Forces, fluctuations, and self-organization in the nucleus

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ABSTRACT **We address several processes and domains in the nucleus wherein holding the perspective of physics either reveals a conundrum or is likely to enable progress.**

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Like its predecessor in 2014, this second special issue of *Molecular Biology of the Cell* (*MBoC*) again engages the cell biology and biophysics community to envision the degree to which physics governs certain domains of cellular function.

As early as a century ago, some leading physicists had envisioned the role their science could play in understanding living systems, as did Erwin Schrödinger with particular cogency (Schrodinger, 1944). Later, in the early 1950s, the physicist Francis Crick did a pioneering study in cell biology (probing the structure of cytoplasm by the resistance it offered to magnetic particles in an applied field) before turning his attention elsewhere (reviewed in Pederson, 2014). In a previous *Perspective* (Pederson and Marko, 2014), the broad outlines of the theme of physics were set forth as regards the nucleus. Here we refine those ideas, focusing on several open problems concerning how the nucleus operates and offering thoughts on how the perspective of physics has been—and will continue to be—useful as we fathom the nucleus.

PASSIVE AND ACTIVE FORCES IN CHROMATIN

From a mechanistic perspective, it is tempting to view the interphase nucleus as being composed of a container—the nuclear envelope—inside of which chromosomes might behave as confined, flexible polymers. However, the flexibility and consequent polymer-like motions of chromatin in the nucleus and the closely related issue of nucleosome folding (beads on a string vs. a 30-nm structure; Maeshima *et al.*, 2014) remain controversial topics. Fluorescence in-situ hybridization studies have provided convincing evidence that the chromosomes occupy discrete, micron-sized "territories" of the nucleus (Cremer and Cremer, 2010). How this large-scale structure is organized by nanometer-sized proteins interacting with chromosomal DNA is a challenging physics problem.

Another important general problem is determining what kinds of biopolymer physical "laws" describe interphase chromatin. It remains an open question exactly how "passive" thermal motions and biomolecule-binding interactions combine with "active" positioning and remodeling processes to determine chromosome locus positions and motions. It is likely that at shorter distances (smaller than 10 nm) more rapid, thermal motions dominate, while at longer distances (longer than 100 nm), slower, actively driven dynamics become more dominant. However, determining exactly how the transition between these regimes occurs will require four-dimensional experiments that can access the large range of time and distance scales relevant to chromosome dynamics.

Chromosome capture techniques such as Hi-C (high-resolution chromosome conformation capture) now provide kilobase-scale detail about relative positions of chromosomal loci in nuclei, including discoveries of transcriptionally active and inactive domains and "fractal globule" nonclassical polymer scaling behavior (Lieberman-Aiden *et al.*, 2009). These new models immediately generate questions of how these structures and nonclassical polymer statistics are generated and maintained, while at the same time permitting genes to change their nuclear positions in response to physiological stimuli (Egecioglu and Brickner, 2011)

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Abbreviations used: FCS, fluorescence correlation spectroscopy; FG, phenylalanine-glycine; NIH, National Institutes of Health; NPC, nuclear pore complex.

and repair of DNA damage (Nagai *et al.*, 2008; Oza *et al.*, 2009; Roukos and Misteli, 2014). A truly remarkable aspect of Hi-C studies is how much structural data can be obtained from them, despite the averaging of many millions of nuclei. A challenge before us is to understand the degree of cell-to-cell variation that is hidden in cell-averaged Hi-C measurements.

Hi-C and other static measurements also suffer from the limitation that they do not provide dynamical information. The question of how chromatin moves in the nucleus is complicated by the possibility that metabolism (ATP)-dependent motion plays a role in locus repositioning (Levi *et al.*, 2005) and the notion that diffusion-like motions can be boosted by metabolic energy (Heun *et al.*, 2001; Weber *et al.*, 2012). Just to consider one example, homologous recombination is a process with potential for roles of both passive thermal and active chromatin dynamics. Sequence "search" and "match" processes of microhomologous DNA regions can proceed by thermal motions in vitro (Qi *et al.*, 2015), but what role—if any—is played in vivo by active processes to bring homologous sequences together in a crowded nucleus is poorly understood.

OUTSIDE-IN: THE "MECHANICAL" NUCLEOCYTOPLASMIC MYSTIQUE

While the intrinsic physical properties of the chromatin polymer exert a strong influence(s) on its dynamics, it is critical to remember that the nucleus is mechanically integrated into the cell (and thereby into tissue). Indeed, mechanical cross-talk between the nucleus and the extracellular environment impacts the cell cycle and stem cell differentiation and feeds back onto the emergent mechanical properties of cells and tissues (Swift *et al.*, 2013; Bellas and Chen, 2014; Pagliara *et al.*, 2014). Although much of this regulation likely occurs through mechanosensitive signal transduction cascades that alter nuclear localization and/or function of transcription factors (Gaspar and Tapon, 2014; Janmey *et al.*, 2013), it is tempting to consider the possibility that forces exerted across the nuclear envelope, likely through the nuclear envelope–spanning LINC complex (Chang *et al.*, 2015), can directly impact genomic processes. Indeed, the cytoplasmic cytoskeleton can drive changes in the dynamics of chromatin through the LINC complex, observed during both meiosis (Hiraoka and Dernburg, 2009; Rog and Dernburg, 2013) and DNA repair (Swartz *et al.*, 2014).

In addition to modulating chromatin dynamics, forces delivered onto chromosomes during meiotic pairing could provide a physical, kinetic "proofreading" of chromosome pairing in which nonhomologous chromosome pairs are disengaged through dynein-dependent forces, while homologous chromosome pairs that can withstand these forces proceed into synapsis (Sato *et al.*, 2009). One can therefore speculate that the LINC complex could serve as a conduit for mechanical information sensed at the cell surface to be rapidly communicated to the nuclear interior (Ho *et al.*, 2013; Swift *et al.*, 2013; Harada *et al.*, 2014; Stewart *et al.*, 2015), perhaps directly driving changes in dynamics, torsion or tension of associated chromatin loci, or even inducing DNA breaks, ultimately leading to an altered topological chromatin state and transcriptional change (Kumar *et al.*, 2014; Teves and Henikoff, 2014; Madabhushi *et al.*, 2015). Failure of the cell to respond appropriately to such mechanical cues may ultimately underlie diseases such as laminopathies and could explain the altered physical properties of metastatic cancer cells (Harada *et al.*, 2014).

Mechanistic study of mechanotransduction across the nuclear envelope will require the development of a quantitative biophysical framework. Adaptation and implementation of tools analogous to those developed to study force transmission across the plasma membrane (traction force microscopy [Harris *et al.*, 1980; Lee *et al.*, 1994]; genetically encoded tension sensors [Grashoff *et al.*, 2010]) will be critical to test the mechanisms and efficiency of force propagation from the cytoskeleton, across the nuclear envelope, and ultimately onto the lamina and chromatin. Real breakthroughs will require the simultaneous establishment of physical models of chromatin topology and the impact that its modulation has on the output of the genome.

THE NUCLEAR TRANSPORT PARADOX

A molecular understanding of transport across the nuclear envelope via nuclear pore complexes (NPCs) continues to present a fascinating problem for physical scientists, particularly as translocation across the NPC is energy-independent (Schwoebel *et al.*, 1998; Englmeier *et al.*, 1999). Although we enjoy an ever-increasing molecular understanding of the core structural scaffold of the NPC (Hurt and Beck, 2015), challenges remain in defining a unified view of the physical nature of the transport channel, which is composed of natively unfolded phenylalanine-glycine (FG) repeat domains of nucleoporins. One of the most outstanding challenges that could be greatly aided by theoretical and experimental approaches from the physical sciences is resolving the "nuclear transport paradox." Macromolecules larger than the diffusion barrier of the NPC channel can selectively permeate the NPC through their binding to nuclear transport factors, which directly bind the FG repeats with high affinity, measured in the nanomolar range in vitro (Ben-Efraim and Gerace, 2001; Gilchrist *et al.*, 2002; Bednenko *et al.*, 2003; Pyhtila and Rexach, 2003; Lott *et al.*, 2010). However, transport across the NPC is extremely fast (estimated transit times of 10–400 ms, perhaps depending on the specific transport factor–cargo complex; Yang *et al.*, 2004; Kubitscheck *et al.*, 2005; Grunwald and Singer, 2010; Smith *et al.*, 2016). Moreover, a single NPC can accommodate the passage of ~1000 molecules/s; such efficiency predicts a maximum affinity between FG repeat-transport factor interactions to be in the micromolar range (Ribbeck and Gorlich, 2001). How these two properties—high-affinity interactions between nuclear transport factors and FG repeats and rapid passage across the NPC—could be physically compatible remains an intriguing mystery. Very recent evidence suggests that rapid exchange of FG–transport factor interactions may be key for the selectivity and rapidity of nuclear transport (Hough *et al.*, 2015). This problem is reminiscent of the concentration-dependent exchange of DNA binding proteins on DNA, where experimentally determined in vitro protein–DNA dissociation rates at low protein concentrations appear, at first glance, to be incompatible with in vivo functional data, due to the strong impact of competing interactions in the latter case (Graham *et al.*, 2011).

THE PHYSICAL NUCLEOPLASM

"Nucleoplasm" is one of the more unfortunate terms in the cell parts list.[1] But nomenclature aside, what do we know about the

[1] "Nucleoplasm," at first blush, seems an etymological cousin of "cytoplasm" and "cytosol" but has fewer credentials. The term "cytoplasm" was coined to combine a perceived slushy "endoplasm" around the cell center and an "ectoplasm" at the cell periphery regarded as a more structured region. In contrast, the term "cytosol" came from biochemists, to denote a nonparticulate cell fraction that was the source of many discoveries, none less important than that of tRNA. (Subsequently, the term "cytosol" crept into the vernacular of in vivo intracellular protein and membrane trafficking, something that a nomenclature committee might have vetoed, had such an authority existed.) In any case, the possible term "nucleosol" thankfully never arrived. The objection to both terms (cytosol and the feared nucleosol that never stuck) is that the suffix implies a true solvent phase in which all molecules and particles are behaving as solutes in a strict sense and where their behavior is that of a true solution embodied in the doxology of physical chemistry.

composition, fluid viscosity and possible organized structure of this intranuclear space? As to its cross-section density, which is confronted by solutes and their motion, we know that the intranuclear diffusion coefficient of GFP, a protein presumed to have minimal interactivity with the chromosomes or other nuclear bodies, is approximately five times lower than if water were the solvent (Wachsmuth *et al.*, 2000). More specifically, this study revealed that the nuclear mobility of GFP displayed a substantial component with a similar diffusion coefficient to that in the cytoplasm (10–12 $\mu m^2/s$), as well as a component with a diffusion coefficient about one-tenth of the former. If the faster nuclear component is assumed to reflect the classical property of fluid viscosity (and that is an assumption), this measured parameter speaks to a high concentration of dissolved proteins and RNA (or other molecules, monomeric or assembled) in the nucleoplasm.

Another property of relevance is the viscoelasticity of the nucleus, which descends from the aggregated individual properties of each constituent in the nucleus. Such measurements are far more technically challenging than those of viscosity, but important data have nonetheless been recorded (Guilak and Tedrow, 2000; Tseng *et al.*, 2004; Pajerowski *et al.*, 2007). As an example of how such measurements of a physical parameter of the nucleoplasm can inform our understanding of larger-scale nuclear phenomena, the Tseng *et al.* (2004) study determined that the nucleoplasm of a mouse fibroblast cell line had a mean shear viscosity of 520 P, and an elasticity of 180 dyn/cm^2 (lower than that of the cytoplasm). From these values, the authors calculated that forces in the range of 3–15 piconewtons are, at minimum, required to move nuclear bodies within the nucleoplasm. To the extent that such movements are known to be driven by thermal energy (i.e., diffusion), the computed kinetic energies would presumably explain the observed velocities of the mean square displacement trajectories these nuclear bodies display.

We also know from studies in which RNA has been tracked as it moves in the nucleoplasm that its trajectory, measured either by fluorescence correlation spectroscopy (FCS; Politz *et al.*, 1998) or by direct imaging of the RNA mobility (Politz *et al.*, 1999), reveals that these RNA–protein complexes enjoy free range throughout the nucleoplasm, using only their inherent kinetic energy to power their diffusive journey, deriving none of the energy balance sheet from metabolic sources. Of course, and as was anticipated, when the data from the FCS study were further decomposed and binned, subpopulations were evident from which it became clear that the reporting molecules were often colliding with less mobile structures (likely most often chromosomes) or were for short periods confined to cul-de-sacs, consistent with the known three-dimensional organization of the genome as one in which curvilinear territories occupied by the interphase chromosomes create such confinement zones as a small fraction of the overall open interchromatin space (Cremer and Cremer, 2010). Thus, when interpreting in situ and in vivo studies of nuclear function, it is to be borne in mind that the nucleoplasm is, from the standpoint of diffusion, a physically heterogeneous domain.

Also to always be considered is the degree to which very high concentrations of RNA or proteins in the nucleoplasm, if they reach such levels, can decrease the effective volume. Such molecular crowding phenomena can change the rates of bimolecular association reactions (Ernst *et al.*, 2012; Hancock, 2014). For example, exposure of K562 cell nuclei to media of low ionic strength leads to a disassembly of nucleoli and promyelocytic leukemia bodies, which can be reversed by restoration of the external osmolarity by inert dextrans (Hancock, 2014). And in previous in vivo experiments with HeLa cells, a similar nucleolar disassembly–reassembly phenomenon was observed as a function of the osmolarity of the medium (Robbins *et al.*, 1970). Beyond these considerations, there are many parameters that we need to determine with respect to the nucleoplasm. What are its pH, ionic strength, RNA concentration, and protein concentration? In addition to their presence in the nuclear envelope, there is evidence that free lipids reside in the nucleoplasm (Cocco *et al.*, 2011), and their likely importance is often not taken into consideration. One wonders as well whether monomeric proteins in the nucleoplasm are governed by the hydrophobic effect, one of the most foundational principles of protein conformation (Tanford, 1978), or whether they deviate to some degree due to some (hypothetical) unusual property of the nucleoplasm (e.g., the degree to which the water concentration may conceivably not be 54.5 M!).

One of the most vexing issues regarding the nucleoplasm has been the issue of whether it may contain some degree of structure at mesoscopic scale (the zone that lies, roughly speaking, between molecules and structures with sizes of a micron or so). Ideas of some kind of an all-nucleus spanning scaffold have been largely dismissed, but are there local sites of organized structure in the chromosome and nuclear body–free regions of the nucleoplasm? The discovery of F-actin in the nucleus (Pederson, 2008; Belin *et al.*, 2013) has brought this idea to the forefront of our thinking about nucleoplasmic "structure."

And then, just when most of us in cell biology weren't looking, an entirely whole new idea arose.

Almost all cell biologists (but perhaps not all biophysicists) think of particles (e.g., ribosomes), membranes (e.g., the nuclear envelope, the endoplasmic reticulum), and organelles (e.g., mitochondria) either as formed or yet to be formed (in their biosynthesis or by replication in the case of mitochondria, those ancient invaders). And although most cell biologists are keenly aware of how dynamic all such parts of the cell are, with their molecular components having either short or long residence times in the steady-state complexes and with the pools of unassembled molecules being large or small and the on and off rates of binding and disassembly being dictated by standard equilibrium principles, few would have quarreled with the notion that these cellular particles and organelles are, as physics, to be regarded as the solid state of matter. (Students sometimes misperceive things like the lateral mobility of proteins in membranes as a "liquid" phenomenon, but it is not. It is the diffusion of a solid within a lipid bilayer that is also a solid.)

At the Marine Biological Laboratory in Woods Hole, three visiting researchers took a look at the extrachromosomal amplified nucleoli in frog oocytes (Brangwynne *et al.*, 2011), observing that their sizes and polymorphism scaled in a way that was best fit by a power law, indicative of a liquid droplet mode of dynamic existence. A subsequent study richly expanded this initial finding (Weber and Brangwynne, 2015). Meanwhile, the laboratory of Steve McKnight reported, in cell-free experiments, that RNA granules, known to exist in many cells, can be phenocopied in vitro based on hydrogels that arise from the physical properties of low-complexity proteins (Han *et al.*, 2012; Kato *et al.*, 2012). These in vitro–assembled RNA particles are not simply a Langmuir layer of adsorbed molecules (classically defined as a single, bound coat) but are seeded in a multivalent mode and thus can have an arborized organization, as do the RNA granules observed in vivo. This concept had been previously suggested on theoretical grounds (Iborra, 2007). It is worth pointing out that, while the liquid droplet–like behavior of nucleoli noted by Brangwynne and collaborators (Brangwynne *et al.*, 2011) and the RNA gels reported by the McKnight group (Kato *et al.*, 2012) appear

to be similar as physical phenomena, we do not presently know this for certain. It is also to be kept in mind that these latter phenomena are distinct from the aforementioned molecular-crowding effects. Molecular crowding impinges on rate laws (i.e., reaction kinetics), whereas phase-shift phenomena relate to intermolecular forces at the solute:solvent interface and are thus inherently thermodynamic in character.

KUDOS FOR BASIC RESEARCH

These recent major heuristic developments about the physical chemistry of the nucleus (nucleoli as liquid-like droplets arising from a phase shift–like principle and RNA gels that accrete RNA-binding proteins into granules reminiscent of those observed in cells) have two characteristics that are to be applauded. Both sets of studies involved experiments that were sheerly curiosity driven and both had elements of serendipity. (Congress and National Institutes of Health please note!) More specifically, both of these studies involved results that at first puzzled the investigators (so often a telltale sign that something profound is lurking). Moreover, these discoveries were made without any special technology or high-throughput machines (important tools, we all agree). Instead, they came from keen observations coupled with the open minds of the observers. Here, in a powerful way, we sense the tradition of physics, wherein a respect for established truth was always balanced by an intrepid awareness that things actually might be even more interesting than first thought, once aptly labeled "the endless frontier."

SUMMARY

In this paper we briefly discuss studies of the nucleus that have been informed by the consideration of physics, adding depth and impact of the findings. We also endeavor to point out unresolved aspects of nuclear dynamics and function, in which analysis of the underlying physics (along with the biology) is likely to lead to further insights.

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