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Structures and functions in the crowded nucleus: new biophysical insights

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Concepts and methods from the physical sciences have catalyzed remarkable progress in understanding the cell nucleus in recent years. To share this excitement with physicists and encourage their interest in this field, this review offers an overview of how the physics which underlies structures and functions in the nucleus is becoming more clear thanks to methods which have been developed to simulate and study macromolecules, polymers, and colloids. The environment in the nucleus is very crowded with macromolecules, making entropic (depletion) forces major determinants of interactions. Simulation and experiments are consistent with their key role in forming membraneless compartments such as nucleoli, PML and Cajal bodies, and discrete "territories" for chromosomes. The chromosomes, giant linear polyelectrolyte polymers, exist *in vivo* in a state like a polymer melt. Looped conformations are predicted in crowded conditions, and have been confirmed experimentally and are central to the regulation of gene expression. Polymer theory has revealed how the chromosomes are so highly compacted in the nucleus, forming a "crumpled globule" with fractal properties which avoids knots and entanglements in DNA while allowing facile accessibility for its replication and transcription. Entropic repulsion between looped polymers can explain the confinement of each chromosome to a discrete region of the nucleus. Crowding and looping are predicted to facilitate finding the specific targets of factors which modulate activities of DNA. Simulation shows that entropic effects contribute to finding and repairing potentially lethal double-strand breaks in DNA by increasing the mobility of the broken ends, favoring their juxtaposition for repair. Signaling pathways are strongly influenced by crowding, which favors a processive mode of response (consecutive reactions without releasing substrates). This new information contributes to understanding the sometimes counter-intuitive consequences and the evolutionary advantages of a crowded environment in the nucleus.

Keywords: nucleus, crowding, entropic forces, nuclear compartments, chromatin loops, fractal globule, signaling, target finding

INTRODUCTION

The nucleus can be viewed as a complex colloidal system of proteins, ribonucleoproteins, and giant charged linear polymers (the chromosomes), confined within the lamina of the nuclear envelope. The measured global concentration of macromolecules exceeds 100 mg/ml (reviewed in [1]); the chromosomes occupy ∼10% of the nuclear volume [2] and total macromolecules between ∼20% [3] and ∼40% [4, 5]. As observed 100 years ago, "physical chemists and biochemists have nowadays come to realize that the most fruitful ground of both chemistry and biology lies in the land of colloids" [6] which Ostwald aptly termed "the world of neglected dimensions" [7].

Thinking in this field has been marked by the realization that entropic forces play major roles in interactions between macromolecules in the nucleus, as they do in colloidal systems [8, 9] and, as noted many years ago, in the cell cytoplasm [10], although they are insignificant in the dilute conditions usually used for molecular biological experiments *in vitro*. Entropic (also termed depletion) forces favor contacts between larger macromolecules or particles in a concentrated mixture, because then the excluded volumes which surround them overlap and more volume is available to smaller molecules [11]. Entropic interactions are highly sensitive to the local shape of macromolecules, conferring a "lock and key" selectivity [12, 13]. A further result of crowding which is likely be important in the nucleus is a significant enhancement of the thermodynamic activity of macromolecules [14] which would allow efficient interactions with fewer members of each species than those required in a dilute medium.

COMPARTMENTS IN THE NUCLEUS

Nuclei contain diverse types of compartments which contain macromolecular complexes with different specialized functions (reviewed in [15]), for example nucleoli where ribosomal RNA is transcribed and other types shown in **Figure 1A**. These

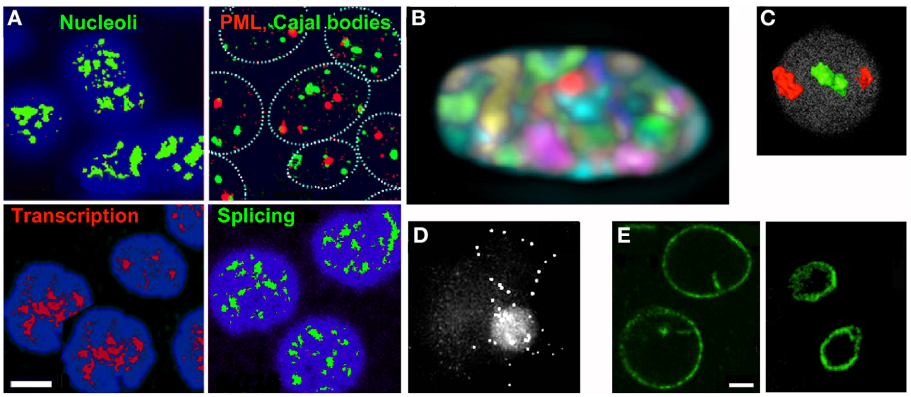

FIGURE 1 | (A–D) Features of the nucleus which can be understood as effects of crowding and entropic forces. **(A)** Compartments visualized by immunofluorescence (reproduced from [20]). **(B)** Discrete territories of the 24 chromosomes in a human fibroblast nucleus labeled with different fluorochrome combinations (reproduced from [21]). **(C)** Gene-poor chromosomes ([18], red) are located more peripherally than gene-rich chromosomes ([19], green) in the nucleus of human lymphocytes (reproduced from [22] by permission from Macmillan Publishers Ltd, 2001). **(D)** A loop of DNA containing ~220 kb of the human dystrophin gene visualized by fluorescence *in situ* hybridization (FISH) (reproduced from [23] by permission of Oxford University Press). **(E)** The nuclear lamina confines and compresses the contents of the nucleus. The nuclear lamina of K562 cells was visualized by immunofluorescence before (left) or after (right) DNA was digested with restriction enzymes and chromatin was removed by electroelution (R. Hancock, unpublished). Scale bars 5 μm.

compartments have no external membrane, and with the exception of chromosomes their macromolecules exchange dynamically with the surrounding milieu (for example [16]), they are mobile [17], and they can divide and fuse [17, 18]. RNAs may be essential structural components of compartments [19]. Despite many descriptive studies, the mechanism by which compartments are formed has been unclear.

Experimental and simulation studies are consistent with a key role for crowding in the association of macromolecules to form compartments. Simulations show that model particles form clusters in crowded conditions (**Figure 2A**) [24]. Cluster formation is observed quite commonly in colloidal systems [25], and the concentration of protein in clusters formed in a crowded solution may reach up to ~700 mg/ml [26] (**Figure 2B**). The formation of compartments can also be regarded as phase separation, where entropic attractions in a mixture of macromolecules result in expulsion of one component as a separate phase [27]. Since entropic effects favor the positioning of particles on a surface [28] they may contribute to the frequently-observed localization of Cajal and PML bodies in contact with chromosomes (for example [29, 30]).

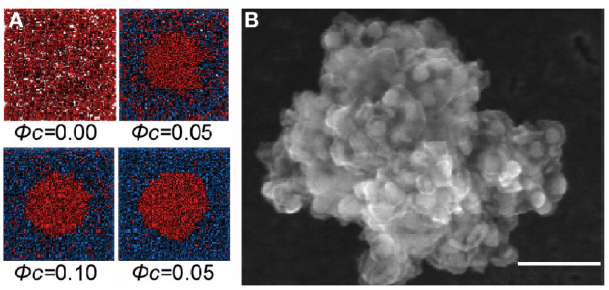

FIGURE 2 | Formation of protein clusters in a crowded solution. (A) Molecular Dynamics simulation of Lennard-Jones particles (red) shows clustering induced by crowding particles (blue) which is more pronounced at higher volume fractions (ϕ_c) of crowder (reproduced from [24] with permission from Elsevier, © 2012). **(B)** A cluster of monoclonal antibody molecules in a solution with trehalose as crowder (scanning electron microscopy); similar clusters form using polyethylene glycol as crowder. Scale bar 100 μm (reproduced from [26] with permission from the American Chemical Society, © 2012).

STRUCTURE AND PACKING OF CHROMOSOMES

CHROMATIN FIBERS

DNA in eukaryotic cells is associated with spherical protein subunits (nucleosomes) as a giant linear polyelectrolyte polymer, and until recently thinking was dominated by the model that this fiber has a regular helical conformation with a diameter of ~30 nm. Nevertheless, irregular conformations were seen quite commonly *in vivo* in studies by electron and optical microscopy (for example [31, 32]) (**Figure 3B**) and were also predicted by simulation of the response to crowding of linear polyelectrolyte polymers [9, 33] (**Figure 3A**) and by considering that chromosomes resemble block copolymers [34, 35] with interspersed regions of repeated DNA sequences [36], methylated cytosine-containing DNA which has particular conformational properties [37, 38], and nucleosomes containing variant or modified histones which influence fiber interactions [39, 40]. These discrepancies have been resolved by recent cryo-electron microscopy and X-ray scattering studies, which show conclusively that chromatin fibers exist *in vivo* in a disordered, interdigitated state resembling a polymer melt [41].

LOOPS IN CHROMATIN FIBERS *IN VIVO*

The existence of loops in DNA *in vivo* has been a common theme in optical and electron microscopy studies of lysed nuclei (for example [42, 43]), and is now realized to be central to understanding chromatin fiber conformations *in vivo* [44]. Looping must be invoked in order to reconcile the spatial distance between two points on a chromosome *in vivo*, measured by

3D fluorescence *in situ* hybridization (FISH), with their linear distance along the chromosome [45, 46]. Simulation shows that spontaneous looping of a polymer is favored by crowding (**Figure 4A**) and is more frequent and persistent in longer chains [47]. These predictions have been confirmed experimentally by mapping the contact points at the base of chromatin loops after crosslinking them *in vivo* (chromosome conformation capture, 3C) which reveals loops a few kb to tens of Mb in length [48, 49]. Looping is at least to some extent a stochastic process, and in the context of gene regulation is clearly an attractive model for bringing regulatory sequences in DNA into proximity to the genes which they control [50, 51] (**Figure 4B**). Nucleoli [52] and transcription factories [53] are proposed to be formed by the assembly of numerous loops.

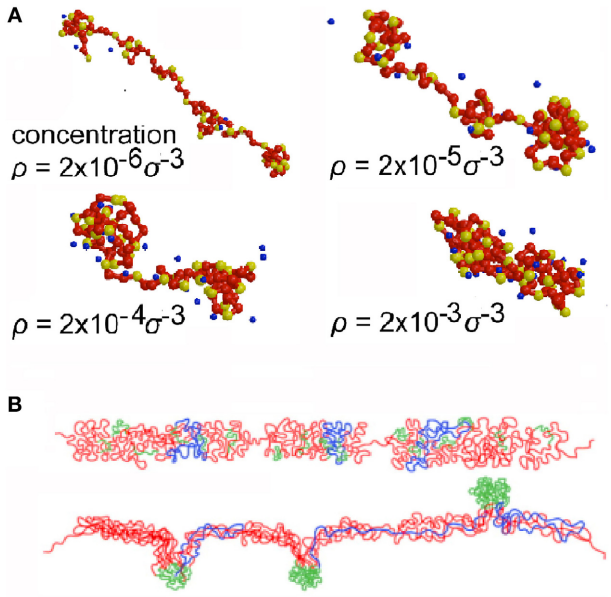

FIGURE 3 | Conformations of chromatin fibers. (A) Simulation by Molecular Dynamics of a linear polyelectrolyte polymer resembling chromatin at increasing concentration (ρ) (self-crowding) (reproduced from [34] with permission of ACS Publications, © 1999). **(B)** Conformations *in vivo* of the DNA of a transgene deduced from studies using FISH; the transgene DNA is red, bacterial artificial chromosome DNA is blue, and lac operator DNA is green (reproduced from [32] by permission of Rockefeller University Press, © 2010).

CHROMOSOME TERRITORIES

Each chromosome is confined to a discrete territory in the nucleus, with little or no intermingling (reviewed in [21, 22]) (**Figure 1B**). Simulations show that this segregation can be understood by the entropic repulsion which occurs between polymers containing loops [44, 54]. The preferential positioning of gene-rich and transcriptionally active chromosomes in central regions of the nucleus while inactive chromosomes are more peripheral [55] (**Figure 1C**) can be explained by entropic effects resulting from a higher frequency of loops in more compact inactive chromatin [52].

PACKING THE GENOME INTO THE NUCLEUS

The ∼2 m of DNA in human cells are packed as chromatin fibers into a nucleus ∼10 μm in diameter, a formidable level of compaction. How this is achieved has been revealed by the seminal simulation studies of the collapse of a linear polymer by Grosberg et al. [57], which show how a compact "crumpled globule" with fractal properties is formed (a notable example of the value of non-translational research). Experiments and simulations strongly support this fractal manner of packing chromatin in the nucleus [58–61] (with the exception of yeast), which was suggested earlier by neutron diffraction studies of nuclei [62]. Data from 3C studies of human chromosomes are consistent with a fractal organization, but not with the alternative "equilibrium globule" conformation [58]. On could speculate that the fractal globule conformation has been selected during evolution because the chromatin fiber does not contain knots or entanglements, DNA is easily accessible, and chromosomes are localized in a

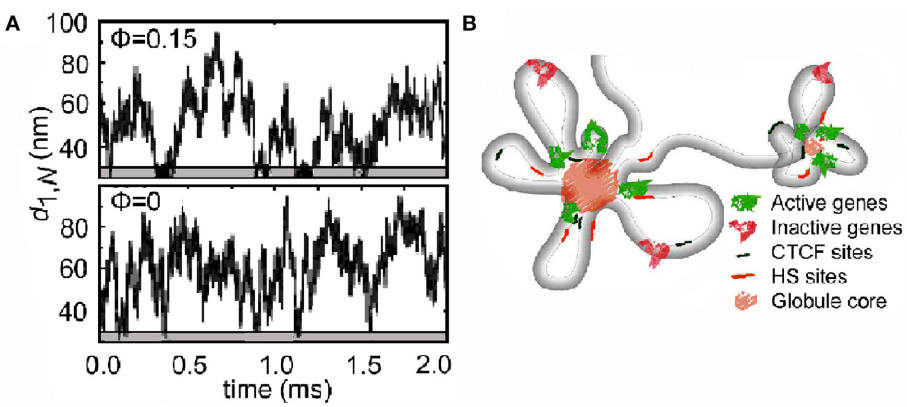

FIGURE 4 | Loops in chromatin. (A) Simulation by Langevin dynamics of the spontaneous formation of loops in a linear polymer. In the presence of crowding particles (upper panel), the minima of the end-to-end distance ($d_{1,N}$) which reflect looping are more frequent and persistent (lower gray areas) (reproduced from [47] with permission from the American Physical Society, © 2006). **(B)** Chromatin loops in the region of the human α-globin gene *in vivo* deduced from chromosome conformation capture (3C) experiments (reproduced from [48] by permission from Macmillan Publishers Ltd, © 2014).

territorial pattern without intermingling (**Figure 5**) [58, 59]. A more evolved model which is consistent with the fractal globule allows dynamic variations of chromatin folding and switch-like changes of genome architecture to be captured [63]. These models will replace the common textbook depictions of chromatin packed through a hierarchical series of largely speculative coiled intermediates.

DIFFUSION AND SIGNALING

Diffusion of molecules is central to all cellular activities, from biochemical reactions to metabolic networks, signaling pathways, and control of gene expression. Diffusion of macromolecules is slowed in the nucleus ([64, 65], reviewed in [66]) probably due to collosions with chromatin and other large obstacles or to viscoelasticity, but nevertheless most macromolecules and multiprotein complexes can explore the entire nuclear volume [67]. Large particles and macromolecules show anomalous diffusion in the nucleus, a lesss-than-linear increase of mean-square displacement with time like that seen in crowded solutions [68]. Remarkably, subdiffusion can increase the probability of finding a nearby target compared to normal diffusion [68–71].

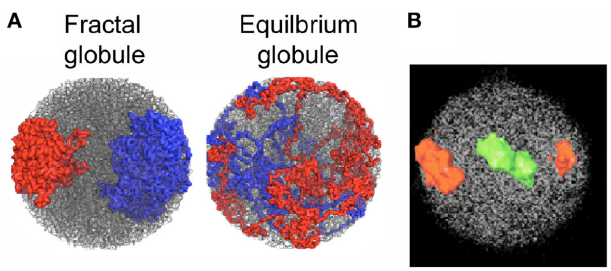

FIGURE 5 | Chromosome territories. (A) Simulation of two polymer chains compacted into a fractal globule shows a territorial organization like that of chromosomes 18 and 19 in a human lymphocyte nucleus **(B)**, whereas they are mixed in an equilibrium globule. **(A)** reproduced from [58], **(B)** reproduced from [22] by permission from Macmillan Publishers Ltd, © 2001.

Signaling pathways depend on diffusion and are therefore influenced by crowding, as illustrated by fascinating recent studies of a step in the Mitogen-activated Protein Kinase (MAPK) pathway which transmits signals from the cell surface to DNA in the nucleus. This has the typical structure of a cascade of kinases in which each kinase phosphorylates the next and activates it; phosphorylation can be reversed by a phosphatase and must occur at two sites for complete activation. In dilute conditions, after phosphorylating its substrate the kinase dissociates leading to a significant probability that a different kinase molecule will phosphorylate the second site, a distributive mode. In contrast, in crowded conditions when diffusion is slower it is more probable that the first kinase molecule will remains bound or close to its substrate while regaining activity by binding ATP and will then phosphorylate the second site, a processive mode (**Figure 6A**) [72]. This prediction has been confirmed experimentally using purified kinases and a crowding agent *in vitro* [73] (**Figure 6B**). Thus responses of pathways of this type appear to be distributive in *in vitro* experiments, but in conditions *in vivo* are actually processive with different downstream responses to signals [74].

FINDING TARGETS IN THE GENOME

Proteins which regulate activities of DNA are believed to find their target in chromatin by facilitated diffusion, a combination of 3-dimensional diffusion in the medium and 1-dimensional sliding on chromatin [75]. Target finding is predicted to be accelerated by crowding [76, 77], and also by DNA looping which facilitates the bypassing of factors which could block sliding [76]. The fractal organization of chromatin [67] also has implications for the kinetics of target finding; chromatin-binding proteins have a long residence time in compact (hetero-) chromatin, suggesting that they bind to all available sites, while on the other hand exploration is faster and less redundant in less compact (eu-) chromatin which offers more exposed DNA, presumably facilitating the detection of less frequent regulatory elements [60].

Target finding is crucial for the survival of a cell when potentially lethal double-strand breaks (DSBs) in DNA must be repaired by rejoining one extremity of the broken DNA correctly

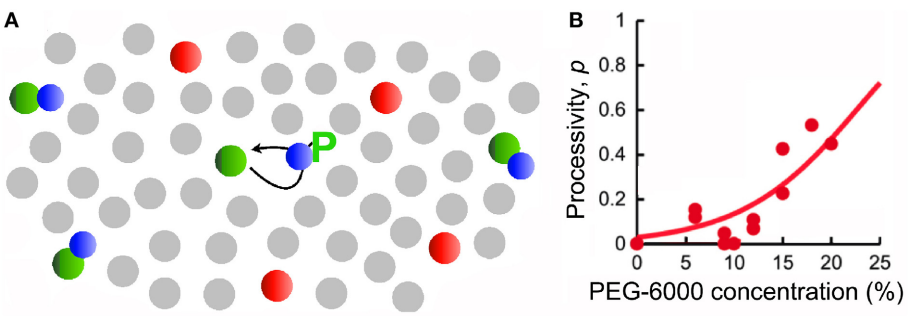

FIGURE 6 | Stimulation by crowding of the processivity of a step in a signaling pathway. (A) In the MAPK pathway MEK (Mitogen/Extracellular signal-regulated Kinase) (green) phosphorylates ERK (Extracellular signal-Regulated Kinase) (blue) at two positions, on tyrosine and threonine. The system also contains phosphatases (red). In the presence of crowders (gray) the reactants diffuse more slowly, and therefore the probability of rebinding and a second phosphorylation of ERK on threonine is increased (reproduced from [72] with permission from Elsevier, © 2014). **(B)** Experimental data using purified kinases *in vitro* showing how the frequency of a second phosphorylation of ERK by the same molecule of MEK (processivity) increases as a function of the concentration of the crowder PEG-6000 (reproduced from [74] by permission from Macmillan Publishers Ltd, © 2013).

to the other extremity. A DSB causes local changes in the mobility of chromatin; modeling predicts an increased mobility due to entropic effects [78], but motion *in vivo* is subdiffusional [79] which reduces the probability of long-range movements. The broken ends adopt more peripheral positions in chromosomes [78], favoring their meeting and rejoining.

FUTURE CHALLENGES AND DIRECTIONS

The new insights discussed here suggest that many features of the nucleus which are apparently complex can be understood by the operation of relatively simple physicochemical principles. Many aspects of the biophysical implications of crowding in the nucleus remain to be explored. Experimentally-accessible questions include:

- the effects of crowding on the structure *in vivo* of chromatin which contains DNA in conformations other than the classical B-form double helix, such as the DNA in telomeres whose conformation *in vitro* is strongly influenced by crowding [80];
- the consequences of crowding for the structures of RNAs and ribonucleoproteins *in vivo*; the folding and stability of RNA *in vitro* are enhanced significantly by crowding [81–83];
- loops in chromatin fibers *in vivo* are usually thought to be stabilized by proteins such as cohesin (for example [84]). Reports that nucleosomes which contain identical DNA sequences can self-associate preferentially [85] raise the possibility that similar interactions could contribute to the formation and stabilization of loops [86]. Could this be one of the still obscure functions of "junk" DNA [87]?

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fphy.2014.00053/abstract

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