

# Chapter 1

## Spatial Organization of the Nucleus Compartmentalizes and Regulates the Genome

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**Abstract** The nucleus must simultaneously orchestrate DNA replication, transcription, splicing, signalling, and directional transport of proteins into the nucleus and RNA out of the nucleus. Yet the nucleus has no internal membranes to compartmentalize these functions as the cytoplasm does. In fact, such compartmentalization would necessarily be detrimental because particular genes at different locations on the linear chromosomes need to be made at different times while others on the same chromosome need to be tightly shut off. Moreover, expressed genes need to be accessible to a feedback mechanism to determine when to modulate transcription. To accommodate these additional needs the nucleus appears to form microdomains by co-assembly of functional complexes. Thus, microdomains can either form around activated regions on a chromosome or regions on a linear chromosome could be fed into such microdomains for activation. Findings that genome encoded regulatory elements such as enhancers can be hundreds of kb and even Mb apart further highlights the need for such a system as these distal elements must come together in the 3D space of the genome for their efficient functioning. While this much is understood, there is much still to be learned about mechanisms that the nucleus uses to regulate the genome and much more to be learned about how these microdomains come into being. As there is no stable structure within the nucleus except for the nuclear envelope, much recent research has been focusing on potential roles of this subnuclear organelle in establishing 3D nuclear architecture and orchestrating the regulation of these various functions.

**Keywords** Nucleus · nuclear envelope · NET · gene position · genome organization.

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## 1.1 Introduction

As the largest organelle within the cell the nucleus was visualized in the earliest days of microscopy over 300 years ago by Dutch microscopist Antonie van Leeuwenhoek, although it was another 100 years before Scottish botanist Robert Brown named it. Both were able to visualize the nucleus with their crude microscopes due to studying organisms with large nuclei such as salmon and plants. As microscopes evolved to reach  $\sim 1 \mu\text{m}$  resolution by the mid-1800s it became apparent that there were substructures inside the nucleus, the most predominant of which are the nucleoli that were first described in the 1830s (Lo et al. 2006). A body of work by several scientists evolving the theory of heredity and connecting this to the distinct worm-like chromosomes observed in mitosis resulted in German zoologist Theodor Boveri's postulating the existence of chromosome territories around the next turn of the century (Boveri 1909). Other indications of nuclear organization came in the same era from Austrian anatomist Carl Rabl's observations that the centromeres of salamander chromosomes were located at the nuclear periphery (Rabl 1885) and Spanish cytologist Santiago Ramon y Cajal's identification of nucleolar accessory bodies subsequently called Cajal bodies (Cajal 1903).

With the advent of electron microscopy (EM) many additional nuclear substructures were observed based on characteristic electron densities. One of the most obvious was the separation of most of the denser staining chromatin, defined at the time as heterochromatin, to the nuclear periphery, particularly in resting lymphoblasts (Mirsky and Allfrey 1960; Hirschhorn et al. 1971). Additionally, Promyelocytic leukaemia (PML) nuclear bodies were identified as electron dense spheres of  $0.1\text{--}1.0 \mu\text{m}$  diameter (de et al. 1960). EM also enabled visualization of processes such as the massive ribosomal RNA transcriptional "trees" of amphibian oocyte satellite DNA associated with the nucleolus (Miller and Beatty 1969). The combination of  $^3\text{H}$ -5-uridine labelling with EM yielded more information about where in the nucleus functions occur, associating more rapidly labelled regions with the perimeter of inter-chromatin granules (Fakan and Bernhard 1971). Finally, the recognition that the nuclear envelope (NE) is a double membrane structure and of the nuclear pore complexes (NPCs) embedded within it first came using EM in large amphibian oocyte nuclei (Callan and Tomlin 1950) and shortly after in mammalian nuclei (Watson 1954). Subsequent work demonstrated the unique eight-fold symmetry of the NPCs (Gall 1967).

At the same time as these early ultrastructural observations there were a number of seminal papers indicating many levels of organization of the genome. For example Huberman and Riggs demonstrated the existence of specific mammalian origins of replication (Huberman and Riggs 1968). Also critical in this period was the development by the team of Joe Gall and Mary Lou Pardue of methods for *in situ* hybridization that have formed the basis for determining the position of specific genes and transcriptional activity in the nucleus (Gall and Pardue 1969; Pardue and Gall 1969). This was quickly used to determine the chromosomal localization of mouse satellite DNA (Pardue and Gall 1970) and subsequently developed to paint whole chromosomes (Lichter et al. 1988). These tools have been used to

compare the positioning of different nuclear structures with respect to one another and also with respect to their positioning within the 3D nucleus as a whole. The much more recent development of genome-wide sequencing technologies quantifying DNA-DNA proximity, such as chromosome conformation capture (Dekker et al. 2002; Lieberman-Aiden et al. 2009) and Genome Architecture Mapping (GAM) (Beagrie et al. 2017), or DNA-protein proximity, such as DNA adenine methyltransferase identification (DamID) (Guelen et al. 2008; Pickersgill et al. 2006), has significantly expanded these visual observations concerning the spatial positioning and/or the three-dimensional organization of the genome. For example, chromosome conformation capture and GAM methods, have revealed that chromosomes fold along their length into delimited structures termed topologically-associated domains (TADs) which subsequently assemble into higher order compartments (Dixon et al. 2012; Nora et al. 2012). Similarly, DamID has revealed the organization of the fraction of TADs at the NE in lamina-associated domains (LADs) (Gonzalez-Sandoval and Gasser 2016; Vogel et al. 2007). Understanding the functions of the spatially distinct regions of the genome has been greatly aided by the ability to investigate the relationship of these genome domains to different proteins in the nucleus. This in turn was greatly aided by the development of antibodies to proteins in these structures that enabled both their labelling by immunogold EM and their visualization under the light fluorescence microscope.

Most of the first antibodies obtained to these structures were fortuitous, coming from autoimmune sera, and thus providing also the first indications of the importance of these nuclear subdomains to human health and disease. Of particular note, serum from patients with the autoimmune disease systemic lupus erythematosus stained nucleosomes (Rothfield and Stollar 1967). Several other nuclear domains and proteins were subsequently found to also be linked to autoimmune diseases such as autoimmune sera from primary biliary cirrhosis patients identifying the first PML/ND10-associated protein Sp100 (Szosteki et al. 1990). At this time, the number of developmental defects and disease states associated with proteins of nuclear substructures are far too great to detail in a single review, but it is worth noting particularly the links between the NE and disease as this can in many ways be linked specifically to nuclear organization. Mutations in the intermediate filament lamin proteins that line the inner nuclear membrane cause several muscular dystrophies and lipodystrophies along with neuropathy, dermatopathy and other disorders including the premature ageing Hutchinson-Hillier Progeria syndrome. Moreover, mutations in NE transmembrane proteins (NETs) that interact with lamins often cause variants of the same diseases (Bonne and Quijano-Roy 2013; Worman and Schirmer 2015). The distribution of the dense peripheral heterochromatin was disrupted in cells from patients with both lamin and NET-linked muscular dystrophy (Sewry et al. 2001; Verga et al. 2003; Fidzianska et al. 1998; Maraldi et al. 2002; Ognibene et al. 1999), progeria (Goldman et al. 2004), mandibuloacral dysplasia (Maraldi et al. 2006) and familial partial lipodystrophy, Dunnigan-type (Maraldi et al. 2006). Differences in chromosome territories and their spatial positions within the nucleus were also observed in cells with specific lamin A mutations (Meaburn et al. 2007; Mewborn et al. 2010) and a progeria

mutation further yielded an abnormal distribution of telomeres and clustering of centromeres (Taimen et al. 2009). The specific organization of the genome with respect to regulatory elements and higher order chromosome structure is also important for human disease as disruption of this organization leads to developmental defects and disease (Guo et al. 2015). As Francis Collins has suggested that most disease-causing mutations yet to be identified likely fall in non-coding regions (Manolio et al. 2009), understanding spatial genome organization and its control are fundamentally important.

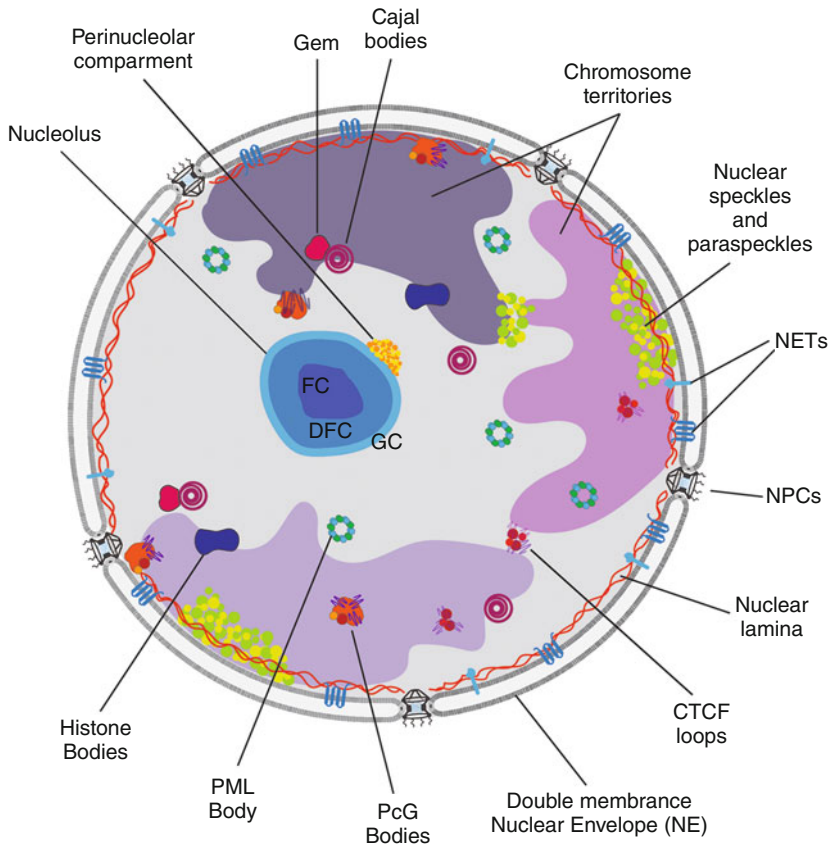
## 1.2 Subdomains of the Nucleus

### 1.2.1 Nuclear Envelope

The NE is a complex system of outer (ONM) and inner (INM) nuclear membranes separated by a ~50 nm lumen in mammalian cells and both connected and perforated at sites of nuclear pore complex (NPC) insertion (Callan and Tomlin 1950). The ONM is continuous with the endoplasmic reticulum (ER) and contains both ER proteins such as ribosomes and also a set of unique NETs, of particular note the KASH domain nesprins that connect the NE to cytoplasmic filaments (Luxton and Starr 2014). The INM harbors its own unique set of NETs (de Las Heras et al. 2013) and the lamins that form a polymer directly under the NE (Gruenbaum and Foisner 2015). Both lamins and most NETs tested bind chromatin proteins (Harr et al. 2016; Kind and van Steensel 2014; Wong et al. 2014). This is particularly noteworthy in context that the lamin polymer confers structural support to the nucleus (Lammerding et al. 2004) and is accordingly the most stable of the nuclear subdomains. With an estimated 9 million copies of lamins per mammalian cell nucleus (Schwanhauser et al. 2013), they are well positioned to serve as the scaffolding of the nucleus. Finally, ONM nesprins interact with INM SUN-domain NETs to connect the lamin polymer to cytoplasmic filaments (Crisp et al. 2006; Padmakumar et al. 2005). This maintains the 50 nm spacing of the lumen and enables force transmission and mechanosignal transduction between the cytoplasm and lamins and associated NETs in the nucleus (Ho et al. 2013; Swift et al. 2013). In addition to its obvious structural function, the NE has been implicated in a wide range of functions that range from the integration of many additional non-mechanical signalling pathways to DNA replication, transcriptional regulation, gene and chromosome positioning, and many others (de Las Heras et al. 2013). It is thus not surprising that the NE is linked to over two dozen diseases (Bonne and Quijano-Roy 2013; Worman and Schirmer 2015) (Fig. 1.1).

### 1.2.2 Nuclear Pore Complexes

The eight-fold symmetrical NPCs are the largest protein complexes in a typical cell, starting at a minimum of >40 MDa in yeast (Rout and Blobel 1993; Yang et al. 1998) and up to 125 MDa in higher vertebrates (Akey and Radermacher 1993; Hinshaw et al. 1992; Reichelt et al. 1990). The diameter of NPCs is ~120 nm



**Fig. 1.1** Overview of nuclear subdomains. Most nuclear subdomains shown occur in the inter-chromatin regions between chromosome territories and are often involved in transcriptional regulation and RNA processing. Some domains such as PcG bodies and CTCF loops are more focused on generating structure within the chromosome. These loop structures can contribute to silencing genes, segregate domains within chromosomes, and promote interactions between domains within or between chromosomes for gene activation. The nucleoli are the largest nuclear subdomain besides the nuclear envelope and is broken up into a fibrillar center (FC), dense fibrillar component (DFC) and granular component (GC)

and not only they span the  $\sim 50$  nm distance between the ONM and INM, but also comprise filaments that extend into the cytoplasm and a more structured nuclear basket extending into the nucleoplasm that make their total length greater than their diameter. Together with an army of transport receptors and other associated proteins, they direct the regulated transport of proteins and RNA in and out of the nucleus (Dickmanns et al. 2015). Transport of soluble macromolecules appears to occur through an  $\sim 39$  nm central channel that is filled with phenylalanine-glycine repeat motifs in unstructured regions of many of the core protein components termed nucleoporins (Rexach and Blobel 1995). However, there are also peripheral channels of  $\sim 10$  nm that have been shown to be involved in transport of

NETs from the ER to the INM (Ohba et al. 2004; Soullam and Worman 1995; Ungricht et al. 2015; Zuleger et al. 2011a). There are roughly 2,000–3,000 NPCs in the nuclei of cycling mammalian cells (Gerace and Burke 1988; Maul and Deaven 1977). Super-resolution microscopy approaches reveal a clear segregation of NPCs from other NE components (Schermelleh et al. 2008; Xie et al. 2016). Nonetheless, NPCs appear to have roles separate from their transport function in genome regulation (Harr et al. 2016; Heessen and Fornerod 2007). Similar to the rest of the NE, an ever-increasing number of NPC proteins are being linked to functions in development and human diseases, particularly cancer (Cronshaw and Matunis 2003; Lupu et al. 2008; Simon and Rout 2014).

### 1.2.3 Chromosomes

Although it is obvious from their condensation in mitosis that chromosomes are discrete gigadalton entities, the idea that they maintain discrete territories in interphase was only theorized for nearly 100 years after Boveri initially postulated this (Boveri 1909). The first proofs of interphase chromosome territories came from a combination of technological advances and creative experimental approaches driven by the brothers Thomas and Christoph Cremer. They clearly showed that interphase chromosomes organize into discrete chromosome territories (Cremer et al. 1982; Schardin et al. 1985). Certain tendencies were subsequently observed such as that active genes tend to be located at the boundary of a chromosome territory that is facing the inside of the nucleus while genes at the boundary against the NE tend to be repressed (Kurz et al. 1996). It was also observed that some small chromosome regions could loop out so that a small portion of a more internal chromosome could touch the periphery (Zink et al. 2004) and that, once established, the positions of chromosomes in interphase tend to be relatively stable (Strickfaden et al. 2010). This led to a whole new field on the relationship between gene position and expression (see below).

### 1.2.4 Centromeres

As the largest individual molecules in the cell, chromosomes are themselves effectively segregated into multiple subdomains. Centromeres occur at the primary constriction of mitotic chromosomes and exhibit specialized chromatin and associated proteins at the kinetochore. This includes the assembly into centromeric nucleosomes of the histone H3 homolog CenpA and assembly of dozens of other centromere proteins into a scaffolding and support structure for the binding of mitotic microtubules (Moreno-Moreno et al. 2017). Some of this structure is dynamically assembled when the chromosomes condense at mitosis while most is maintained in both mitosis and throughout interphase. Centromeres range in size from several Mbp out of a total of ~50–250 Mbp on a typical mammalian chromosome to 125 bp in the much smaller chromosomes of the yeast *Saccharomyces cerevisiae*.

### **1.2.5 Telomeres**

The ends of the linear chromosome are capped by telomeres. These range typically from several kbp in humans to 300 bp in *S. cerevisiae*. They are known to be functionally important for maintaining the integrity of chromosomes, but they shorten as cells divide so that loss of gene contents can more easily occur from the chromosome ends in “older” cells (Wu et al. 2017).

### **1.2.6 Nucleolus**

The nucleoli were the first noted nuclear substructure because they can be observed by light microscopy as visible circular structures within the nucleus by their darker appearance and large size (from 0.5 to 8  $\mu\text{m}$  in diameter). This darker appearance correlates with their dense staining in EM. There can be multiple nucleoli in a nucleus and they form around tandem repeats of ribosomal DNA (rDNA). They function primarily in synthesis of rRNA and in ribosome biogenesis, first clarified with the identification of a *Xenopus laevis* mutant lacking nucleoli that also lacked rRNA synthesis (Brown and Gurdon 1964). Recent studies suggest that nucleoli have additional roles in RNA transport, RNA modification, and cell cycle regulation (Stepinski 2016).

### **1.2.7 Perinucleolar Compartment**

The perinucleolar compartment (PNC) is a dynamic compartment that forms in cancer cells adjacent to nucleoli and much smaller at 0.2–1  $\mu\text{m}$  in diameter. It was first identified from observations that the pyrimidine tract-binding protein (PTB) accumulated adjacent to nucleoli (Ghetti et al. 1992). It was subsequently named the PNC after the discovery that it also contains several polymerase III RNAs (Matera et al. 1995). However, its composition appears to be highly dynamic with both RNAs and proteins rapidly exchanging between the compartment and the surrounding nuclear area and its function has not been fully elucidated (Pollock et al. 2011).

### **1.2.8 Cajal Bodies**

Cajal bodies tend to be in the range of 0.3–1.0  $\mu\text{m}$  in diameter, but can be much smaller or get as big as 2  $\mu\text{m}$  (Cioce and Lamond 2005). They were first identified by Santiago Ramón y Cajal in 1903 and called nucleolar accessory bodies. Their characterization much later by EM led to the new name of Coiled bodies because the higher resolution yielded the appearance of a coiled string and subsequently they came to be referred to as Cajal bodies in honor of their discoverer. However, the

core protein constituent was named p80/coilin based on the Coiled body name. A typical nucleus contains 1–10 Cajal bodies with the larger numbers found in metabolically active cells, but they are most prevalent in highly proliferative cells such as embryos and tumors. Cajal bodies are principally involved in assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs), but have many different roles relating to RNA processing including also small nucleolar RNA (snoRNA) maturation, histone mRNA modification and telomere maintenance (Cioce and Lamond 2005).

### ***1.2.9 Gemini of Coiled Bodies***

Gemini of Cajal bodies, also called gems for short, are “twins” to Cajal bodies with similar size and shape discovered by Liu and Dreyfuss in 1996 (Liu and Dreyfuss 1996). However, unlike Cajal bodies, gems do not contain snRNPs. They are readily distinguished by immunofluorescence microscopy because Cajal bodies are positive for both coilin and the survival of motor neuron (SMN) protein, while gems are SMN positive and coilin negative (Navascues et al. 2004). Gems are believed to be involved in pre-mRNA splicing and assist Cajal body function.

### ***1.2.10 PML/ND10 Bodies***

Promyelocytic leukaemia bodies (PML bodies) are known by many names including nuclear domain 10 (ND10), Kremer bodies, and PML oncogenic domains (Bernardi and Pandolfi 2007). They range in size from 0.1–1.0  $\mu\text{m}$  in diameter and a typical nucleus has between 5 and 30. Their primary name comes from their primary component, the PML protein, though there are also non-PML body stores of PML protein (De Vos et al. 2011). There are many functions ascribed to PML bodies, but these all seem to reflect a core unifying function contributing to cellular responses to stress. In keeping with this the PML protein appears to be dispensable under normal cellular conditions as PML knockout mice develop normally in the absence of major stress conditions. In addition to PML there are a few other core components of PML bodies including Daxx (Ishov et al. 1999), Sumo (Muller et al. 1998) and Sp100 (Szostecki et al. 1990). Interestingly, PML bodies accumulate at centromeres through an interaction with CenpC during viral infection (Everett et al. 1999) and can also associate with telomeres (Chung et al. 2011) and may have functions with other nuclear subdomains.

### ***1.2.11 Speckles***

Speckles contain pre-messenger RNA splicing factors and are located in interchromatin regions. Their association and function with splicing has resulted in their also being called splicing speckles, nuclear speckles, splicing factor compartments,



interchromatin granule clusters (IGCs), and B snurposomes. They were first visualized by EM as clusters of interchromatin granules and by fluorescence microscopy appear highly irregular, typically varying in size from 0.8–1.8  $\mu\text{m}$  and so being one of the largest structures in the nucleus after the chromosomes themselves and the nucleoli (Lamond and Spector 2003). They are highly dynamic and their composition and location changes in response to modifications in mRNA transcription (Handwerger and Gall 2006). These structures are also reported to function as storage sites for splicing factors (Matera et al. 2007).

### **1.2.12 Paraspeckles**

The interchromatin space contains irregularly shaped structures that tend to be in close proximity to splicing speckles named paraspeckles (Fox et al. 2002). These are generally observed in human tissues and studies in HeLa cells show that they are highly dynamic. Paraspeckles existence depends on RNA Pol II transcription and these structures change rapidly in response to changes in cellular metabolism (Fox et al. 2005). Typically 10–30 paraspeckles can be observed in a HeLa cell nucleus. Several roles have been suggested for paraspeckles, including regulation of apoptosis and mitosis (Gao et al. 2016) and RNA sequestration in the nucleus (Hu et al. 2015) and DNA damage response (Gao et al. 2014). However, their protein composition would suggest RNA editing and regulation as their core function. Paraspeckles differ from most nuclear subdomains in having a more regular size of  $\sim 0.5 \mu\text{m}$ .

### **1.2.13 Histone Bodies**

Also called histone locus bodies, histone gene synthesis occurs in these bodies formed around the histone gene cluster. Histone transcription is unusual in that histone genes produce the only known cellular mRNAs that are not polyadenylated and instead form a conserved stem loop (Duronio and Marzluff 2017). Histone bodies contain scaffolding protein NPAT, FLASH and U7 SnRNP and are 0.2–1.2  $\mu\text{m}$  in diameter (Sleeman and Trinkle-Mulcahy 2014). NPAT is a substrate for Cyclin E/Cdk2 phosphorylation to activate histone synthesis at the G1/S transition and interacts with the small heat shock protein Cpn10/HSPE for its function. Loss of Cpn10 specifically disrupts these bodies and histone transcription without affecting neighboring nuclear bodies (Ling Zheng et al. 2015).

### **1.2.14 Polycomb Bodies**

Polycomb proteins assemble visible structures involved in mediating gene pairing and silencing called Polycomb group (PcG) bodies. These structures are 0.3–1  $\mu\text{m}$  in diameter and are defined by the presence of Bmi1 and Pc2 proteins. Loci

targeted to PcG bodies become compacted, presumably because of the functions of polycomb repressive complex methylation of histone H3K27 (Margueron and Reinberg 2011). However, PcG bodies also appear to contribute to long-range chromosomal interactions through maintaining multi-looped chromatin structures (Tolhuis et al. 2011; Bantignies et al. 2011). Unlike other nuclear subdomains, however, PcG bodies appear to be visible more due to the local concentration of chromatin fibers rather than protein and RNA assemblies (Smigova et al. 2011).

### 1.3 Composition of Nuclear Structures

As noted above, the first identifications of protein components of nuclear subdomains came from autoimmune sera that were used to identify the proteins and to reveal their association with a particular structure by immunoelectron microscopy. The standard approach was then to use various means ranging from 2-hybrid studies to co-immunoprecipitations to identify other components. Sometimes this was revealing for the function of these bodies. For example, in Cajal bodies, Coilin and its interaction partners were found to associate with pre-mRNA splicing, pre-ribosomal RNA processing, and histone pre-mRNA 3' maturation, increasing our understanding of the function of these bodies in assembly of spliceosomal snRNPs and small nucleolar ribonucleoproteins (snoRNPs) (Gall 2000; Matera 1999).

In other cases identification of subdomain components led to even more confusion. PML/ND10 bodies are an example of this situation. Once the first core components including PML protein, Sp100, and Daxx had been discovered, there were over 100 more to follow such as TDG, PIASy, HIPK2, LEF1, p53, Akt, ChK2, p53, multiple poly-glutamine proteins and many viral proteins. These proteins suggested functions ranging from replication and transcriptional regulation, to cell signaling, cell cycle, anti-viral responses and apoptosis (Lallemand-Breitenbach and de The 2010). The finding that sumoylation regulates the intranuclear partitioning of PML (Muller et al. 1998) and other cofactors such as Daxx (Ishov et al. 1999) shortly led to the hypothesis that PML/ND10 bodies functioned as a nuclear trash depot for proteins that needed to be degraded (Negorev and Maul 2001). This view dominated the field for several years until many studies revealed that there were distinct PML/ND10 bodies with different composition beyond their core components that have individual functions (Lallemand-Breitenbach and de The 2010). These functions include, stress responses to upregulation of interferons, heavy metals, proteasome inhibition, and DNA damage among others.

#### 1.3.1 *Mass Spectrometry Opens a Whole New Dimension in Defining Nuclear Subdomains*

Though many nucleolar proteins had been identified over the years, it was not until 35 years later that a comprehensive proteomic analysis of the nucleolus was

undertaken. Careful isolation of nucleoli from HeLa cells and analysis by mass spectrometry revealed 271 proteins make this complex structure (Andersen et al. 2002). Roughly 30% of these proteins were previously uncharacterized, raising the possibility that there might be additional functions for nucleoli. A subsequent study increased the number of nucleolar proteins to 489 (Andersen et al. 2005). Treatment of cells with metabolic inhibitors caused changes in the relative amounts of many of the identified nucleolar proteins accompanied by changes in nucleolar morphology (Andersen et al. 2005). When nucleosome-association was tested for identified proteins, the Lamond group found that one uncharacterized protein targeted to a new nuclear subdomain that they called paraspeckles, thus naming this protein paraspeckle protein 1 (PSP1) (Fox et al. 2002). They later identified additional components, such as PSP2 and p54/nrb, that also localized to paraspeckles and found an average of 10 to 20 of these subdomains in a typical HeLa cell nucleus.

The NPC proteome turned out to be a little less exciting, but this is in large part because the structure of the NPC made it easier to study and accordingly more of its constituent proteins called nucleoporins were identified prior to the application of proteomics. The first nucleoporin discovered was the transmembrane gp210 (originally called gp190) that was isolated from a crude NE/NPC fraction and used to generate antibodies that labeled NPCs by immunogold EM (Gerace et al. 1982). Many other nucleoporins were soon identified largely because of their abundance and easy fractionation from *Xenopus* oocytes. Once the first nucleoporins were identified, the discovery that they tend to form subcomplexes rapidly facilitated further identifications by co-immunoprecipitation e.g. Nup62 was identified due to its abundance and strong antigenicity (Starr et al. 1990) and Nup54 and Nup58 were found by their interaction with Nup62 (Hu et al. 1996; Kita et al. 1993). In the two decades between the first identification of gp210 and the first proteomic study in yeast, 26 nucleoporins had already been identified. The yeast proteomics made 174 protein identifications of which many were transport factors, chaperones and obvious contaminants, but it increased the number of nucleoporins to 30 (Rout et al. 2000). Shortly thereafter, the Matunis laboratory determined the composition of the mammalian NPC that revealed the remarkable conservation of this nuclear structure (Cronshaw et al. 2002). The results of both studies were a surprise to many as the general expectation had been that, there would be 50–100 nucleoporins, based on the mass estimated by cryo-EM (Akey and Radermacher 1993; Hinshaw et al. 1992; Reichelt et al. 1990). However, relative protein abundance estimated by spectral counts suggested that while some nucleoporins may be only represented in eight copies due to the eight-fold symmetry of the NPC (Gall 1967), others have 16 or 32 copies (Cronshaw et al. 2002; Rout et al. 2000). The number of copies estimated for nucleoporins does not account for the total predicted mass: 44 MDa was accounted for yeast out of 55–72 MDa expected (Rout and Blobel 1993; Yang et al. 1998) and ~60 MDa accounted in mammals out of 125 MDa expected (Akey and Radermacher 1993; Reichelt et al. 1990). A subsequent study of the whole NE proteome (see below) additionally found a third transmembrane nucleoporin, NET3/Ndc1, most likely because of special approaches applied for the identification of membrane proteins (Schirmer et al. 2003; Mansfeld et al. 2006). It

is likely that the rest of the mass is provided by a combination of transport factors and proteins in transit *i.e.* the NPC reflects a general characteristic of nuclear subdomains in that they are largely dynamic in structure and composition and highlights the importance of establishing the core for each nuclear body/subdomain.

In contrast to the NPC, the NE proteome was greatly expanded by proteomics. A study using the multi-dimensional protein identification technology approach (MudPIT; (Washburn et al. 2001, 2003; Wolters et al. 2001)), that avoids gel separation steps used in an earlier study (Dreger et al. 2001), increased the number of proteins associated with this nuclear subdomain by roughly 10-fold (Schirmer et al. 2003). Subsequent testing of individual proteins identified for NE targeting confirmed the vast majority, but also revealed that many also have other subcellular localizations (Malik et al. 2010). This is somewhat consistent with the dynamic behavior of PML/ND10 bodies, nucleoli and the NPC.

## 1.4 Self-Assembly of Nuclear Structures

The entire nucleus disassembles and reassembles in each mitosis of higher organisms (Schellhaus et al. 2016). While this dynamic behavior could be viewed as an obstacle to maintaining spatial genome organization, it is also an opportunity for a cell to change its genome organization by starting from scratch. In post-mitotic cells larger genome organization changes such as the inversion of heterochromatin that occurs in retina cells takes weeks (Solovei et al. 2013), but with the disassembly and rebuilding of the nucleus that occurs in mitosis large-scale global repositioning of whole chromosomes can take place in under an hour. For some whole chromosomes, such changes can occur by an interaction with the NE (Finlan et al. 2008; Reddy et al. 2008). But, many nuclear subdomains are placed in between chromosome territories and far away from the NE and need an independent mechanism for self-assembly.

With its eight-fold symmetry and composition of over 30 different nucleoporins in copies from 8 to 32, the NPC is probably the most complex individual structure in biology. The identification of nucleoporin interaction partners revealed that all nucleoporins associate into specific subcomplexes (Alber et al. 2007). These subcomplexes self-assemble and are stable enough to obtain both EM and crystal structures. The subcomplexes can in turn self-assemble to form the whole NPC in a stepwise LEGO-like fashion (Floch et al. 2014). Interestingly, many nucleoporins have been shown to have separate roles in mitosis. For example, Nup107 associates with the spindle assembly checkpoint protein MAD1 and recruits also one of its subcomplex members, Nup133 to the kinetochore in mitosis (Rodenas et al. 2012). It would be interesting if these separate mitotic roles evolved as effective “place holders” to partly maintain subcomplexes in an inactive state until breaking these interactions would allow reassembly of the NPCs.

Many other individual nuclear subdomains assemble due to specific affinity interactions between components. For example, with PNCs the three core

components mitochondrial RNA-processing (MRP), PTB and CUG-binding protein (CUGBP) have all been shown to interact by pulldown assays (Pollock et al. 2011). In the case of paraspeckles, multiple components share the characteristic of having complexity prion-like domains that promote their association (Hennig et al. 2015). In this respect they could be argued to be more prone to aggregate than to assemble. Nonetheless, this particular method of interaction might have the advantage of segregating paraspeckle assembly from adjacent splicing speckle assembly.

In other cases, assembly may combine specific affinity interactions with a driver process, such as transcription. For example Cajal bodies require active transcription for the U1, U2, U4/U6 and U5 snRNPs to assemble and colocalize in the structures (Carmo-Fonseca et al. 1992). The dynamics of these bodies and association with chromatin also requires ATP (Platani et al. 2002). Likewise, the structural cohesion of the nucleolus depends on its activity and, accordingly, inactivation of rDNA results in a loss in the cohesion of nucleolar structures (Hernandez-Verdun 2006). A similar concept may apply for the different types of PML/ND10 bodies. Some PML/ND10 bodies appear to be associated with transcriptional foci. An elegant live-cell imaging study showed a modified gene locus and a PML/ND10 body moving until they co-localized. The gene locus then changed from a condensed to an open structure and transcription was observed to initiate (Tsukamoto et al. 2000). PML/ND10 bodies have been found to also associate with telomeres, where the PML and Sp100 proteins appear to similarly form a ring wrapping around the telomere. Details on how these structures are nucleated and whether their assembly differs significantly from standard PML/ND10 body nucleation remain unclear. It is thought that free PML and Sp100 might bind particular proteins on the telomeres such as TRF1/2, the DNA repair protein NBS1, or the SUMO ligase MMS21 to initiate assembly of these structures (Chung et al. 2011).

## 1.5 Genome Organization Patterns

Mirroring the compartmentalization of the nuclear structures described above, the genome is non-randomly organized within the three-dimensional space of interphase nuclei (Bickmore 2013; Bickmore and van Steensel 2013; Lanctot et al. 2007; Zuleger et al. 2011b). This organization exists at multiple scales, ranging from whole chromosomes to individual genes, and is defined by the relative spatial proximity of specific DNA sequences to distinct nuclear structures and/or to each other.

### 1.5.1 *Loops and Topologically-Associated Domains*

At a locus level, the transcriptional output of specific genes can be controlled by regulatory elements hundreds of kilobases or even megabases distal along linear chromosomes through physically looping through nuclear space (Dekker and

Mirny 2016). Such looping provides additional regulatory information to gene promoters in a manner necessary to achieve the diversity of transcriptional outputs required in complex multicellular organisms (Rubinstein and de Souza 2013). Much of this looping occurs locally and is the basis of forming so-called TADs. Chromosomes are divided along their length into TADs defined by preferential local interactions that were determined using the chromosome conformation capture (C) and GAM technologies (Beagrie et al. 2017; Dixon et al. 2012; Sexton et al. 2009). Like LADs, the organization of the genome into TADs appears to be conserved from flies to humans, and perhaps also budding yeast, (Dixon et al. 2012, 2015; Eser et al. 2017), where they function to limit the interactions of enhancers to only their target genes and to create independent regulatory domains. Indeed, genes within TADs display coordinated expression changes between different cell types and cell states (Flavahan et al. 2016; Nora et al. 2012; Shen et al. 2012). Consequently, disruption of TAD boundaries induces ectopic interactions between enhancers and inappropriate genes, resulting in gene misexpression and disease (Hnisz et al. 2016; Flavahan et al. 2016; Ibn-Salem et al. 2014). For example, a duplication, inversion or deletion encompassing the boundaries of the *Epha4* TAD results in a limb bud-specific enhancer inappropriately associating with and activating the *Pax3*, *Ihh* or *Wnt6* loci that are normally located in neighboring TADs. This misregulation ultimately results in limb malformations (Lupianez et al. 2015). As a result of these critical functions it is perhaps not surprising that breaks in synteny between species are frequently found proximal to TAD boundaries in a manner that conserves TADs during evolution (Vietri Rudan et al. 2015). This, combined with the observation that the majority of TADs are conserved between different cell types (Dixon et al. 2012; Fraser et al. 2015), suggests that TADs represent a fundamental unit of genome organization. Supporting this, significant overlap is observed between TADs and other organizational units of the genome, including isochores (Jabbari and Bernardi 2017), LADs (Dixon et al. 2012; Fraser et al. 2015), and replication timing domains (Pope et al. 2014). It is also important to note that these are likely not fixed structures because, although their external boundaries appear largely invariant, their internal configurations can undergo significant remodelling during development (Andrey et al. 2017; Javierre et al. 2016).

### 1.5.2 Compartments

TADs that share similar functional states can also be adjacent in the higher order compartmentalization of the genome, even when separated by megabases along the same linear chromosome or when occurring on different chromosomes (Lieberman-Aiden et al. 2009). Initially, these preferential associations between TADs identified in Hi-C contact maps were delimited into two regimes, referred to as A and B, that correlate with active and repressed chromatin, respectively (Lieberman-Aiden et al. 2009). However, analysis on a higher resolution Hi-C contact map further

segregated these compartments into the A1, A2, B1, B2, B3 and B4 sub-compartments, each of which shares a unique set of properties and spatial localizations (Rao et al. 2014). For example, while all the B sub-compartments represent heterochromatic states displaying diminished gene expression, the B1 compartment is specifically enriched for H3K27me3 polycomb domains, the B2 compartment for LADs and nucleolar-associated domains, and the B3 compartment specifically for only LADs. Similarly, while enriched for active chromatin modifications and transcribing genes, the A sub-compartments can be differentiated with respect to their distance to lamina-associated regions with A2 regions being significantly closer to LADs along linear chromosomes than A1 regions (Robson et al. 2017). Intriguingly, the presumed spatial segregation of such compartments has also been confirmed independently using super-resolution FISH microscopy and single-cell Hi-C combined with 3D-modelling (Stevens et al. 2017; Vietri Rudan et al. 2015). Significantly, the composition of compartments, in contrast to that of TADs, is altered significantly during processes such as differentiation and senescence and between different cell types in a manner correlated with gene activity (Criscione et al. 2016; Dixon et al. 2015; Fraser et al. 2015). While the fundamental associations within TADs and compartments appear to be independent of spatial position within the nucleus, this compartment switching appears to be associated with the spatial repositioning of loci and occurs concomitantly with altered lamina-association during differentiation (Fraser et al. 2015). Hence, the genome is spatially organized into functionally distinct genomic regions that can be reorganized to accommodate changes to gene activity when necessary.

## 1.6 Structure-Function Relationships

### 1.6.1 *Layers of Functional Separation*

Early immunogold-EM studies revealed that the PML protein yields a ring-like staining around the PML/ND10 bodies (Weis et al. 1994) that appears donut shaped by high-resolution immunofluorescence microscopy (Boisvert et al. 2000). The application of super resolution 4Pi microscopy revealed that major components PML and Sp100 protein occur in largely distinct alternating patches in the outer shell of PML/ND10 bodies and that this shell is 50–100 nm thick (Lang et al. 2010). It appears that the integrity of the interactions forming the shell depends on sumoylation of the proteins. Moreover, some of the sumo modifications face inwards toward the center of the shell, presumably to associate with proteins in the core and further facilitate assembly, amongst which heterochromatin protein 1 (HP1) was observed. FRAP and FCS studies further demonstrated that the outer shell did not prevent the diffusion of proteins through the PML structure because GFP conjugated to an NLS was able to freely move through PML/ND10 bodies (Lang et al. 2010). However, the GFP-NLS failed to concentrate on the inside of the PML/Sp100 ring whereas HP1 did. These data argue that specific interactions

build the PML/ND10 bodies in layers with the outer layer relatively constant while the inner material varies. It remains unclear whether the ring forms around the inner material or the inner material is recruited into an already formed ring.

Immunogold EM staining for other nuclear subdomains reveals a similar layered organization. The advent of EM further enabled the subdivision of the nucleolus into fibrillar centers (FCs) that are surrounded by the dense fibrillar component (DFC), which in turn has the granular component as the outermost layer. Finally these three rings are surrounded by perinucleolar heterochromatin (Nemeth and Langst 2011). It is thought that this organization enables the sequential processing of RNAs through the nucleolus. When there is high rDNA transcription, multiple FCs can be observed embedded in the DFC, which helped ascertain that transcription preferentially occurs in the FC subregion. More processed rRNAs accumulate in the outermost subdomains suggesting that their processing occurs sequentially as they move from the FCs outward through the other nucleolar regions (Lamond and Sleeman 2003). The nucleolus initially forms around genome regions containing the genes encoding the large ribosomal RNAs, so-called nucleolar organizing regions, suggesting that the layers also reflect a mechanism for self-association in their assembly. Nuclear speckles also clearly have a layered structure (Nemeth and Langst 2011) (Fig. 1.1).

### ***1.6.2 Loops***

TAD formation appears to be driven by the interactions of proteins present on chromatin. In mammals, many TAD boundaries are demarked by a number of classical insulator elements, including interspersed repeats of the SINE family, the promoters of housekeeping genes, cohesin, and the zinc finger protein CCCTC-binding factor (CTCF) (Dixon et al. 2012; Sexton et al. 2009). Of these boundary elements CTCF is the most studied with deletions or modifications abrogating individual CTCF sites causing a collapse of TAD boundaries and/or creation of novel domains and, in some cases, gene miss-expression (Dixon et al. 2012; Downen et al. 2014; Guo et al. 2015; Lupianez et al. 2015). The mechanism of TAD formation by CTCF is yet to be fully demonstrated, however, an increasing body of evidence supports a model of loop extrusion. In this model loop-extrusion factors such as cohesin or RNA polymerase II, continuously produce increasingly large DNA loops that ultimately stall at boundary elements such as CTCF (Fudenberg et al. 2016). This model is supported by its ability to computationally recapitulate a number of TAD structures as well as numerous data including a recent single-cell Hi-C study demonstrating significantly greater variability of TADs between cells than LADs or compartments (Stevens et al. 2017; Flyamer et al. 2017). Consequently, across short time scales, TADs represent population and temporally averaged ensembles of multiple loops forming and collapsing within the confines of these boundaries. By contrast, LADs and compartments represent less dynamic and more uniform spatially segregated domains within a cell population.



Although the boundaries of TADs correspond significantly to specific CTCF sites, many contacts observed within TADs or between compartments do not, suggesting different additional regimes of chromatin folding may exist. Supporting this, a growing body of evidence now suggests such physical CTCF-independent proximities are driven instead through homotypic interactions between regions possessing similar chromatin states. For example, during limb development and haematopoiesis a number of tissue-specific and CTCF-independent looping interactions were instead enriched for shared active histone modifications such as H3K27ac and H3K4me2 (Andrey et al. 2017; Javierre et al. 2016). As many of these tissue-specific associations were between enhancers and promoters, it is tempting to think that such changing interactions may be integral to the regulation of gene activity. Suggesting a common fundamental property of genome organization, similar repressive associations have also been observed for loci possessing PRC1 and PRC2 polycomb proteins together with several repressive chromatin marks such as H3K27me3 and HP1 (Schoenfelder et al. 2015; Sexton et al. 2012; Tolhuis et al. 2011; Wijchers et al. 2016) and for transcriptionally active genes and enhancers at transcriptional hubs (Beagrie et al. 2017; Schoenfelder et al. 2010). Of particular note, one study targeted transcriptional factor Nanog, repressor SUV39H1 or boundary histone EZH2 to a lacO array inserted into a TAD by fusing them to Lac repressor and found that changing epigenetic marks was sufficient to redirect the locus to a different chromosome compartment (Wijchers et al. 2016). Taken together, these data support a model where genome organization is driven by the self-association of genomic regions that are biochemically similar or share similar components for their function. Such associations likely have the effect of localizing and thus effectively concentrating factors required for specific functions, thus improving efficiency. However, if correct, such a model presents a fundamental question: how is the self-association of the genome limited to prevent its complete non-functional aggregation? In this regards, anchoring of genomic regions such as LADs and nucleolar-associated regions to fixed nuclear structures may be the answer.

### 1.6.3 Scaffolds

The NE is probably the most important structure from which the genome is spatially organized. Early EM revealed an asymmetric distribution of chromatin within nuclei, with euchromatin dominating the nuclear interior and electron dense heterochromatin dominating regions proximal to the NE and nucleoli (Moses 1956). The advent of fluorescence in situ hybridization (FISH) subsequently revealed that individual loci, differently timed replication origins, and even certain whole chromosomes also have preferred radial positions with respect to the NE (Bickmore 2013; Zink et al. 1999; Zuleger et al. 2011b). Interestingly, the preference towards positioning whole chromosomes at the nuclear periphery corresponded to their gene density (Croft et al. 1999). Together with the concentration of heterochromatin at the NE, this suggested a relationship between locus position and their distinct

transcriptional requirements. Entire chromosomes were also found to reposition during differentiation and to exhibit tissue-specific variation in radial position, possibly reflecting differences to the transcriptional output of their constituent genes (Kim et al. 2004; Parada et al. 2002, 2004; Szczerbal et al. 2009).

It remains unclear if changes to the radial positioning of a locus direct alterations to gene expression at that locus or *vice-versa*. On the one hand, anchoring of specific genomic regions to the periphery through artificial DNA-NE interactions was sufficient to induce repression of tethered genes (Finlan et al. 2008; Reddy et al. 2008). However, different experiments using the same system yielded considerable differences in transcriptional effects (Finlan et al. 2008; Kumaran and Spector 2008; Reddy et al. 2008). On the other hand, targeted transcriptional activation and local chromatin unfolding using viral proteins was sufficient to release individual loci from the periphery suggesting the chromatin state can in some cases determine the position of a gene (Chuang et al. 2006; Therizols et al. 2014; Tumber and Belmont 2001; Tumber et al. 1999). Hence, evidence supports both an affinity-tethering mechanism and a gene activation/chromatin unfolding mechanism. Whether different genes use distinct mechanisms or these mechanisms function redundantly is not known.

The advent of high-throughput genome-wide technologies enabled taking these initial EM and FISH-based observations and interrogating their global use. One such technology is DamID, which employs the bacterial Dam methylase. When fusing the Dam methylase to lamin B1 to precisely and globally map DNA regions within tens of nanometres from the NE (Vogel et al. 2007), it was found that ~35–45% of the genome is positioned at the NE in discrete blocks termed LADs. These LADs were found to be largely conserved between cell types and mammalian species (Meuleman et al. 2013; Peric-Hupkes et al. 2010; Robson et al. 2016; Robson et al. 2017). Although depleted in genes relative to non-LADs, both endogenous and ectopically introduced reporter genes present within LADs display 5–10 fold lower expression compared to their non-LAD counterparts (Akhtar et al. 2013; Guelen et al. 2008), supporting EM and FISH studies demonstrating the repressive capacity of the NE. Correspondingly, LADs are significantly depleted of genes, early-replication domains, and active histone modifications such as H3K4me2, H3K4me3, H3K9ac, H3K27ac and H3K36me3, while they are enriched in late-replication domains, pericentric satellite repeats, A- and T-rich sequences, and repressive modifications such as H3K9me2, H3K9me3 and H3K27me3 (Guelen et al. 2008; Pope et al. 2014). Accordingly, loss of repressive histone modifications, such as H3K9me2/3 and H3K27me3 disrupts lamina association (Demmerle et al. 2013; Harr et al. 2015; Kind et al. 2013; Zullo et al. 2012).

The logical mechanism for establishing such genome organization patterns would be the affinity of NE proteins for these silencing marks. Biochemical evidence for the physical interaction of chromatin with the NE goes back over three decades (Bouvier et al. 1985). The loss of certain NE proteins specifically disrupts the NE positioning of specific loci (Robson et al. 2016; Zullo et al. 2012). The NET LBR, for example, binds to HP1 (Ye and Worman 1996) and the depletion of LBR or lamin A from rod cells of nocturnal mammals causes a complete inversion of heterochromatin clusters with their repositioning to the nuclear interior and

euchromatin accumulation at the NE (Solovei et al. 2009; Solovei et al. 2013). As a number of NETs display interactions with enzymes that add heterochromatic modifications to chromatin (Demmerle et al. 2012; Somech et al. 2005) it seems likely that this peripheral association is self-propagating/re-enforcing.

The finding of specific NETs involved in tethering specific genes to the NE enabled testing the effects of gene positioning on gene regulation without artificial systems. Specifically manipulating the position of endogenous genes by manipulating levels of NETs that direct their positioning revealed that peripheral association contributed roughly 50% of the repression normally observed (Robson et al. 2016). However, these gene expression changes were only observed when releasing a locus from the periphery by NET knockdown in differentiating cells. When NET overexpression was performed in a system where the NET was not normally expressed, it inappropriately recruited a locus to the periphery but it did not change the expression, suggesting that gene regulation is a complex interaction of specific transcriptional regulators and the gene repositioning (Robson et al. 2016).

While it is easy to understand how recruitment of a locus and transcriptional regulators to the generally silencing environment of the periphery could contribute to locus repression, there are also more complex effects of the NE on genome organization and regulation. When focusing on their organization into territories, TADs and compartments, it is easy to forget that chromosomes are even more fundamentally linear strands of DNA. As such, a LAD tethering part of a chromosome at the periphery can restrict the nuclear position of genes further down the linear length of the chromosome. As there are many LADs along the linear chromosome, one could view inter-LAD regions as loops reaching into the nucleoplasm from the NE of differing sizes according to the linear length of DNA between the LADs. How particular genes can find one another within the large 3-dimensional space of the nucleus is a major question for the field, especially as modelling studies indicate that it is unlikely for loci to find each other in a single cell cycle by diffusion alone if they are 10 Mb apart and thus potentially  $>1 \mu\text{m}$  away from one another (Dekker and Mirny 2016). In contrast, a  $0.5\text{--}0.8 \mu\text{m}$  space could be sampled by a locus in 1 h. In a recent study identifying LADs that change during lymphocyte activation, it was noted that loci that were released from changing LADs, but flanked by LADs that were maintained, remained typically much less than  $0.8 \mu\text{m}$  from the periphery (Robson et al. 2017). Critically, multiple loci with similarly flanked LADs that were kept apart when at the periphery were able to find one another upon this “constrained release” from the NE. When comparing the LAD data with compartment data it was found that the released genes associate in the similarly regulated A2 subcompartment (Robson et al. 2017). Thus the limited space to sample increases the likelihood of incorporation of a released locus into a similarly regulated active compartment.

In addition to heterochromatin and gene interactions, telomeres and centromeres also can be oriented to the NE. This was first noted with the Rab1 conformation of chromosomes that is often directed at supporting chromosome alignment for synaptonemal complex associated recombination in meiosis (Scherthan et al. 1996). While telomeres associate at least transiently with the NE in organisms

from yeast to man, the mechanisms appear to be somewhat varied. In the budding yeast *S. cerevisiae* the Ku proteins on the telomeres were first implicated in the association (Laroche et al. 1998) followed by the NPC protein TPR (Galy et al. 2000). Subsequently, SUN domain NETs were found to also contribute to the telomere association with the periphery in yeast (Antoniacci et al. 2007; Bupp et al. 2007; Chikashige et al. 2006); however, this association is slightly more complex in mammals. In spermatocyte meiosis, one study implicated SUN1 (Ding et al. 2007) while another implicated SUN2 (Schmitt et al. 2007), possibly indicating that the multiple SUN proteins of higher organisms have partially redundant and partially distinct functions. While SUN proteins appear to be the one unifying player, there are likely to be significant differences in telomere tethering complexes because budding yeast maintain telomeres at the NE while, apart from spermatocytes, most mammalian cells just anchor telomeres transiently in meiosis. Nonetheless, there are other specialized NE-telomere interactions in mammals. For example, telomeres are tethered to the NE during post-mitotic NE reassembly and this association involves both SUN1 and the shelterin subunit RAP1 (Crabbe et al. 2012). A specialized interaction with lamins is also indicated due to the abnormal distribution of telomeres in cells expressing a lamin mutation that causes Hutchison-Gilford progeria syndrome (Taimen et al. 2009).

#### **1.6.4 Boundary Elements**

NPCs in yeast contribute to genome regulation by creating boundary elements where NPC connections segregate active and silent regions (Ishii et al. 2002). It appears that associations of certain nucleoporins with the specific silencing-associated histone variant H2AZ form regions of transcriptionally repressed DNA, but directly adjacent to these regions there are also transcriptionally active regions. Yeast nucleoporins also bind transcription factors and thus the large size and complex structural organization of the NPC could segregate the silenced regions from regions with transcription factors that promote gene activation (Schmid et al. 2006; Taddei et al. 2006). Mammalian nucleoporins also have both silencing and activating interactions with chromatin. However, some of the historical findings about interactions are confounded by the fact that some nucleoporins have separate nucleoplasmic pools that are not integrated into the NPC structure and can have distinct effects on genes in the nucleoplasm (Capelson et al. 2010; Kalverda et al. 2010).

### **1.7 General versus Tissue- or State-Specific Functions**

A number of developmentally regulated loci reposition to or from the nuclear periphery concomitantly with changes to expression during differentiation. For example, the *MyoD* locus repositions from the nuclear periphery to the nuclear

interior when it becomes transcriptionally active during myogenesis (Meister et al. 2010; Yao et al. 2011) as does the *IgH* (Kosak et al. 2002) and the *Mash1* loci (Williams et al. 2006) during B cell and neuronal differentiation, respectively. Conversely, *Nid1* and *c-maf* reposition to the periphery upon transcriptional repression during myogenesis (Robson et al. 2016) and T-cell differentiation (Hewitt et al. 2004), respectively. Hence, the radial position of a locus is frequently related to its transcriptional state, suggesting a relationship. The application of DamID to systems following differentiation and changes in cell states has also revealed changes in ~5% of LADs. DamID on cells during neurogenesis, myogenesis and T-cell activation all revealed that a small but significant fraction of the genome enriched in developmentally regulated genes display gain or loss of lamina-association in a manner correlating with transcriptional activation and repression, respectively (Peric-Hupkes et al. 2010; Robson et al. 2016, 2017). These findings indicate that differentiation involves the selective reorganization of specific developmentally regulated genes in a manner that correlates with changes to their transcriptional status.

In the case of myogenesis, it was further shown that tissue-specific NETs direct changes in genome organization. Interestingly, it was found that the NE proteome is highly tissue-specific. Strikingly, studies of the NE proteomes of liver (Schirmer et al. 2003), lymphocytes (Korfali et al. 2010) and skeletal muscle (Wilkie et al. 2011) showed that, only ~17% of the proteins identified were shared by all three tissues (liver, lymphocytes and muscle). Moreover, many of these proteins were uniquely expressed in the particular tissue investigated (Korfali et al. 2012). Thus, tissue-specific NETs likely direct many aspects of tissue-specific genome organization patterns.

Nucleolar composition also varies with the metabolic condition of the cell and throughout different interphase cell cycle stages (Leung and Lamond 2003). It also changes composition during adenovirus infections with 24 nucleolar proteins out of 351 identified by mass spectrometry showing a greater than two-fold change in abundance based on SILAC ratios. Fifteen out of these 24 proteins were directly tested by immunofluorescence microscopy and of these eleven also exhibited altered localization during adenovirus infection (Lam et al. 2010).

## 1.8 Conclusions and Open Questions

While much has been learned about nuclear subdomains and genome organization there are many open questions for the field. For example, while recent studies demonstrate the existence of tissue-specific differences in both the nuclear subdomains and in genome organization, very few tissues have been analyzed. Establishing if tissue differences reflect common or distinct mechanisms and identifying the relevant players in these processes will require sampling of a much greater set of tissues and should be investigated for all nuclear subdomains.

### ***1.8.1 Mechanism for Establishment and Function of Different Organizational Patterns***

There is clear evidence for general heterochromatin interactions directing aspects of spatial genome organization and there is also clear evidence for tissue-specific NETs directing a subset of tissue-specific aspects of spatial genome organization. However, there is no understanding currently of whether the two interact or one directs the other. For example, a high-affinity tissue-specific interaction set up during NE reassembly could position chromosomes so that heterochromatin facing the periphery subsequently engages with LBR and lamin A while that facing the nuclear interior engages with the nucleolus. Alternatively, heterochromatin interactions could facilitate the tissue-specific genome organization patterns or both may work together. Testing such issues will require developing methods not yet in existence for measuring kinetics and relative binding affinities of all the proteins involved. Also, though it is clear that lamins and NETs can contribute to spatial genome organization through tethering chromatin at the periphery, the relative requirements for withstanding forces from gigadalton chromosomes have not been worked out and there may be many additional proteins involved that have yet to be identified.

There are also many standing inconsistencies in the literature such as lamin A plays a major role in the radial organization of heterochromatin (Solovei et al. 2013), but at the same time, though LADs are identified by lamin interactions, LAD organization was mostly unaffected with lamin knockout (Amendola and van Steensel 2015). Thus, there are likely many other factors contributing to these processes that have yet to be identified. We have only just begun investigating the relationship between radial genome organization (LADs) and how it can influence the organization of TADs into compartments.

### ***1.8.2 Zip Codes and Dynamics***

One of the biggest outstanding questions is how proteins are targeted within the nucleus and how their dynamics are controlled. While much is now known about how affinity interactions direct self-assembly of complexes, this information is only available for a tiny subset of the many nuclear proteins and subdomains. Though it has been nearly two decades since observations of the *gypsy* insulator DNA sequence being able to re-target an internal locus to the periphery (Gerasimova et al. 2000), questions remain for what DNA sequences contribute to most other aspects of genome organization.

What is clear is that nuclear subdomain and genome dynamics are considerable and likely needed for most of these self-assembly and other aspects of nuclear subdomain function. Individual loci in the nuclear interior can move rapidly

over large distances during interphase, especially when they become activated (Chuang et al. 2006). In one elegant study live cell co-labeling of PML bodies, a gene locus, and its product revealed that upon transcriptional activation the locus decondensed and moved until it associated with PML bodies to maximize transcriptional output (Tsukamoto et al. 2000). It is noteworthy that the different classes of PML bodies include a subclass that moves by a metabolic-energy-dependent mechanism (Muratani et al. 2002).

NE contacts appear to at least partly inhibit such mobility as loci at the nuclear periphery tend to be much less mobile (Chubb et al. 2002). Though whole chromosome territories are generally maintained during interphase, there is greater loss of defined photoactivated regions in the interior compared to the periphery (Strickfaden et al. 2010). Nonetheless, some dynamic exchange of peripheral loci occurs with recently developed single-cell DamID approaches revealing that only 2/5 of LADs are universally observed and some LADs actively change during interphase (Kind et al. 2013). In fact, LADs may move up to 1  $\mu\text{m}$  away from the periphery over 5–20 hours and many LADs were observed to shuffle between the NE and nucleoli. NETs might contribute to this as FRAP and photoactivation experiments on NETs involved in chromosome repositioning revealed that some are very dynamic while others are not (Zuleger et al. 2011a).

### 1.8.3 Conclusions

The dynamics of nuclear subdomains makes particular sense in conjunction with the absence of intranuclear membranes. The same genes need to be active or shut down both temporally and in different tissues and different genes on the same linear chromosome need to be active or repressed. How would one segregate the same chromosome into distinct compartments? While it might be possible to segregate RNA processing into a membrane bound compartment, there are aspects of quality control that appear to occur co-transcriptional that could not be engaged if this process were segregated by membranes. It makes more biological sense to dynamically move self-assembling factories around the nucleus to where they are needed when they are needed in each cell type.

Other critical questions remaining include what are the fundamental principles of nuclear assembly and re-assembly after each mitosis? How do mutations in the vast majority of non-coding genome affect genome structure and subsequently cause disease? How universal are nuclear organization patterns and mechanisms? When and how did it arise in evolution? Is it a fundamental property of complex multicellular organisms? It is interesting in this regard that the tissue-specific NETs tend to be poorly conserved in evolution (de Las Heras et al. 2013) and thus it could be speculated that the appearance of nuclear subdomains and their variation may have enabled an ever increasing complexity in genome regulation that has driven tissue-specification in evolution.

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