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Mini-Review

Genome 3D-architecture: Its plasticity in relation to function

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The genome of higher eukaryotes is non-randomly organized in the interphase nucleus. However, notwithstanding the absence of membrane bound sub-compartments, the nucleus coordinates a number of functions largely by organizing chromatin in a non-random but dynamic manner. The plasticity of chromatin structure and function relies on epigenetic modifications as well as its association with nuclear landmarks such as the nuclear envelope, nuclear lamina, nuclear pore complex and nuclear bodies such as the nucleolus among others. In the absence of membrane-bound compartments, cells and the nucleus, in particular, employ phase-separation, which unmixes phases that constrain biochemical reactions in complex non-membranous sub-compartments such as the nucleolus or even the heterochromatin. This review attempts to provide a glimpse into the microcosm of phase-separated nuclear sub-compartments, that regulate nuclear structure–function relationships.

Keywords. Chromatin; CTCF; euchromatin; heterochromatin; nucleolus; nucleoporins; nucleus; phase separation

1. Introduction

The nucleus is perhaps one of the most exciting and enigmatic of cellular organelles, considering how little we know about it. In addition to nuclear entities collectively referred to as nuclear bodies, the nucleus houses chromatin. What is particularly remarkable is the absence of membrane bound sub-compartments within the nucleus. How does the nucleus achieve separation of function in the absence of membrane bound sub-compartments? The largest non-membranous body in the nucleus is the nucleolus formed around five pairs of human chromosomes around the regions that code for ribosomal DNA (rDNA). The nucleolus is much like a liquid droplet or 'liquid-like' state, which is phase-separated and sub-compartmentalized within the nucleus (Hyman *et al.* 2014; Hult *et al.* 2017). This is facilitated by self-organizing and oligomerizing nucleolar factors such as Nucleolin and Nucleophosmin that bind to ribosomal DNA and RNA. Interestingly, nuclear Lamin B2 also keeps nucleoli discrete and separated from one another, since the depletion of Lamin B2 results in large nucleolar aggregates in the nucleus (Sen Gupta and Sengupta 2017). Investigating the molecular mechanisms of how the nucleolus phase separates and regulates rDNA chromatin organization and function will be crucial in order to further our understanding of protein synthesis.

2. Chromosome territories

In eukaryotic cells, chromosomes typically assume a unique sub-volume in the interphase nucleus, referred to as chromosome territory – a term coined by Theodor Boveri more than a century ago! Interestingly, the conformation that each chromosome territory assumes in the nucleus correlates with transcriptional activity. The topology of chromosome territories have been elucidated to an unprecedented resolution by calculating chromatin contact frequencies as revealed by chromosome conformation capture (3C) and Hi-C analyses (Belaghzal *et al.* 2017). Furthermore, high-resolution imaging of fluorescently labelled chromosome territories have unraveled the architecture of chromosome territories, which reiterate that the conformation of chromosomes territories correlates with function. For instance, the gene-rich human chromosome 19 territory, assumes a more open conformation than the gene-poor chromosomes (Guelen *et al.* 2008). This correlates with the significantly higher transcriptional activity of chromosome 19 than the gene-poor chromosome 18.

3. Lamina-associated domains and heterochromatin

Gene expression is also modulated by the relative proximity of chromatin to the nuclear lamina. The gene-poor human chromosomes are closer to the nuclear periphery

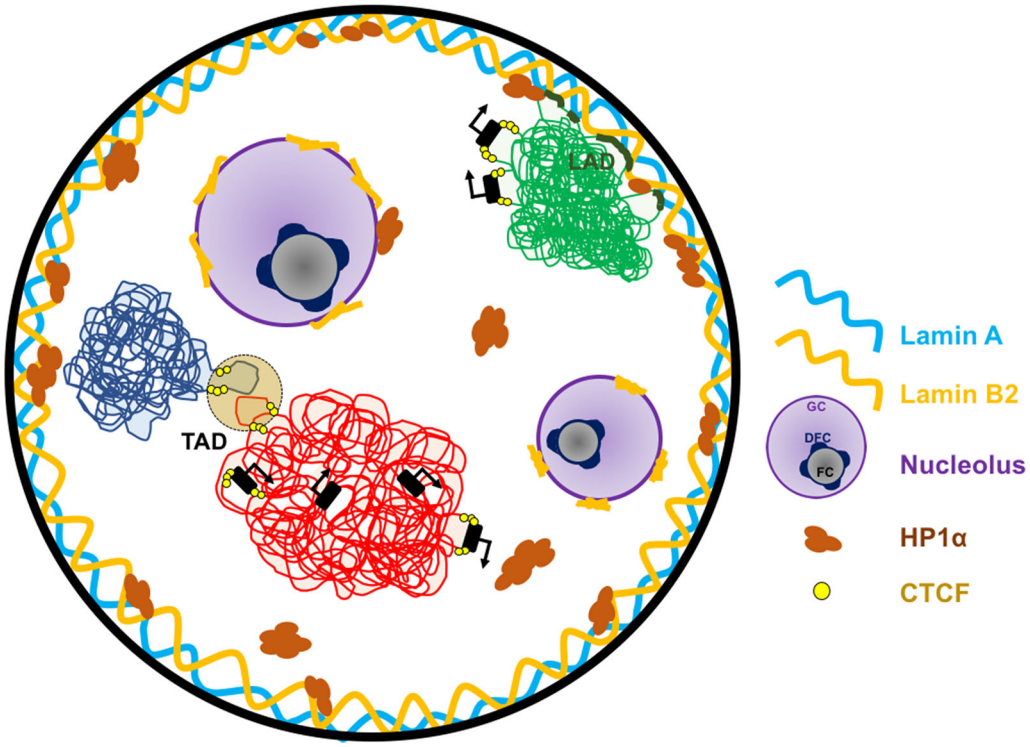

Figure 1. Nucleus is a highly crowded milieu. For better illustration of the same, only select few chromosome territories and nuclear bodies are depicted. The nucleolus is the largest phase separated sub-compartment within the nucleus, essential for the synthesis of ribosomal RNA (rRNA). Chromosome territories (depicted in red, green and blue) occupy distinct sub-volumes in the interphase nucleus. Neighbouring chromatin interacts within Topologically Associated Domains (TADs). The gene-rich chromosome territories (red) near the nuclear interior are relatively more open and active, while the gene-poor chromosome territories (green) near the nuclear periphery contact the nuclear lamina in lamina-associated domains (LADs), are relatively compact and repressed. The nuclear periphery is highly enriched with the lamin meshwork composed of A and B type lamins. Furthermore, HP1α (heterochromatin protein 1α) associates with heterochromatin towards the nuclear periphery and sequesters chromatin as a phase separated complex. CTCF is a chromatin organizer which is localized at the boundary of chromatin domains that controls the extent of heterochromatin spreading, thereby modulating gene expression.

and are enriched in lamina-associated domains (LADs), while gene-rich chromosomes closer to the nuclear interior have reduced LAD association (Guelen *et al.* 2008). Interestingly, LAD association correlates with gene repression. Chromatin in close proximity associates into topologically associated domains (TADs). Interestingly, TAD association can be tighter or weaker and is reversible, which further contributes to the remarkable plasticity of chromatin in different nuclear microenvironments. TAD organization is inherently flexible, and maintains an ∼70% conservation in stem and differentiated cells, while the non-conserved subsets further contribute to chromatin plasticity (Rowley *et al.* 2017) (figure 1).

In essence, chromatin is maintained in a uniquely balanced state of compaction, albeit in a cell-type-specific manner. Chromatin architecture is remarkably well regulated such that chromatin can compact to a significant extent that represses gene expression as in the highly condensed chromosomes at metaphase. Euchromatin in gene-rich chromosomes at the nuclear interior is relatively more open to allow transcriptional activation and gene expression. In addition to chromatin conformation emerging as an important regulator of chromatin activity and function, heterochromatin assumes a unique organization in the nucleus, wherein heterochromatin is bound by Heterochromatin Protein (HP1α). Recent studies reveal that phosphorylated HP1α forms liquid droplets, sequestering chromatin and excluding other factors from such a phase separated complex thereby maintaining chromatin in a repressed state preferentially at the nuclear periphery (Larson *et al.* 2017) (figure 1).

4. CTCF – A chromatin organizer

Just as heterochromatin achieves phase separation, actively transcribing genes phase separate into a unique milieu conducive for transcription with enhancers, super-enhancers, transcription factors and RNA *Pol*II. Interestingly, both

experimental and simulation studies, agree on the phase separation of actively transcribing gene loci. The well-known chromatin organizer – CTCF (CCCTCF binding factor) – may well participate in phase separation, the mechanisms of which remain unclear. CTCF is localized at boundaries of chromatin domains and modulates gene expression by regulating the extent of heterochromatin spreading (Ong and Corces 2014). The HOXA gene cluster is a remarkable example of a chromatin sub-domain organized into loops held by CTCF. In the context of transcriptionally active genes such as oncogenes, a tempting proposition is the potential sequestration of oncogenes, into phase separated clusters of higher expression. Such phase separated oncogenes, could potentially serve as therapeutic targets for the attenuation of expression levels of oncogenes in the foreseeable future (figure 1).

5. DNA damage repair in the nucleus

Chromatin plasticity is a prerequisite for cells as they continually encounter and rapidly respond to various external stressors such as DNA damage and heat shock among others. Chromatin at the nuclear periphery resides in a strikingly different microenvironment as compared to the nuclear interior. Recent studies suggest the recruitment of repair machinery to damaged DNA, differentially at the nuclear interior and the periphery. It is likely that these repair proteins function in conjunction with the nuclear envelope and linker to nucleoskeleton and cytoskeleton (LINC) factors localized at the interface of the cytoskeleton and the nucleus (Kalousi and Soutoglou 2016).

6. Non-coding RNA modulates chromatin organization

It is perhaps not surprising that the role of non-coding RNA features prominently in modulating genome plasticity. The gene locus Bcl11b switches from a repressive to a more euchromatic or permissive environment in developing thymocytes. A novel non-coding RNA – ThymoD untethers Bcl11b gene locus from the nuclear lamina to the nuclear interior. Interestingly, this mechanism closely cross-talks with genome organizers such as CTCF and Cohesins that further regulate the organization of chromatin loops. These findings also implicate transcription factors such as GATA, and TCF in facilitating enhancer promoter associations in a phase separated complex (Isoda *et al.* 2017).

In summary, we are in an exciting phase of our understanding of the structural implications of chromatin on its functional manifestations. However, how chromatin seamlessly transits across multiple states of compaction, decompaction and through phase transitions albeit in diverse cell types, will prove to be crucial for us to address and unravel fundamental molecular underpinnings underlying chromatin architecture and function in development and disease.

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