inward and outward forces on the NE resulting in a situation like a stretched balloon governed by the Young-Laplace law:

$$\Delta p = 2\sigma_n H,$$

where Δp is the local pressure difference across the NE, σ_n is the tension in the NE and H is the local mean curvature. The relevant pressures on the NE include osmotic pressures of proteins both inside and outside the nucleus that are unable to diffuse across the barrier (small ions and macromolecules that can diffuse, will equalize their concentrations, and not contribute), pressure due to the confined chromatin, osmotic pressure of the chromatin counterions and forces from the cytoskeleton. The authors carefully consider these forces and by making simple approximations (e.g. ideal gases of counterions/proteins, confinement pressure of a single excluded volume polymer (chromatin)), estimate rough bounds for these contributions.

Table 1. Order of magnitude estimates of the contributions of different nuclear and cytoplasmic components to the inward and outward pressures that are exerted on the nucleus of nonstriated muscle cells

Species/source of pressure	Chromatin polymer, Pa	Chromatin counterions, Pa	Cytoskeletal elasticity, Pa	Cytoplasmic proteins, Pa	Nucleoplasmic proteins, Pa
Human	30	300	500	8,000	8,000
Fission yeast	1.5	20	500	8,000	8,000

Order of magnitude estimates of the contributions of different nuclear and cytoplasmic components to the inward and outward pressures that are exerted on the nucleus of nonstriated muscle cells. In striated muscle cells, the cytoskeletal stress is directional and may exceed hundred of kilopascals during contraction, making it the dominant contribution in that case, which is outside of the scope of this paper.

Table 1 clearly shows that, by far the most dominant contribution is from cytoplasmic/nuclear proteins that rely on active transport. For most (but not all) cases of interest, the NE tension is negligibly small ($\sigma_n \approx 0$; relaxed balloon) resulting in an equalization of pressure ($\Delta p \approx 0$), which sets the nuclear size. In the limit that the non-diffusive components of the nucleus and cytoplasm take up negligible volume (which the authors argue for) and pressures being proportional to protein concentrations, the pressure balance condition requires equal concentrations of nuclear and

cytoplasmic proteins. This simply yields a ratio of nuclear to cytoplasmic volumes (N/C) that is equal to the ratio of the numbers of nucleoplasmic and cytoplasmic proteins that are localized to each side. This ratio therefore should be quite insensitive to almost anything except alteration to nucleocytoplasmic transport or differential production of said proteins, explaining the remarkable robustness of N/C ratios.

The second recommended paper by Lemiere *et al* [3], coincidentally almost picks up where the first paper leaves off. They hypothesize that the nuclear size is set by the osmotic pressures of the localized proteins and proceed to experimentally test the predictions of such a model in yeast cells. While the first paper also serves as an instructive example of research using simple approximations and estimates leading to insight, the second is an excellent example of careful experimental validation of a physical theory in a biological system. First, the authors subject yeast cells and protoplasts (cells with cell walls digested away) to a wide range of both hyperosmotic and hypoosmotic conditions. They show that nuclei swell and shrink exactly as one would predict and maintaining the N/C ratio, showing that the NE tension was indeed small, and that the presence of the cell wall tension did not affect the ratio (incidentally a prediction of the first paper as well). They then performed the critical experiment of inhibiting nuclear export (using a drug leptomycin treatment), which led to an accumulation of large cargo within the nucleus and an increase in the N/C ratio just as predicted. The other critical experiment was to change global production of proteins. If nuclear and cytoplasmic proteins are proportionally affected the ratio would be insensitive to this perturbation. Using a (cycloheximide) treatment that slows down protein production, they observed that the yeast cells grew slower but maintained N/C ratio. Finally, and perhaps most interestingly, they investigate whether this passive osmotic model can give rise to homeostasis in growing cells, allowing cells with aberrant N/C ratios to correct themselves. Assuming that the growth of both nucleus and cytoplasm are governed by production of proteins in the cytoplasm that are destined for the nucleus and cytoplasm respectively yields a simple law for the dynamics of the N/C ratio that exponentially approaches the fraction of the total protein synthesis destined for transport to the nucleus (~8%). Their observations of the dynamics of the N/C ratio in growing mutant cells which start out with widely different N/C ratios (due to asymmetric division) match their model with no adjustable parameters, providing further validation for a passive, osmotic homeostasis mechanism.

While both papers discuss a few non-ideal cases where, for example, the NE tension is non-zero and the N/C ratio is therefore not maintained, overall, they point to the intrinsic robustness of the N/C ratio. Deviations of N/C ratio from what it should be for that cell type require severe disruptions in nucleocytoplasmic transport and/or protein production and are usually markers for serious disease such as cancer. The fact that the N/C ratio is not actively regulated by the cell but is rather obtained for "free" due to entropic forces is intriguing. This points to the possibility that many more such homeostasis mechanisms could be obtained from purely physical forces, as long as they have no negative effects (and potentially some positive effects) that do not have to be actively mitigated by the cell.

References:

1. Cantwell H., Nurse P., Unravelling nuclear size control. Curr. Genet. 65, 1281–1285 (2019)

2. Deviri D., Safran S., Balance of osmotic pressures determines the nuclear-to-cytoplasmic volume ratio of the cell, PNAS, 119(21) e2118301119 (2022)
3. Lemiere J., Real-Calderon P., Holt L., Fai T., Chang F., Control of nuclear size by osmotic forces in Schizosaccharomyces pombe. bioRxiv [Preprint] (2021).