

Chromatin at the nuclear periphery and the regulation of genome functions

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Abstract Chromatin is not randomly organized in the nucleus, and its spatial organization participates in the regulation of genome functions. However, this spatial organization is also not entirely fixed and modifications of chromatin architecture are implicated in physiological processes such as differentiation or senescence. One of the most striking features of chromatin architecture is the concentration of heterochromatin at the nuclear periphery. A closer examination of the association of chromatin at the nuclear periphery reveals that heterochromatin accumulates at the nuclear lamina, whereas nuclear pores are usually devoid of heterochromatin. After summarizing the current techniques used to study the attachment of chromatin at the nuclear lamina or the nuclear pores, we review the mechanisms underlying these attachments, their plasticity and their consequences on the regulation of gene expression, DNA repair and replication.

Keywords Fluorescence in situ hybridization · Light microscopy · Nuclear pore · Nuclear lamina · Super-resolution microscopy

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Introduction

The organization of chromatin into higher-order structures provides the full level of compaction necessary to fit the genome into the nucleus. Even prior to the discovery of the DNA double helix and the nucleosome, the first observation of higher-order chromatin compaction came from electron micrographs showing that chromatin had a distinct appearance in certain regions of the nucleus, described in (Passarge 1979). Heterochromatin, in contrast to euchromatin, was defined as regions refractory to electrons. Heterochromatin and euchromatin distinguish two different states of compaction (with heterochromatin being more compacted) and transcriptional activity (with heterochromatin being silent).

Beside the different compaction states of chromatin, the organization of different loci within the 3D space of the nucleus constitutes another level of regulation of genome function. Indeed, the position of a specific locus within the nucleus is not random and several rules govern this. In this review, we focus on the association of the genome with the periphery of the nucleus—the nuclear lamina and the nuclear pores—and the possible functional consequences of this organization.

Staining methods to identify DNA in the nucleus in the modern era

The staining method developed by Robert Feulgen a century ago remains an important quantitative method for assessing DNA in the nucleus. However, it is not able to identify specific genomic regions or to discriminate different chromatin or activity states.

The study of replication timing allowed for the visual detection of early and late replicating genomic domains in the nucleus. Cells are pulse labeled—typically with a halogenated thymidine analogue (5-bromo-2-deoxyuridine/BrdU, chlorodeoxyuridine/CldU or iododeoxyuridine/IdU) and the sites of incorporation detected by immunofluorescence (Yokochi and Gilbert 2007). This approach was used to show that replication timing domains form fundamental units of chromosome structure, 100 kb–1 Mb in size, that are maintained through cell division (Zink et al. 1999).

Large chromosomal regions can also be visualized, and in living cells, by the photoactivation of fluorescently (GFP/RFP) tagged histones. Generally, it has not been possible to identify which regions of the genome are being photoactivated, but this was overcome by the activation of a specific chromosome at mitosis, whose identity was guided by the detection of an inserted lac operator array—using a lac repressor–GFP fusion protein (Müller et al. 2010).

Genomic regions that are associated with specific proteins can be visualized in cells using a derivative of the DNA adenine methyltransferase identification (DAM-ID) method. The *E. coli* DNA adenine methyltransferase (Dam) is fused to a protein of interest and methylates adenines within GATC sequences that are in close proximity to the protein to which it is fused. Adenine methylation does not occur in mammalian cells and therefore marks the genomic-binding sites of the protein of interest. The methylated sequences can then be isolated and identified, e.g., by sequencing, but they can also be visualized in situ using a GFP-tagged protein that binds to GA^mTC. This approach was used to assess cell to cell variability and the dynamics of genome associations with the nuclear lamina (Kind et al. 2013).

The most common method for visualizing specific genomic loci or chromosomal domains in the nucleus is fluorescence in situ hybridization (FISH). Depending on the nature of the probe used, whole chromosomes (chromosome paints), specific subsets of loci (Boyle et al. 2011) and individual loci can be detected—but only in fixed cells. Whereas FISH allows the direct visualization of, for example, the spatial proximity between two sequences for just for a few loci at a time, molecular techniques derived from chromosome conformation capture (3C) might enable assessment of the 3D folding and spatial organization of the entire genome in the nucleus, either in single cells or in a population of cells. 3C allows measurement of the frequency at which sequences are ligated together after formaldehyde cross-linking of cells and is generally thought to reflect spatial proximity between two sequences. Therefore, theoretically FISH and 3C should give similar results, but this is not always the case suggesting that, until there is a better understanding of the parameters underlying both

of these methods, studies of spatial genome organization should be validated by different independent methods (Williamson et al. 2014).

The use of super-resolution microscopy to visualize nuclear organization

In addition to technical developments in the ability to label and detect specific genomic regions in fixed or live cells, optical microscopy resolution has been considerably improved so that it is now possible to study nuclear organization at a resolution below the diffraction limit of light (~200 nm). The three main super-resolution techniques used are structured illumination microscopy (SIM), stimulated emission depletion (STED) and photoactivation localization microscopy/stochastic optical reconstruction microscopy (PALM/STORM). In SIM, the sample is illuminated with spatially patterned light, and the information contained in these patterns (Moiré fringes) used to extract details in the image that are too small to be resolved by the microscope, increasing *xy* resolution by a factor of 2 (Carlton 2008). SIM is beginning to be used more widely to image nuclear ultrastructures and chromosomal topography. SIM has, for example, been successfully used to resolve structures at the nuclear periphery such as the nuclear pore complex and the nuclear lamina (Schermelleh et al. 2008) (Fig. 1). The demonstration that 3D-FISH preserves chromatin features even at the resolution used by SIM (Markaki et al. 2012) opens up the possibility of combining FISH with SIM to study chromatin organization at high resolution (Giorgetti et al. 2014).

FISH using oligo-paint probes has been recently combined with STORM imaging (Beliveau et al. 2015), as has the visualization of tagged histone molecules (Ricci et al. 2015). It will be important to establish how consistent the views of chromatin organization obtained with these different super-resolution imaging modalities are, with respect to each other.

Chromosome territories

FISH with chromosome paints revealed the existence of chromosomal territories—i.e., the fact that each interphase chromosome occupies a distinct portion of the nucleus (Stack et al. 1977). This observation is confirmed by Hi-C analyses of all metazoan genomes (Kalhor et al. 2012; Lieberman-Aiden et al. 2009; Sexton et al. 2012; Zhang et al. 2012). Most of the captured cross-linked interactions are in *cis* rather than in *trans*. However, in *trans* associations are also captured, albeit at low frequencies (Hakim et al. 2011; Kalhor et al. 2012; Lieberman-Aiden et al. 2009; Simonis et al. 2006; Yaffe and Tanay

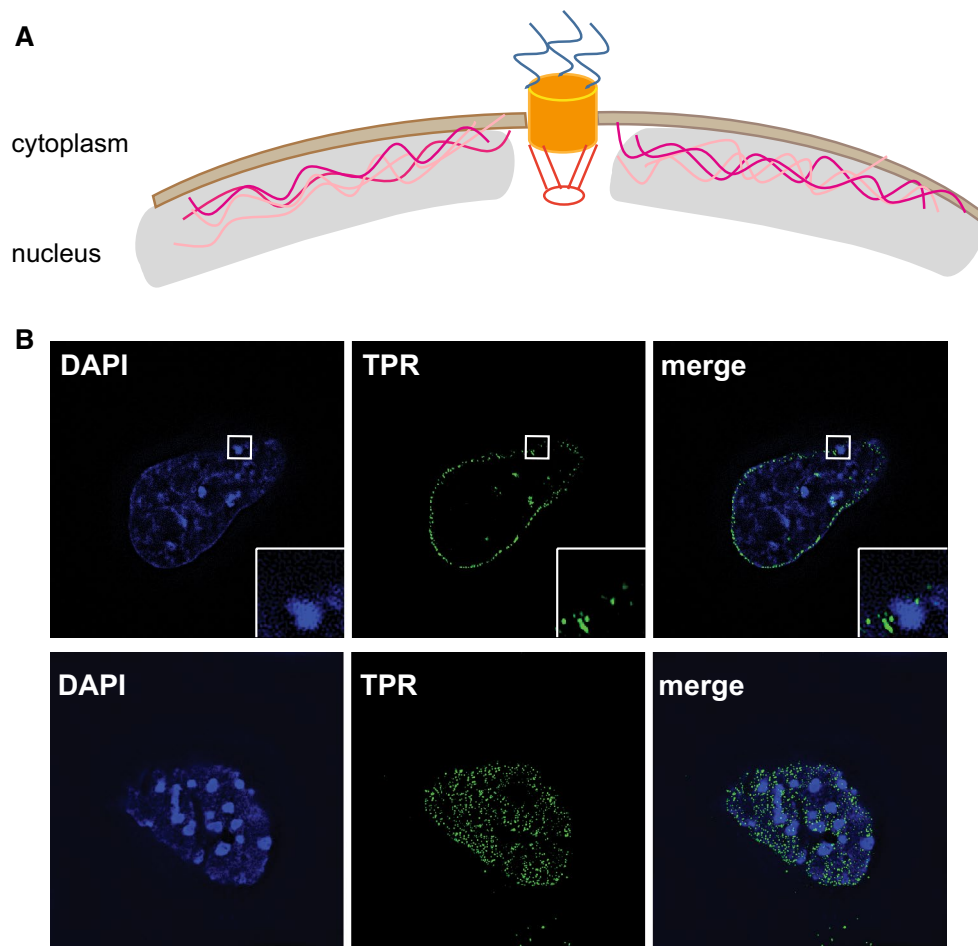


Fig. 1 **a** Schematic representation of the nuclear envelope. Heterochromatin (in *gray*) is associated with the nuclear lamina (in *pink*) and is absent from nuclear pores (*orange*). **B** Micrographs of mouse embryonic stem cells obtained by structured illumination microscopy (SIM), showing immunofluorescence for TPR (*green*) and

DAPI staining (*blue*). *Upper panel* shows a middle section through the *z*-axis, the *lower panel* shows a *z* plane from the *bottom* of the nucleus. When a chromocenter is present at the nuclear periphery, nuclear pores are seen to be excluded

2011). The regions that associate *in trans* are usually gene rich and transcriptionally active and correspond remarkably well to the loci that are known to visibly loop outside of their own chromosome territory (Mahy et al. 2002). Live cell analysis reveals that this looping out is dynamic (Müller et al. 2010). Consistently, gene-dense chromosomal regions decorate the outside of their own chromosome territories (Boyle et al. 2011) (Fig. 2). The functional consequences of looping out are unclear, although it was hypothesized to favor gene activation by allowing genes to interact with transcription factories or nuclear speckles (Brown et al. 2008b; Schoenfelder et al. 2010; Sutherland and Bickmore 2009). However, DNA FISH coupled to the analysis of gene expression by RNA FISH suggests that the alleles located more to the exterior of chromosome territories are those that were already active and already associated with transcription factories before they ‘looped out.’ Hence, active gene regions may loop

out from chromosome territories because they can do, not because they need to in order to facilitate gene expression (Morey et al. 2009).

Radial chromosome organization

Chromosomes are not randomly positioned in the nucleus, but have instead a preferred position relative to the nuclear periphery or interior. In the majority of mammalian cells studied, FISH and Hi-C analyses show that gene-dense chromosomes tend to position in the nuclear interior, whereas gene-poor chromosomes are positioned closer to the nuclear periphery (Bolzer et al. 2005; Boyle et al. 2001; Cremer et al. 2001; Kalhor et al. 2012; Küpper et al. 2007). However, a given chromosome does not seem to consistently have a specific neighbor (Kalhor et al. 2012; Walter et al. 2003). Therefore, the position of a chromosome or gene is neither random nor fixed, but rather is probabilistic.

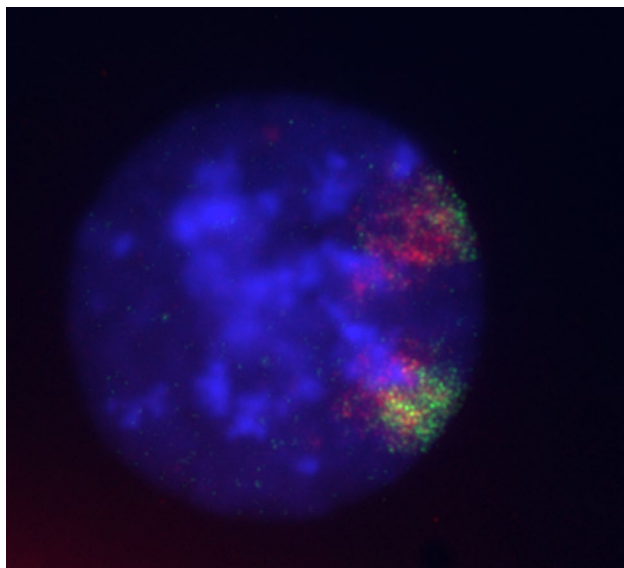


Fig. 2 Fluorescence in situ hybridization (FISH) image of a mouse nucleus stained with DAPI (*blue*) and hybridized with a probe that detects a whole mouse chromosome (*green*) and another oligo-probe pool (*red*) that detects just the exons from that chromosome. The polar organization of the chromosome is revealed with the gene (exon)-dense portion of the chromosome oriented away from the nuclear periphery. Image courtesy of Shelagh Boyle (Boyle et al. 2011)

Within a specific chromosome, chromatin regions are also polarized, with gene-poor regions preferentially oriented toward the nuclear periphery, whereas gene-rich regions are oriented toward the nuclear interior (Boyle et al. 2011; Küpper et al. 2007) (Fig. 2). Furthermore, DamID allowed the identification of genomic regions directly associated with proteins of the nuclear lamina (lamina-associated domains—LADs) (Guelen et al. 2008). These are generally gene poor and associated with low levels of gene expression—consistent with the results from microscopy. The nuclear lamina therefore constitutes a nuclear structure that has a major influence on the global 3D organization of the genome. A second important nuclear structure that binds specific genomic regions is the nucleolus. In addition to rDNA loci, regions that co-purify with the nucleolus substantially overlap with LADs (van Koningsbruggen et al. 2010; Németh et al. 2010). Therefore, the nuclear lamina and the nucleolus might provide two alternative locations where the same repressive genomic domains can partition. Indeed, analysis of mother and daughter cells through mitosis reveals that loci originally at the nuclear periphery in a mother cell can relocate either to the nuclear periphery or to the periphery of the nucleolus in the daughter cells (Thomson et al. 2004; Kind et al. 2013).

Attachment of chromatin to the nuclear periphery

Association of chromatin with the nuclear lamina

The nuclear lamina is constituted by the type V intermediate filaments proteins lamin A/C and lamin B that assembles in a meshwork of 10-nm filaments underneath the inner nuclear membrane (INM) (Fig. 1a). Lamin B is expressed in all somatic cells and is tightly bound to the INM via a stable C-terminal farnesyl modification, whereas A-type lamins are expressed only in differentiated cells and are present in the INM as well as in the nucleoplasm. Several integral nuclear membrane proteins associate with the lamins including the lamin B receptor (LBR) which, together with lamin A, is necessary for maintaining heterochromatin at the nuclear periphery (Solovei et al. 2013). Mapping of genome interactions by DamID and chromatin immunoprecipitation (ChIP) allowed the identification of 1100–1400 LADs in mammalian cells that range in size from 10 kb to 10 Mb and that collectively cover approximately 40 % of the genome. They may correspond to large blocks of heterochromatin at the nuclear periphery that were first observed by electron microscopy (e.m.) (Akhtar and Gasser 2007; Belmont et al. 1993). The exact mechanism of chromatin tethering to the nuclear lamina is still under investigation. Different mechanisms might contribute, including sequence-specific interactions, tethering by chromatin components and involvement of specific nuclear periphery proteins. One of the characteristics of LADs in embryonic stem cells is the presence of long stretches of DNA of high A/T content, but this is less clear in differentiated cells (Meuleman et al. 2013). A nuclear lamina interaction motif, enriched in (GA)_n repeats, was identified in some mouse LADs, and an artificial array of this motif was sufficient to promote nuclear lamina targeting. This element could be recognized by the cKrox transcriptional repressor, in complex with HDAC3 and the nuclear lamina protein Lap2 β (Zullo et al. 2012). However, (GA)_n repeats are not enriched in LADs (Guelen et al. 2008), which suggests that additional motifs or additional mechanisms might be involved in the tethering of LADs to the nuclear lamina.

The exact role of lamin proteins in chromatin organization remains unclear. Although mutations of lamin A are reported to lead to reduced interactions with chromatin in some but not all studies (Kubben et al. 2012; McCord et al. 2013) and depletions of lamin B1 lead to alterations of chromatin organization and chromosome positioning (Malhas et al. 2007; Shimi et al. 2008), triple lamin knockout murine embryonic stem cells (mESCs) are viable and the organization of LADs as detected by DamID for the nuclear lamina protein emerlin remains unchanged (Amenola and van Steensel 2015), suggesting that lamins are not

necessary for tethering of LADs at the nuclear lamina in mESCs. LBR is a possible candidate for mediating LADs interactions with the nuclear periphery. Removal of both LBR and lamin A/C proteins causes inversion of the normal radial organization of heterochromatin, with heterochromatin accumulating in the center of the nucleus instead, mimicking the organization observed in rod cells of nocturnal animals (Solovei et al. 2013). It would be interesting to use DamID to study the interactions of LADs with the nuclear lamina in the absence of LBR, or of both LBR and lamin A. Other interesting candidates include the INM protein emerin, in complex with HDAC3 or other nuclear envelope transmembrane proteins (NETs), many of which are tissue specific. Some of these NETs were shown to control the positioning of individual chromosomes relative to the nuclear periphery (Zuleger et al. 2013).

Interestingly, lamin A and B form distinct subdomains at the nuclear periphery (Shimi et al. 2008) and whether LADs interact similarly to both types of domains still remains to be investigated. Recently, ChIP-seq experiments showed differences between sequences associated with lamin A and lamin B, with lamin A being able to bind more open chromatin. However, lamin A is localized both at the nuclear periphery and in the nucleoplasm and it is still unclear whether these differential interactions happen at the nuclear periphery or in the nuclear interior (Lund et al. 2015). Further studies by immunoFISH will be needed to answer this question.

Some repressive histone marks have been implicated in LAD formation. In *C. elegans*, the H3K9 histone methyltransferases (HMTs) MET-2 and SET-25 are necessary to allow positioning of heterochromatin at the nuclear periphery (Towbin et al. 2012). In mammalian cells, the loss of H3K9me1 results in the disruption of the nuclear lamina (Pinheiro et al. 2012) and H3K9me2 and H3K9me3 are involved in the targeting of the human beta globulin locus to the nuclear periphery (Bian et al. 2013). Furthermore, the G9a HMT, that is responsible for H3K9me2, also promotes the interaction of LADs with the nuclear lamina (Kind et al. 2013). Ying Yang 1 (YY1) has also recently been shown to act in concert with lamin A/C, and H3K27me3 and H3K9me2/3 to promote association and maintenance of some LADs at the nuclear lamina (Harr et al. 2015).

Association of chromatin with nuclear pore complexes

Nuclear pore complexes (NPCs) are large transmembrane complexes, consisting of about 30 different proteins called nucleoporins (Nups). Each nucleoporin occurs in multiple copies, resulting in 500–1000 protein molecules in the fully assembled NPC (Cronshaw et al. 2002). They form a ring-shaped structure of eightfold rotational symmetry. NPCs consist of a membrane-embedded scaffold build around a

central transport channel, a cytoplasmic ring, a nuclear ring and eight filaments attached to each ring. The nuclear filaments are connected to a distal nuclear ring to form the nuclear basket of the NPC. NPCs mediate transport of macromolecules to and from the nucleus. A barrier allowing for the diffusion of molecules of up to ~40 kDa (Ma et al. 2012) is formed by unstructured nucleoporins segments that contain numerous phenylalanine–glycine (FG) repeats. Nups carrying 4–48 FG repeats fill the central channel of the NPC and form a meshwork determining the pore permeability limit (D’Angelo and Hetzer 2008). Transport of molecules bigger than 40 kDa necessitates their binding to transport receptors. FG repeats serve as docking sites for transport receptors and the complex between cargo and transport receptor can then move through the diffusion barrier of the pore (Raices and D’Angelo 2012).

Besides their role in nucleocytoplasmic transport, nuclear pores are also involved in mitosis, chromatin organization, regulation of gene expression and DNA repair. While in yeast, most of the interactions between nucleoporins and chromatin occur at the nuclear pores, in metazoans, there are two different pools of nucleoporins—at the nuclear pores and in the nucleoplasm (Rabut et al. 2004). In *Drosophila*, DamID with the nucleoporins Nup98, Nup50 and Nup62 provided evidence for two different patterns of genome interaction: one occurring at the NPC but the majority (80 %) of interactions occurring with the nucleoplasm pool of Nups (Kalverda et al. 2010). The latter were mainly active regions of the genome, whereas at the NPC interactions were equally with intergenic regions or genes, without enrichment for active genes but with an enrichment for chromatin insulators (Kalverda and Fornerod 2010).

In mammalian cells, ChIP for NUP93 identified inactive parts of the genome—similar in characteristic to those found by lamin-DAM—but the double cross-linking methods used raise some concern about indirect cross-linking (Brown et al. 2008a). However, a recent DAMID-Nup153 study also implicated this nucleoporin in transcriptional repression, by the polycomb complex PRC1, and immunoFISH confirmed the Nup153-dependent localization of some of these loci at the nuclear periphery (Jacinto et al. 2015).

Dynamics of chromatin association with the nuclear periphery

Many regions of the genome—constitutive LADs (cLADs)—interact with the nuclear lamina independently of cell type. In contrast, for facultative LADs (fLADs), interaction with the nuclear lamina is dynamic during development. In the inner cell mass of the blastocyst, chromatin fibers are dispersed and undergo massive reorganization at the epiblast stage after implantation, including

formation of large heterochromatic domains at the nuclear periphery as identified by electrosopic e.m. (Ahmed et al. 2010). Concomitantly, hundreds of genes relocate to, or move away from, the nuclear periphery during early development correlated with changes in gene expression, genes at the nuclear lamina being repressed, whereas the genes moving away from the nuclear lamina are activated (Peric-Hupkes et al. 2010). This raises the question of whether chromatin compaction or transcription could drive movements to and from the nuclear periphery during development. Indeed, changes in chromatin condensation have been recently shown to result in relocalization of fLADs away from the periphery (Therizols et al. 2014). Therefore, global changes in chromatin state during blastocyst implantation may be crucial for the regulation of fLADs location.

Besides their dynamics during differentiation, FISH and live cell imaging have shown the stochasticity of LADs in single cells. Only a fraction of the LADs of a mother cell return to the nuclear lamina in the daughter cells. Although the mechanisms underlying this redistribution are not fully understood, the dimethylation of H3K9 by G9a appeared to be important in regulating attachment to the nuclear lamina (Kind et al. 2013).

During the differentiation of human cells, Nup98 associates with developmentally regulated genes. Two modes of interactions were identified: On the one hand, genes could interact with Nup98 at the NPCs and, on the other hand, genes that were highly induced interact with Nup98 in the nucleoplasm (Liang et al. 2013). However, overall, the dynamics of chromatin attachment to nuclear pores or nucleoporins is little explored.

Regulation of gene expression

Most genes in LADs are transcriptionally silent and associated with repressive histone marks (Guelen et al. 2008; Pickersgill et al. 2006; Wen et al. 2009), and the nuclear lamina itself seems to directly contribute to gene repression. Genes associated with the nuclear lamina in flies lacking one of the lamins are derepressed (Shevelyov et al. 2009), whereas in flies and mammalian cells, artificial tethering of certain genes to the nuclear periphery can lead to gene repression (Dialynas et al. 2010; Finlan et al. 2008; Reddy et al. 2008). Furthermore, the analysis of thousands of random genomic integrations of reporter genes into the mouse genome showed a lower level of transcription in mouse LADs compared to non-LADs (Akhtar et al. 2013).

Several mechanisms have been proposed to mediate gene repression at the nuclear lamina. Targeting of lamin A—and not lamin B—to promoters can cause transcriptional repression (Lee et al. 2009). Additionally, the lamin-B receptor (LBR) binds directly to heterochromatin protein

1 (HP1), suggesting that it might play a role in the regulation of gene expression at the nuclear lamina (Ye et al. 1997). Another transmembrane protein of the nuclear lamina that might be involved in the regulation of gene expression is RFBP, an ATPase protein that interacts directly with a potential chromatin remodeler related to the SWI/SNF (Mansharamani et al. 2001). Furthermore, the LEM domain proteins mediate binding to barrier to autointegration factor (BAF) (Furukawa 1999), involved in higher-order chromatin organization, transcription regulation and nuclear envelope assembly (Margalit et al. 2007). Additional evidence for a role of INM proteins in the regulation of gene expression is the binding of EMD and LAP2 β to the histone deacetylase HDAC3 that increases its enzymatic activity (Demmerle et al. 2012; Nili et al. 2001). Furthermore, repression of genes artificially tethered at the periphery was shown to be dependent on the action of HDACs (Finlan et al. 2008).

Both e.m. (Akhtar and Gasser 2007) and super-resolution optical microscopy (Schemmelleh et al. 2008) reveal that there is no heterochromatin in the vicinity of NPCs. In line with this, yeast genes interacting with the NPCs are active and this interaction is important for gene expression (Brickner and Walter 2004; Casolari et al. 2004; Taddei et al. 2006). However, in *Drosophila*, active genes interact with the nucleoplasmic pool of Nups—and not with the NPC (Kalverda et al. 2010). Consistent with a role for nucleoporins in the regulation of gene expression away from the NPCs, the mobility of Nup153, Nup98 and Nup50 is dependent on RNA PolII activity (Buchwalter et al. 2014; Griffis et al. 2002). In *Drosophila*, depletion of Nup98 leads to the downregulation of the interacting genes and its overexpression induces the upregulation of the same genes (Kalverda et al. 2010). Similarly, silencing of the nuclear basket nucleoporin Nup153 leads to the repression of thousands of genes (Vaquerizas et al. 2010). However, the exact mechanisms by which the nucleoporins regulate transcription remain poorly understood. Nup98 FG repeats can recruit histone acetyl transferases or HDACs (Bai et al. 2006; Kasper et al. 1999; Wang et al. 2007) and are involved as translocation-associated fusion proteins in acute myeloid leukemia and are thought to inappropriately trigger gene activation (Franks and Hetzer 2013).

In *Drosophila*, the nucleoporins Nup153 and TPR/Mtor associate with the hyperactive dosage-compensated male X chromosome (Mendjan et al. 2006; Vaquerizas et al. 2010). However, their role in dosage compensation seems to be independent of their localization at the nuclear pores (Vaquerizas et al. 2010), further suggesting a role for the nucleoplasmic pool of Nups in the regulation of gene expression.

A few studies suggest that some nucleoporins can be involved in transcriptional repression. *Drosophila* Nup88 is

reported to associate with transcriptionally inactive genes (Capelson et al. 2010). Similarly, Nup93 interacting chromatin seems to be enriched in repressive histone marks (H3K27me3 or H3K9me3) in human cells (Brown et al. 2008a). Furthermore, it was recently shown that Nup153 participates in the downregulation of developmental genes in ESCs, therefore contributing to the maintenance of pluripotency, by promoting the recruitment of the polycomb-repressing complex PRC1. Depletion of Nup153 in ESCs led to the deregulation of these genes and loss of stem cell identity. This role for Nup153 in the regulation of gene expression could be dissociated from its localization at the nuclear pore, and target genes were positioned either at the nuclear periphery or in the nucleoplasm (Jacinto et al. 2015).

Regulation of DNA repair

When considering the role of the nuclear periphery on DNA repair, two different questions can be asked: Whether the proteins of the nuclear periphery play a role in DNA repair, independently of the position where it happens in the nucleus, or whether the position of a lesion at the nuclear periphery influences its repair.

DNA repair at the nuclear periphery

Studies in yeast showed that persistent double-strand breaks (DSBs) or telomeres are anchored at the nuclear periphery, both at the INM and at the pores (Kalocsay et al. 2009; Khadaroo et al. 2009; Nagai et al. 2008; Therizols et al. 2006). Whereas anchoring at nuclear pores was proposed to allow recombinational repair of persistent DSBs in a SUMO-dependent pathway (Nagai et al. 2008), anchoring at the INM was proposed to enable the irreparable breaks and the telomeres to be repaired by alternative pathways (Oza and Peterson 2010). Recent work showed that relocation of persistent DSBs to the nuclear periphery involves different mechanisms. Whereas the SWR-C chromatin remodeling complex is necessary for the relocation of persistent DSBs at both the nuclear pores and the INM, INO80 was shown to be necessary only for relocation at the pores (Horigome et al. 2014). Furthermore, breaks relocated at the two compartments are repaired by different pathways; breaks located at nuclear pores are repaired by break-induced repair or microhomology-mediated error-prone mechanisms, whereas breaks associated with the INM are repaired by the error-free homologous recombination (HR) pathway (Horigome et al. 2014).

In mammalian cells, the positional stability of DSBs (Soutoglou et al. 2007) suggests that relocation of persistent breaks might not occur and that cells need to ensure

efficient DNA repair, regardless of the location of the lesion. Accordingly, the efficiency of DSB repair was shown to be similar at the nuclear lamina, at the nuclear pores or in the nucleoplasm. However, whereas repair at the nuclear pores or in the nuclear interior occurred through the classical repair pathways—HR and non-homologous end joining (NHEJ)—the nuclear lamina appeared to be refractory to HR and to allow repair by the error-prone alternative end-joining mechanism (Lemaître et al. 2014).

Role of proteins of the nuclear periphery in DNA repair

Mutations of lamins are associated with several degenerative disorders and premature aging syndromes (including the Hutchinson–Gilford progeria syndrome—HGPS), features that are often associated with increased genomic instability. Indeed, fibroblasts from HGPS patients, and from a mouse model of progeria lacking the *Zmpste24* protein that is necessary for lamin A maturation (*Zmpste24*^{-/-}), showed increased DNA damage and increased sensitivity to damaging agents (Liu et al. 2005; Varela et al. 2005). Similarly, fibroblasts from laminopathy mandibuloacral dysplasia type A patients as well as *Lmna*^{-/-} MEFs show increased genomic instability and increased unrepaired DSBs, as visualized by γ H2AX foci (Gonzalez-Suarez et al. 2009; di Masi et al. 2008). Mechanistically, lamin A has been proposed to be involved in DSB repair by both HR and NHEJ (Redwood et al. 2011). A direct demonstration that the integrity of the nuclear lamina is important for maintaining genome stability comes from the massive DNA damage that occurs in the micronuclei associated with cancer cells and that have disrupted nuclear lamina (Hatch et al. 2013). This may underlie the phenomenon of chromothripsis (Zhang et al. 2015).

In yeast, five core nucleoporins—Nup84, Nup120, Nup133, Nup170 and Nup188—have been implicated in the repair of damage caused by ionizing radiation (Bennett et al. 2001). Additionally, Mlp1 and Mlp2, two nucleoporins from the nuclear basket, were also identified as regulators of DSB repair. Indeed, deletion of both proteins or of Nup60, which allows their anchoring to NPCs, leads to accumulation of Rad52 foci, a marker of DSB (Palancade et al. 2007). The mechanisms by which nucleoporins participate in DSB repair seem sumoylation dependent. The Nup84 complex and Nup60 are required for the recruitment of the SUMO protease Ulp1 to NPCs (Zhao et al. 2004) and depletion of these nucleoporins result in the displacement of Ulp1 from the NPCs and affect cellular sumoylation patterns (Palancade et al. 2007). Ulp1 mutants that cannot localize to the NPCs show similar phenotypes to nucleoporins mutants with regard to DSB repair, whereas Ulp1 overexpression can partially rescue this phenotype

(Palancade et al. 2007), suggesting that the role of nucleoporins in DSB repair is via their function in Ulp1 recruitment at NPCs. A proposed target of this regulatory mechanism is yKu70, which shows decreased sumoylation levels in nucleoporins mutants (Palancade et al. 2007). However, a large number of proteins are sumoylated during the DNA damage response and DSB repair (Dou et al. 2011) and other targets of Ulp1 might be important for repair. In mammalian cells, the presence of NPC-associated SUMO-regulating proteins is conserved (Palancade and Doye 2008; Zhang et al. 2002); however, no role for nucleoporins in DSB repair linked with the SUMO pathway has been described yet.

The only mammalian nucleoporin identified so far as a regulator of DNA repair is Nup153, depletion of which leads to impaired nuclear import of 53BP1 (Lemaître et al. 2012; Moudry et al. 2012). However, Nup153 also seems to promote repair by NHEJ through a mechanism independent of 53BP1 import (Lemaître et al. 2012). Therefore, additional studies are required to understand the exact role of Nup153 in DNA repair.

Regulation of DNA replication

Apart from gene expression and DNA repair, the other major transaction that has to take place on the genome is DNA replication. Replication timing profiles reveal the presence of megabase-long regions with similar replication timing, called replication domains, that replicate either early or late during S phase (Desprat et al. 2009; Hiratani et al. 2008, 2010; Woodfine et al. 2004). Later replicating DNA is preferentially located at the nuclear or nucleolar peripheries (Dimitrova and Gilbert 1999; O’Keefe et al. 1992) and overlaps well with LADs. Consistent with this, the movement of fLADs during differentiation correlates with changes in their replication timing (Hiratani et al. 2008; Williams et al. 2006). Genome-wide analysis of replication timing reveals major developmental changes in replication timing, including a set of early to late changes, during the post-implantation epiblast stage coincident with their repositioning toward the nuclear periphery and accumulation of heterochromatin at the nuclear periphery (Hiratani et al. 2010). Conversely, for loci that change from late to early replication, this often coincides with their movement away from the periphery.

These correlations raise the question of as to whether nuclear position can directly influence replication timing. To test this, synthetic activators have been used to induce relocalization of several loci from fLADs away from the nuclear envelope in mESCs (Therizols et al. 2014). Interestingly, when the synthetic activator contained a domain activating transcription of the target loci, replication timing

was shifted from late to early replication. However, when the synthetic factor only induced chromatin decondensation and relocalization, replication timing was unchanged—thus uncoupling nuclear positioning from the direct control of replication timing.

More globally, the importance of the nuclear lamina in DNA replication is shown by the absence of DNA replication in micronuclei that lack lamin B (Okamoto et al. 2012). The correlation between active chromatin and early replicating domains suggests that chromatin associated with the nuclear pores might replicate early. However, chromatin tethering and transcriptional activity at the nuclear pore complex were suggested to be a barrier for the replication fork that increases topological tension (Bermejo et al. 2011). Interestingly, some nucleoporins, such as Nup153 and Nup98, were identified as potential targets of the S-phase checkpoint proteins ATR/Chk1 (Blasius et al. 2011; Matsuoka et al. 2007), which could suggest a regulation of chromatin–nuclear pore association during replication.

Conclusion

The association of chromatin with different nuclear compartments allows an optimal regulation of DNA metabolism, including transcription, replication and repair. Although being in a very close proximity, the nuclear pores and the nuclear lamina constitute two different compartments with very distinct functions in the regulation of genome function. However, a regulatory role of one compartment on the other one cannot be excluded. For example, the nucleoporin Nup153, which is able to bind lamins and is therefore thought to allow anchorage of nuclear pores in the nuclear envelope, was shown to be necessary for a proper nuclear lamina organization (Zhou and Panté 2010). This implies that the composition or the density of nuclear pores could influence the organization of the nuclear lamina and the functions of LADs. Interestingly, NPC composition is not constant during differentiation (D’Angelo et al. 2012) and it will therefore be interesting to study the influence of nuclear pores composition on the organization of LADs during differentiation.

The initial observation of heterochromatin accumulation at the nuclear periphery triggered a lot of efforts to identify the regions attached to the nuclear envelope. Interestingly, LADs seem to have properties that differentiate them from the rest of the chromatin: They replicate later, their transcription is repressed, and DNA repair occurs through different mechanisms from chromatin in the nuclear interior. Although the general conclusions made from genome-wide studies by DamID allowed for the identification of general features of LADs, emerging evidence from

microscopy-based studies demonstrates variability between cells. This observation emphasizes the need to study nuclear organization and genome function at the scale of individual cells in tissue and organs, and despite the development of other molecular methods, microscopy will continue to be an important tool to use for this purpose.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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