Chapter 2
The Molecular Composition and Function of the Nuclear Periphery and Its Impact on the Genome

C. Patrick Lusk and Megan C. King

Abstract The nuclear periphery is an essential element of nuclear architecture that contributes to the organization and function of the genome. Over the last few decades, remarkable molecular insight from many model systems has contributed to a dynamic and nuanced view of the nuclear periphery, which had previously been considered a static, transcriptionally-silent nuclear subcompartment. While modern genomic analyses have confirmed that the nuclear periphery is home to repetitive, gene-poor chromatin rich in repressive histone marks, specific genic regions either leave or associate with the nuclear periphery in response to external environmental or developmental inputs in a way that correlates with transcriptional output. Recently, work suggests surprisingly that transcription per se is not a determinant of gene position in relation to the nuclear periphery; an emerging view instead supports that peripheral tethering may reflect mechanisms to promote genome stability while being dispensable for gene silencing. Here, we review our current understanding of the molecular components that form the nuclear periphery, including integral inner nuclear membrane proteins and the nuclear lamins, while overviewing the key studies that are contributing to our evolving view of this important nuclear subcompartment.

Keywords Inner nuclear membrane · lamina · LADs · heterochromatin · histone · genome stability

2.1 The Conservation of the Spatial Positioning of the Genome Across Eukaryotes

The genome is encased in the nuclear envelope (NE) – a double membrane that is contiguous with the endoplasmic reticulum (ER). The biochemical (and thus,

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functional) specialization of the NE is conferred by a discrete proteome that includes nuclear pore complexes (NPCs) and specific membrane and membrane-associated proteins that directly interface with the genome and the inner nuclear membrane (INM; Fig. 2.1). Indeed, it is widely accepted that the nuclear periphery is a major component of nuclear architecture that contributes to the non-random organization of chromosomes within "territories" in the nucleus, a term first coined by Boveri in 1909 (Boveri 1909) but which was not directly visualized until many decades later (see (Cremer and Cremer 2010) for a more extensive historical overview). Moreover, most eukaryotic cells display a distinct segregation of (largely) transcriptionally silent heterochromatin at the nuclear periphery with more active euchromatin within the interior. This observation, made first by Rabl over 130 years ago (Rabl 1885), has been revisited time and again with ever increasing technological advances in many model organisms.

The obvious tethering of heterochromatin to the nuclear periphery from yeast to man has captivated our imaginations and given rise to long-standing hypotheses that posit a central role for peripheral tethering in regulating gene expression, whether to "gate genes" (Blobel 1985) or (conversely) to silence gene expression. Perhaps not surprisingly, decoupling gene recruitment to the nuclear periphery and processes linked to transcriptional up or down-regulation has proven extremely challenging. Nonetheless, work over the last several decades has delineated mechanisms by which the nuclear periphery acts as a critical platform for modulating transcriptional output, maintaining genome stability and regulating coordinated differentiation programs during development in multicellular eukaryotes. Here, we will overview the proteome and interactome of the NE and describe our molecular understanding of how the nuclear periphery, particularly the INM, impacts these critical genomic processes.

2.2 Integral INM Proteins

The nuclear periphery can be conceptualized as being "built" upon the resident components of the INM. It is well understood that the INM has a distinct proteome made up of integral membrane and membrane-associated proteins (Fig. 2.1), although a complete cataloguing of the INM has remained elusive. This is due to several experimental limitations: (1) the continuity of the NE and ER make biochemically isolating the INM a so-far insurmountable hurdle; (2) the relatively low abundance of many integral INM proteins, which is exacerbated by their insolubility; and (3) the likely ability of many ER proteins to sample the INM, without accumulating or functioning there (Deng and Hochstrasser 2006; Smoyer et al. 2016). As a consequence, a major fraction of the INM proteome at steady state is likely identical to that of the outer nuclear membrane (ONM)/ER, making even successful subtractive proteomics approaches (Schirmer et al. 2003) ineffective at conclusively differentiating between ONM and INM without additional experimental evidence. Indeed, while hundreds of NE transmembrane

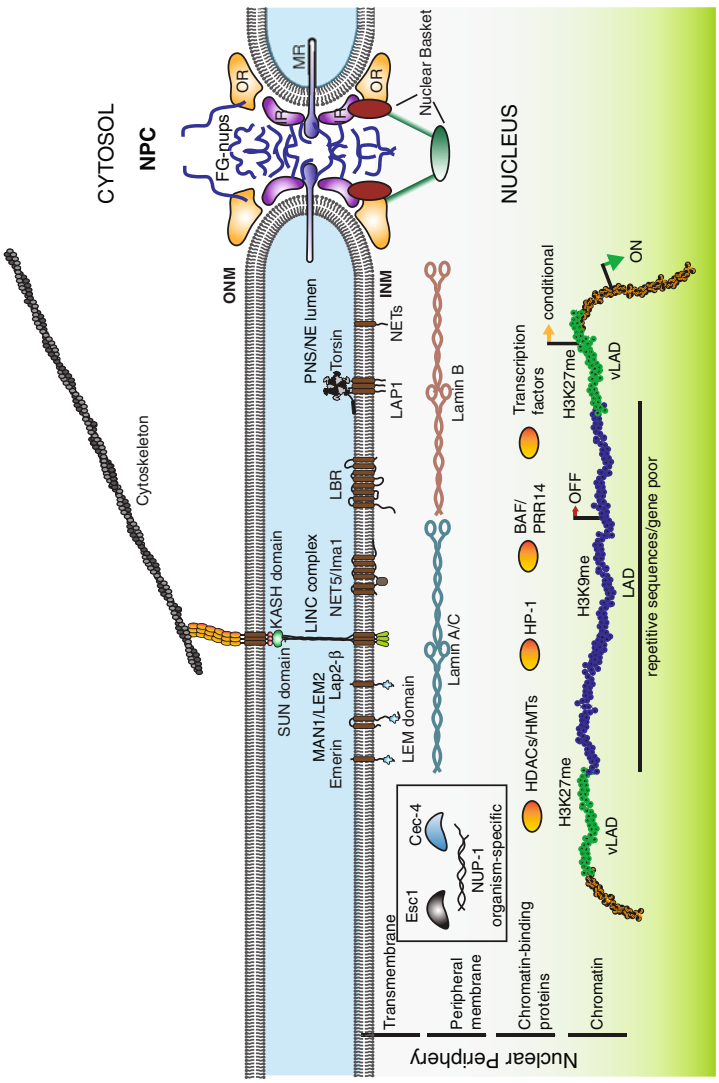

Fig. 2.1 Schematic of major components of the nuclear periphery required for genome tethering. The nuclear periphery is built on the inner and outer nuclear membranes (INM and ONM) with embedded nuclear pore complexes (NPC). NPCs are lined with FG-rich proteins scaffolded by subcomplexes like the outer ring (OR), inner ring (IR) and membrane ring (MR); the nuclear basket extends into the nucleoplasm. Well established integral INM proteins are shown including the SUN and KASH domain proteins that make up the linker of nucleoskeleton and cytoskeleton (LINC) complexes that span the perinuclear space (PNS)/nuclear envelope (NE) lumen and the Lap2-Emerin-MAN1 (LEM) domain proteins. Several peripheral membrane proteins have been identified, the most notable of which are the A- and B-type lamin networks. There are also other peripheral tethers in disparate model systems (Esc1, *Saccharomyces cerevisiae*, Cec-4, *Caenorhabditis elegans*, NUP-1, *Trypanosoma brucei*). The tethering and/or recruitment of lamina-associated domains (LADs) requires several factors including histone methyltransferases (HMTs) and/or histone deacetylases (HDACs), heterochromatin-binding proteins (HP-1; which might also require binding to PRR14), transcription factors like YY1 or cKROX and barrier to autointegration factor (BAF). In general LADs are transcriptionally silent and rich in H3K9me marks (blue) with H3K27me (green) at LAD borders. The LAD borders are often conditionally associated with the nuclear periphery and have been termed variable (v) LADs

proteins (NETs) have now been identified, some of which show tissue-specific expression (Schirmer et al. 2003; Korfali et al. 2010, 2012; Wilkie et al. 2011), there remains a laborious task of testing how many of these ultimately localize (and function) at the INM. The latter is particularly challenging as reagents such as specific antibodies are largely unavailable, necessitating a reliance on heterologous tagging/overexpression strategies that often lead to aberrant accumulation of the excess protein in the ER, therefore altering its steady-state distribution. Moreover, many heterologous tags, particularly large proteins such as GFP, can often interfere with membrane integration, and/or their targeting to the INM (Khmelinskii et al. 2014). Most critically, standard immunofluorescence microscopy cannot discern the INM from the ONM due to the inherent diffraction limited resolution of light microscopes, making immunoEM the gold standard for confirming INM localization (which is itself difficult and far from perfect given the decrease in effective localization accuracy when using secondary gold conjugated antibodies). Fortunately, technological innovations such as single molecular FRAP (Mudumbi et al. 2016) and super-resolution microscopy promise to supplant immunoEM to precisely localize integral membrane proteins (Korfali et al. 2016), suggesting that our understanding of the INM proteome will likely continue to expand with time.

2.3 A Brief History of INM Targeting

Like most subcellular compartments, the biochemical identity of the INM is assured by mechanisms that control protein targeting and quality control to turnover damaged or mistargeted proteins (Boban et al. 2014; Webster et al. 2014; Foresti et al. 2014; Khmelinskii et al. 2014; Turner and Schlieker 2016; Webster and Lusk 2016). While various hypothetical models for accumulating integral membrane proteins at the INM have been proposed (see (Katta et al. 2014) for discussion), there is a general consensus that membrane proteins travel along the continuous bilayer from the ONM/ER across the nuclear pore membrane to the INM (Lusk et al. 2007; Antonin et al. 2011; Laba et al. 2014) (Fig. 2.2). For example, several studies support that disruption of specific NPC components (nucleoporins/nups) influences the kinetics and/or steady-state distribution of integral INM proteins (Ohba et al. 2004; King et al. 2006; Deng and Hochstrasser 2006; Theerthagiri et al. 2010; Mitchell et al. 2010; Zuleger et al. 2011; Meinema et al. 2011; Boni et al. 2015; Ungricht et al. 2015; Lokareddy et al. 2015).

But, what is the mechanism of INM targeting? Moreover, do all INM proteins utilize the same mechanism or do subsets of INM proteins access distinct mechanisms? Early studies examining the localization of the Lamina Associate Polypeptide-1 (LAP1) and others revealed that binding to elements of the nuclear architecture (particularly nuclear lamins) plays a critical role in determining the steady-state distribution and immobilization of most integral membrane proteins at the INM (Powell and Burke 1990; Smith and Blobel 1993; Soullam and Worman

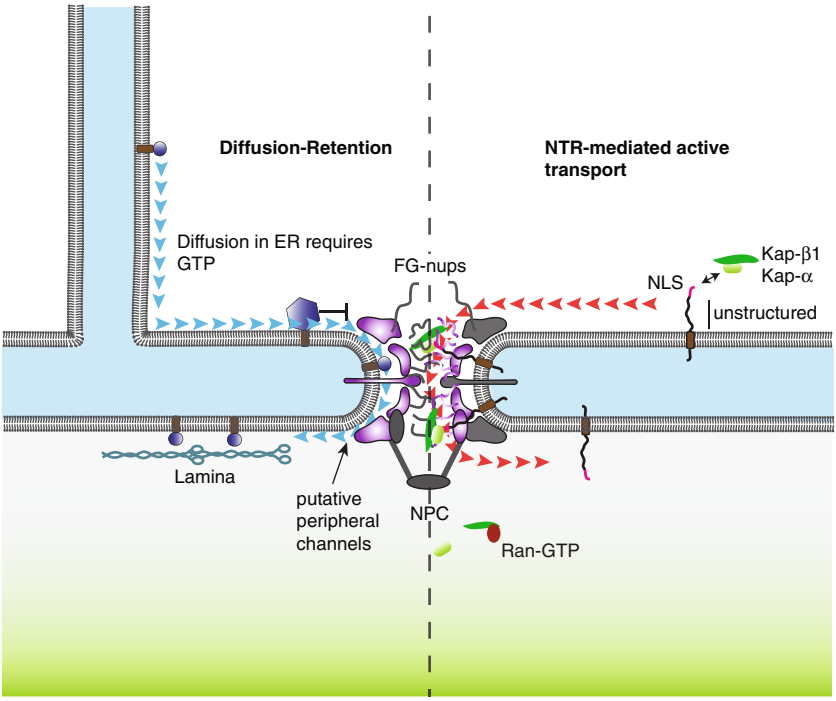

Fig. 2.2 Models of integral membrane protein targeting to the INM. In the diffusion-retention model (left; blue arrows), GTP is required to remodel the ER in a way that promotes membrane protein diffusion and thus the probability of reaching the nuclear pore membrane is increased; passage by the NPC is likely through putative peripheral channels that impose a molecular weight cut-off (small blue circles pass whereas large hexagons do not). Retention is mediated by binding to nuclear factors like the lamins or chromatin. In the NTR-based model, GTP is required for the Ran cycle with Ran-GTP dissociating INM protein cargo from the NTRs Kap-β1/Kap-α in a mechanism directly analogous to soluble nuclear transport. Such a model requires that extralumenal domains reach into the central transport channel of the NPC to allow NTR binding to FG-nups. This is thought to be achieved by a long ~120 amino acid unstructured region that is capped by a high affinity NLS. NPC subcomplexes shaded purple have been shown to be required for either diffusion-retention or NTR-mediated transport. Key in Fig. 2.1

1993, 1995; Ellenberg et al. 1997; Ostlund et al. 1999; Vaughan et al. 2001; Gruenbaum et al. 2002; Ohba et al. 2004). These data, in combination with those remarking on the free diffusion of viral proteins that access the INM (Torrisi et al. 1987; Torrisi et al. 1989), were suggestive of a model of INM accumulation in which "diffusion-retention" is sufficient for INM targeting (Fig. 2.2). In such a model, a molecular weight cut-off of ~60 kD is established for extralumenal domains of membrane proteins (likely imposed by steric hindrance by the scaffold of the NPC); exposure of nucleoplasmic domains to the nuclear interior then allow for subsequent binding to (and retention by) a nuclear factor (typically thought to be nuclear lamins or chromatin). However, the simplicity of such a mechanism was challenged by work supporting the existence of an energy-dependent targeting

step; the requirement for energy was postulated to be essential to remodel the NPC scaffold to allow passage of membrane proteins along constrictive peripheral channels that line the nuclear pore membrane (Ohba et al. 2004).

The energy-requirement to accumulate a reporter at the INM stimulated consideration of potential active INM targeting pathways. As membrane proteins must pass the NPC, a logical hypothesis was that Ran-GTP and nuclear transport receptors (NTRs; a.k.a. karyopherins/importins/exportins) might, in addition to supporting soluble nuclear transport, also promote membrane protein targeting. Indeed, it was recognized that the integral INM protein lamin B receptor (LBR) has a nuclear localization signal (NLS; (Soullam and Worman 1993, 1995), as do many other INM proteins (Lusk et al. 2007)) that could, in principle, be recognized by NTRs. However, the classical SV40 large T-antigen NLS (recognized by the karyopherin/importin α/β1 heterodimer) or the nucleoplasmin NLS (recognized by transportin/karyopherin β2) fused to a heterologous type II ER membrane protein was insufficient to confer INM localization (Soullam and Worman 1995), putting this idea aside until the discovery of conserved integral INM proteins of the LAP2, emerin, MAN1 (LEM) family in budding yeast, Src1/Heh1 and Heh2 (King et al. 2006). Importantly, molecular insights into the ability of NTRs to promote targeting of yeast LEM domain proteins across the NPC explain the failure of these engineered constructs to localize to the INM (Meinema et al. 2011).

A stand out feature of Heh1 and Heh2 is the presence of a bipartite NLS just downstream of the conserved LEM domain. The Heh2 NLS directly binds the NTR Kap α in the absence of Kap-β1 (King et al. 2006), an atypical result for NLSs transported by the Kap α/β1 heterodimer, which usually require Kap-β1 to bind and remove an inhibitory domain of Kap-α that prevents NLS binding (Rexach and Blobel 1995; Fanara et al. 2000). Thus, this observation suggested that the Heh2-NLS binds to Kap-α with an unusually high-affinity that can effectively compete with its inhibitory domain, a hypothesis that was directly confirmed by subsequent biochemical and structural analyses (Lokareddy et al. 2015). In addition, this NLS is required for Heh2 to gain access to the INM; the molecular necessity of this high-affinity, Kap-α-specific NLS for INM targeting remains enigmatic to this day.

Consistent with the ability of an NLS and NTRs to promote the efficient targeting of LEM domain proteins to the INM in yeast, genetic ablation of Ran, Kap α/β1 and several nups also inhibited INM targeting of Heh2 (King et al. 2006; Meinema et al. 2011). Together these results support a model in which Heh2 uses the soluble transport machinery to gain access to the INM, a confounding result when one considers that the nuclear domains of Heh1 and Heh2 are 40–50 kD; the addition of Kap-α (and its binding partner Kap-β1) would contribute an additional ~200 kD of mass, making passage through the size-restricted channel along the nuclear pore membrane likely impossible.

Interestingly, consistent with earlier studies in mammalian cells (Soullam and Worman 1995), an NLS fused to a transmembrane domain was also insufficient to drive INM accumulation in yeast, suggesting that other sequence determinants are required to get membrane proteins, including Heh1 and Heh2, across the nuclear

pore membrane (Meinema et al. 2011). Surprisingly, virtually the entire N-terminal domain of Heh2 is necessary for efficient INM targeting; a breakthrough was the recognition that this domain is largely unstructured (Meinema et al. 2011) (Fig. 2.2). Indeed, sufficiency of targeting a multipass transmembrane component of the ER translocon to the INM could be achieved by addition of a high affinity NLS coupled to an extended, completely artificial unstructured linker of at least 120 amino acids (Meinema et al. 2011). Thus, the passage of this ~200 kD complex through the NPC is likely facilitated by the ability of the unstructured linker to cut through the pore membrane-proximal scaffold, with the NLS-associated NTR moving through the central transport channel (Fig. 2.2).

But, how universal is an active INM-targeting mechanism? Apart from a mammalian nuclear pore membrane protein (POM121) that shares a similar NLS-requirement for INM accumulation (Doucet et al. 2010; Funakoshi et al 2011; Kralt et al. 2015), this question remains to be fully answered. For example, a recent study where interpretation of an extensive analysis of the steady state and kinetics of the localization of multiple reporters (both modeled on native integral INM proteins and completely artificial reporters) in a permeabilized mammalian cell system firmly supports a diffusion-retention model (Ungricht et al. 2015). Similarly, the results from an RNAi-based screen examining the genetic requirements that contribute to the kinetics of INM targeting (with mathematical modeling) were again most consistent with diffusion-retention being the major determinant of INM protein distribution, although NTRs were among those factors identified that influenced INM targeting kinetics, albeit potentially indirectly (Boni et al. 2015). Moreover, the energy requirement for INM targeting could be attributed to the necessity of energy-dependent ER dynamics required for the lateral mobility of membrane proteins to increase the likelihood that they reach the nuclear pore membrane (Ungricht et al. 2015).

How can we reconcile these two, potentially antagonistic, views of the INM targeting pathway? One possibility is that with the evolution of an open mitosis (which might allow larger extralumenal domains access to the nucleus without having to travel past NPCs) coupled to a more elaborate nuclear architecture that includes the lamina, the advantages of an active targeting pathway were supplanted by other functional priorities. In addition, while the NPC itself is compositionally near-identical from yeast to man (Rout et al. 2000; Cronshaw et al. 2002), it is much larger in vertebrates (Yang et al. 1998) owing to a doubling of the stoichiometry of the scaffold nups (Bui et al. 2013). Indeed, while the major "Y-complex" is likely organized in a single head-to-tail ring in the yeast NPC (Alber et al. 2007), the second "Y" in humans overlaps the other resembling a brick wall (Bui et al. 2013; von Appen et al. 2015). Thus, it is possible that any plasticity that might allow an unfolded peptide to weave through the scaffold in yeast was lost as the NPC became more elaborate through evolution. Lastly, there may be specific proteins, such as the LEM domain proteins of yeast, for which a rapid, active mechanism is beneficial to prevent these factors from residing in the cytoplasmic compartment; in this context, such a pathway may not be a requirement for passage through the NPC, but instead a mechanism to promote import efficiency

immediately upon biosynthesis. Importantly, no one has yet directly visualized the passage of INM proteins across the nuclear pore membrane in yeast or in mammalian cell lines, leaving the door open for additional new discoveries.

2.4 The Nuclear Lamina

Virtually all the well-determined integral INM proteins in metazoans interact with the lamin network that lines the INM, made up of A- and B-type lamin proteins, which are members of the type V intermediate filament family (Fig. 2.1). The A-type lamins, lamin A and lamin C, are derived by alternative splicing of the single *LmnA* gene (Burke and Stewart 2013; Gruenbaum and Foisner 2015), while the broadly expressed B-type lamins, lamin B1 and lamin B2, are encoded by separate genes. While most lamins associate with the INM, there is also a soluble pool of A-type lamins within the nucleoplasm. INM association of lamins can be reinforced by post-translational farnesylation at their C-terminal CAAX box, although in lamin A additional processing by the protease Zmpste24 removes the C-terminus, leading to production of "mature" lamin A (Davies et al. 2009; Burke and Stewart 2014). The function of the processing of lamin A remains ill-defined, as a mouse model expressing only the (unmodified) lamin C splice variant is viable and without phenotype (Sullivan et al. 1999; Fong et al. 2006), while mutations that disrupt maturation leading to constitutive farnesylation of lamin A (a form of the protein called progerin) lead to severe human disease (Burke and Stewart 2013). In contrast, the farnesylation of lamin B1 is required for its function and contributes to its association with the INM (Moir et al. 2000; Burke and Stewart 2014).

Until recently, our understanding of the organization of the lamin network was derived from iconic electron microscopy images of an interlocking 10 nm-diameter thick orthogonally-organized filament network in frog oocytes observed over 30 years ago (Aebi et al. 1986). More recently, with the advent of super resolution light microscopy and the revolution in detector technology that has improved the resolution of cryo-EM, we are approaching a clearer in situ picture of the lamina in model systems and in human cells. For example, super-resolution microscopy studies provide a compelling description of distinct (yet interdependent) A- and B-type lamin filament networks (Shimi et al. 2015; Xie et al. 2016), which had been inferred from lower resolution approaches (Shimi et al. 2008; Taimen et al. 2009; Kolb et al. 2011). These networks, while distinct, rely on each other to form a cohesive nuclear lamina, although how they interface with one another remains unclear (Shimi et al. 2015). In addition, it is not well understood how other NE landmarks like NPCs might contribute to the formation and/or organization of distinct lamin networks. Intriguingly, NPCs are specifically recruited to filaments formed through the overexpression of lamin C (in a lamin A null background), but not lamin A, suggesting a specific molecular link between lamin C and the NPC, perhaps through the nuclear basket component, Tpr (Xie et al. 2016).

Direct physical links between the lamina and NPCs have been suggested by several studies (Smythe et al. 2000; Hawryluk-Gara et al. 2005; Al-Haboubi et al. 2011), and are likely visualized by cryo-EM views in which lamin filaments appear to directly contact NPCs (Grossman et al. 2012). Very recently, cryoelectron tomography has revealed that the nuclear lamina is predominantly composed of lamin tetramers that give rise to a meshwork of 3.5 nm filaments in somatic cells; this suggests that the organization of the lamins is morphologically distinct from all cytoplasmic cytoskeketal elements (Turgay et al. 2017).

The interdependence of the lamin filament networks supports a model in which the nuclear lamina provides mechanical support to the nucleus. This function is well established, with A-type lamins contributing substantially to nuclear rigidity when subjected to large deformations, which is likely most critical in "stiff" tissues where mechanical strain on the nucleus would be predicted to be high (Davidson and Lammerding 2014). Consistent with this idea, lamin A levels scale with tissue stiffness (Swift et al. 2013) and many of the diseases (the laminopathies; discussed in detail elsewhere (Burke and Stewart 2014)) associated with lamin dysfunction manifest as defects in nuclear shape/integrity. In response to small deformations another lamina component, the chromatin, and particularly the heterochromatin associated with the nuclear periphery, also contributes to the mechanical response of nuclei (King et al. 2008; Schreiner et al. 2015; Furusawa et al. 2015; Stephens et al. 2017). This network not only provides a bulwark that ensures nuclear integrity in cell culture (De Vos et al. 2011; Vargas et al. 2012; Hatch et al. 2013; Maciejowski et al. 2015), but most dramatically serves critical functions as migrating cells move through confined spaces either in vivo or in vitro (Denais et al. 2016; Raab et al. 2016).

These studies highlight that the nucleus is far from an island, but is instead mechanically integrated into the cell (and tissue) in which it resides. A growing body of work demonstrates that the nucleus and its interactions with the cytoskeleton through the Linker of Nucleoskeleton and Cytoskeleton (LINC) complexes, which bridge both INM and ONM to mechanically couple the nuclear lamina to the cytoplasmic cytoskeleton (Fig. 2.1), play important roles in responding to and coordinating forces generated by cells and tissues (Lombardi and Lammerding 2011). Tension exerted onto LINC complexes in isolated nuclei can drive changes in the post-translational modifications of key lamina components such as emerin (Guilluy et al. 2014), suggesting a potential role for the LINC complex in mechanotransduction (although the transcriptional outputs of such a cascade remain poorly defined in vivo). Further, several recent studies highlight that nuclear lamina components, including the conserved integral INM (Sad1p, UNC-84; SUN) proteins that comprise the inner aspect of LINC complexes, play important (and unexpected) roles in regulating cell-matrix and cell-cell adhesions (Stewart et al. 2015; Thakar et al. 2017). Taken together, these studies suggest that signaling between cell junctions at the cell surface, and LINC complexes, which can be envisaged as NE "junctions," given that they couple two lipid bilayers that span a lumenal or extracellular space (Blobel 2010), may provide mechanisms for mechanical communication to (and from) the nucleus that awaits further investigation.

2.5 Lamina Associated Domains

In addition to providing a mechanical scaffold that supports nuclear integrity, the lamins (with INM proteins) organize a network of interactions that both promote the formation of heterochromatic domains and physically link these domains to the INM in most differentiated cell types where it has been examined (Fig. 2.1). A key technological breakthrough that revolutionized our understanding of the chromatin interface with the nuclear periphery was the development of the Dam-ID approach, which relies on the expression (at low levels) of Lamin A or B fused to the bacterially-derived Dam-methylase (Pickersgill et al. 2006; Vogel et al. 2007); Dam specifically methylates adenines within the sequence GATC. Until very recently, adenine methylation within eukaryotic DNA was thought to be completely absent; while it is now recognized that this modification does take place at very low levels (Wu et al. 2016), the Dam-ID approach continues to provide advantages for the characterization of some protein-DNA interactions, such as for the lamina. Sites of adenine methylation can be identified genome wide using microarray chip technologies, or, more recently, next generation sequencing.

As might be expected from the physical enrichment of heterochromatin at the nuclear periphery, lamina-associated domains (LADs) are 0.1–10 Mb chromosomal regions rich in repetitive gene-poor "deserts" covering ~30% of the genome (Guelen et al. 2008). Clues to what might define LADs can be found by analyzing their boundaries, which contain binding sites for insulator elements like CTCF, CpG islands and active promoters transcribing away from the border (Guelen et al. 2008). The enrichment for CTCF is particularly interesting as it is postulated to bring sequence specificity to organize architectural proteins of the SMC family (cohesin and condensin) to define so-called Topologically Associated Domains (TADs; (Dixon et al. 2012; Jin et al. 2013; Fudenberg et al. 2016)) revealed by chromosome conformation capture methods that identify genomic regions that are proximal in space (reviewed in (Dixon et al. 2016)). Indeed, at least in some contexts (like the X-chromosome) it seems clear that LADs likely represent a subset of TADs, and therefore are defined by some of the same topological determinants (Nora et al. 2012).

Interestingly, actively transcribed genes can also be found in LADs, suggesting that peripheral tethering, per se, is not sufficient to inhibit transcription ((Wu and Yao 2013) and more on this below). However, as NPCs have been established to be linked to active transcription in a variety of model systems (see Chap. 3), an alternative possibility is that Dam-ID may not have sufficient resolution to distinguish the differential association of a gene promoter with NPCs rather than the lamina. Lastly, it is important to consider that most Dam-ID (and ChIP) experiments result in population-based metrics, which average out single-cell variability. To this point, the development of single-cell Dam-ID reveals that the majority of LADs are stochastically associated with the nuclear periphery (Kind et al. 2013), with only ~15% of LADs establishing more stable "backbone" interactions (Kind et al. 2015).

2.6 LADs as Developmentally Regulated Regions

The observation that many LADs display high variability within cell populations could be interpreted in two very different contexts. In the first, this observation could reflect a highly stochastic aspect of nuclear compartmentalization, suggesting that gene regulation may, in many cases, be independent of gene position. Indeed, recent studies in *Caenorhabditis elegans* showing that peripheral tethering of heterochromatin is not essential to maintain gene silencing, supports this point of view ((Gonzalez-Sandoval et al. 2015); more below). However, a compelling case can also be made for the second context, in which subnuclear compartmentalization leads to direct functional consequences. Indeed, a wealth of studies have documented that altered subnuclear localization of a given locus (or group of loci responsive to a given input) occurs concurrent with the execution of differentiation programs in cells and organisms; individual examples include the immunoglobulin heavy-chain (IgH)(Kosak et al. 2002; Reddy et al. 2008), β-globin (Ragoczy et al. 2006) and CFTR (Zink et al. 2004) loci. However, altered subnuclear compartmentalization is likely to be much more extensive in some contexts; indeed, genome-wide studies using Dam-ID reveal that two thirds of LADs dissociate from the nuclear periphery upon murine stem cell differentiation (Peric-Hupkes et al. 2010). Such changes can also be recapitulated using repetitive transgenes in multicellular models like *C. elegans*, which are released from the nuclear periphery in fully differentiated tissues (Meister et al. 2010). How the observed changes in subnuclear distribution mechanistically impact gene output in many of these cases still remains largely enigmatic. However, insights can be gleaned from the investigation of the *MyoD* locus, which is released from the nuclear periphery during myogenesis. In this case, regulatory transcription factors exhibited distinct steady-state distributions with respect to the nuclear periphery, which correlated with their occupancy on the *MyoD* promoter during differentiation (Yao et al. 2011). These data suggest that release from the periphery might promote encounters with distinct transcriptional environments.

It may well be that satisfying universal rules for how subnuclear position and transcription are related across the genome will never arise. For example, several landmark studies exploited conditional genomic tethering systems to directly test whether peripheral tethering was sufficient to infer transcriptional silencing (Kumaran and Spector 2008; Reddy et al. 2008; Finlan et al. 2008). While these studies generally support a model in which peripheral tethering leads to a down-regulation of transcription at genic regions surrounding the tether (Reddy et al. 2008; Finlan et al. 2008), it is clear that transgenes driven by high level promoters can be insensitive to repression at the nuclear periphery, as assessed by the recruitment of RNA Pol II and the kinetics of transcriptional activation (Kumaran and Spector 2008). Taken together, these results suggest that gene tethering at the nuclear periphery might be an initial step that provides a platform for the subsequent recruitment of other factors that ultimately confer silencing on specific genes with (potentially) specific promoters.

Consistent with the concept that peripheral tethering is simply a first step in a more elaborate gene inactivation program, conditional tethering of transgene loci resulted in the local recruitment and accumulation of integral INM proteins like LAP2, lamin B and Emerin (Kumaran and Spector 2008; Reddy et al. 2008). As these factors also bind to chromatin modifying enzymes like histone deacetylases (HDACs; (Somech et al. 2005)), it is easy to imagine a scenario in which transcriptionally active acetylated chromatin is locally deacetylated as a transition to a more silenced state. Consistent with such a model, inhibition of deacetylases led to the reversal of the tethering-induced transcriptional down-regulation (Finlan et al. 2008). And indeed, HDAC3 can target LADS (Zullo et al. 2012). Thus, silencing is likely determined by local chromatin structure; consistent with this, the introduction of local chromatin decondensation is sufficient to induce dissociation of genetic loci from the NE (Therizols et al. 2014).

2.7 Histone Modifications at the Nuclear Periphery

In addition to the nuclear periphery being relatively free of "active" histone marks, it is also rich in silent epigenetic signatures like histone 3 lysine 9 (H3K9) and H3K27 methylation (me; Fig. 2.1); H3K27 tri-methylation (me3) tends to be more enriched at LAD borders (Pickersgill et al. 2006; Guelen et al. 2008; Ikegami et al. 2010; Towbin et al. 2012; Kind et al. 2013; Bian et al. 2013), which are more variably associated with the nuclear periphery (so called variable "v" or facultative LADs; Fig. 2.1). As most heterochromatin is rich in H3K9/K27me, this result is not overly surprising. However, a more functional connection is suggested by the observation that the silent epigenetic signature is likely itself essential for the physical association of heterochromatin with the nuclear periphery. For example, while random insertion of the β-globin locus resulted in its targeting to the nuclear periphery, this peripheral association could be prevented by co-inhibition of the methylases required for both H3K9me2 and H3K9me3 (G9a and Suv39H1/2, respectively; (Bian et al. 2013)). Similar results were observed for specific vLAD-sequences (Harr et al. 2015). Indeed, the requirement for H3K9me is likely a conserved feature of genome-INM contacts in all Metazoa; for example, hundreds of tandem arrays of transgenes associate with the INM and accumulate H3K9me and H3K27me marks in *C. elegans*, which also provided a genetic platform to probe the requirements for INM association in a multicellular genetic model (Towbin et al. 2010).

Interestingly, while genetic screens in *C. elegans* identified dozens of factors that could de-repress transgene arrays, only the knockdown of two near-identical S-adenosyl methionine synthetases (SAMs) resulted in both array de-repression and de-localization from the nuclear periphery (Towbin et al. 2012), supporting a critical role for histone methylation as the key nexus of these two aspects: gene output and subnuclear compartmentalization. Consistent with the theme that transcription does not influence nuclear position relative to the periphery,

de-repression of the arrays was not sufficient, nor was it required for peripheral release. Interestingly, the effects of SAM inhibition could be recapitulated by the specific knockdown of two histone methylases, SET-25 (the homologue of mammalian G9a and SUV39h1/2) and MET-2 (homologue of SETDB1), which both target H3K9. Consistent with the idea that H3K9 was the essential histone modification that conferred INM tethering, only H3K9me2 and me3 were globally reduced in set-25/set-2 animals while H3K23, K27 or K36 were largely unaffected. Furthermore, a careful analysis of methyl marks after individual deletion of SET-2 and SET-25 supported a step-wise model, with SET-2 providing the H3K9mono and di-methyl substrate for SET-25. Interestingly, SET-25 itself is enriched at the nuclear periphery through (likely) indirect interactions with the H3K9me3 marks that it produces (Towbin et al. 2012), supporting a model of action in which local tri-methylation is amplified by a self-reinforcing cycle of SET-25 recruitment and catalysis.

2.8 Peripheral Tethers

The growing functional links between H3K9me and peripheral tethering supports the existence of INM proteins capable of mediating direct physical interactions with specific chromatin domains either through histone modifications, transcription factors and/or direct binding to DNA sequence elements (Fig. 2.1). While there is some evidence that lamin A/C might directly bind to DNA ((Kubben et al. 2012) and references therein), it is likely that much of the defective heterochromatin tethering to the nuclear periphery observed in lamin A/C-null cells is due to mislocalization of other integral INM proteins like the LEM proteins, which themselves might directly or indirectly (through effectors like BAF) interact with DNA (Brachner and Foisner 2011). Interestingly, the contribution of lamin A to heterochromatin tethering activity appears to be at least partially redundant with that of LBR (Solovei et al. 2013). This redundancy was elegantly illustrated in specialized murine retinal cells that possess an "inverted" nuclear architecture with heterochromatin concentrated at the center of the nucleus; this adaptation is thought to help focus light to improve night-vision (Solovei et al. 2009). Indeed, many nocturnal animals exhibit this change in global chromosome organization and these morphological changes are correlated with a repression of both lamin A and LBR expression in these cells. Moreover, the experimentally controlled up-regulation of LBR could mitigate these effects (Solovei et al. 2013) supporting a direct role for LBR as a peripheral tether. Indeed, in addition to binding to lamin B, LBR also directly interacts with the H3K9me3-binding protein HP-1 and has a Tudor domain that recognizes the silencing H4K20me2 modification (Hirano et al. 2012).

The redundancy between LBR and lamin A with respect to maintaining heterochromatin at the nuclear periphery could reflect the critical importance of this aspect of nuclear organization; redundancy is also reflected in the evolutionary expansion in the number of the LEM domain protein paralogues (and likely

others) (Brachner and Foisner 2011; Barton et al. 2015). Additional tethers have also been recently identified like proline-rich protein 14 (PRR14), a dynamic soluble protein that serves as a bridge between the lamins and HP-1(Robson et al. 2016), and there are also NETs that modulate peripheral anchoring in specific tissues (Zuleger et al. 2013). Dealing with this level of complexity (and integration) provides a challenge to cleanly defining a function for chromatin tethering to the nuclear periphery, making simpler genetic model systems essential to define the mechanistic paradigms. For example, in *C. elegans* a genetic screen to identify a specific H3K9me2/3-tether of an integrated repetitive transgene array identified the novel factor Cec-4. Cec-4 is not an integral INM protein but nonetheless specifically associates with the INM through a lamin-independent mechanism that remains to completely defined (Gonzalez-Sandoval et al. 2015) (Fig. 2.1). Consistent with its tethering activity, Cec-4 contains a chromodomain that specifically recognizes H3K9me3; mutation of this domain leads to a loss of the peripheral array position and, most importantly, globally affects chromosome tethering to the INM. Interestingly, however, in differentiated larva the effects of Cec-4 depletion on chromosome position were more muted, suggesting that there are likely multiple redundant tethers in differentiated cells of *C. elegans*, perhaps a reflection of a nuclear architecture that becomes more cemented to ensure the maintenance of cell fate. Consistent with this idea, depletion of Cec-4 (and thus peripheral tethering) did not maintain an artificially induced muscle-cell fate in embyros (Gonzalez-Sandoval et al. 2015).

2.9 Lamina Associated Sequences

In a scenario in which there are multiple INM tethers, some of which are tissue specific, one strategy might be to approach mechanisms of peripheral tethering intrinsic to the DNA sequence itself. While "DNA zip codes" that are sufficient to confer gene localization to the nuclear periphery have been long identified in unicellular models like yeasts (Ahmed et al. 2010), these sequences have been more challenging to identify in multicellular eukaryotes. Nonetheless, by focusing on the developmentally regulated regions of LADs that are variably associated with the lamina, some sequence elements sufficient to confer lamina association have been identified. These sequences are rich in GA dinucleotides (unlike most of LADs that are A/T rich) and have been termed lamina associated sequences (LASs) (Zullo et al. 2012; Harr et al. 2015). Interestingly, like in budding yeast, in which nuclear peripheral targeting of the DNA zip codes is conferred by direct binding to transcription factors (Brickner et al. 2012), the GAGA transcription factor cKROX (Zullo et al. 2012) and also "Ying Yang 1" (YY1) (Harr et al. 2015) were identified as key factors that could direct LASs to the nuclear periphery, perhaps (and very intriguingly) specifically through binding to lamin C (Harr et al. 2015). In addition, cKROX association with LASs persists throughout mitosis, suggesting that LAS binding by soluble elements of the transcription machinery

are likely early events that help re-establish nuclear organization as the NE reforms at mitotic exit (Zullo et al. 2012), thus providing compelling evidence for a concept proposed by Blobel 30 years ago (Blobel 1985).

2.10 Beyond Silencing: The Periphery and Genome Integrity

Given the putative myriad of (often redundant) chromatin tethers and the likely multifactorial nature of sequence elements and gene-specific binding proteins in tying gene position with gene output, defining explicit function(s) for anchoring of chromatin to the nuclear periphery will remain a persistent challenge. Moreover, even in systems like *C. elegans*, in which it is possible (at least in early development) to release heterochromatin from the periphery through deletion of Cec-4, this chromatin nonetheless remains silenced in the nuclear interior (because it retains H3K9me2/3 marks; (Gonzalez-Sandoval et al. 2015)). Perhaps most shockingly, however, is the finding that the abrogation of H3K9 methylation, which can be achieved in *C. elegans*, gives rise to completely viable animals, with only rare, mild phenotypic abnormalities (Towbin et al. 2012; Zeller et al. 2016). Thus, H3K9 methylated heterochromatin, much of which is associated with the nuclear periphery, is dispensable for the development of a multicellular organism. Interestingly, however, after a few generations the worms became completely sterile; this arises due to massive, p53-dependent apoptosis in the germline, suggesting persistent DNA damage (Zeller et al. 2016). Consistent with this, use of a reporter construct in somatic cells from these animals revealed an increase in insertion-deletion (indel) rates specifically within a heterochromatic chromatin environment. Taken together, these data suggested that H3K9me (and perhaps peripheral tethering) is perhaps most important for maintaining genome stability within silenced regions of the genome.

2.11 Repetitive DNA and the Nuclear Periphery

Looking back, the connection between repetitive DNA, the nuclear periphery, and genome stability first arose in pioneering work in the relatively "simple" unicellular yeast models. While budding yeast do not utilize the H3K9me modifications, they nonetheless compartmentalize repetitive regions of their genomes at the nuclear periphery, most notably the ribosomal DNA (rDNA) repeats housed in the peripheral nucleolar compartment (Taddei and Gasser 2012). Indeed, the concept that the INM could promote genome stability was first established in work examining the stability of rDNA repeats (Mekhail et al. 2008). rDNA repeats are tethered to the nuclear periphery by a complex of proteins called Chromosome Linkage Inner nuclear membrane Proteins (CLIP). The INM tether for this complex is the conserved integral INM protein Heh1/Src1 (a member of the LEM

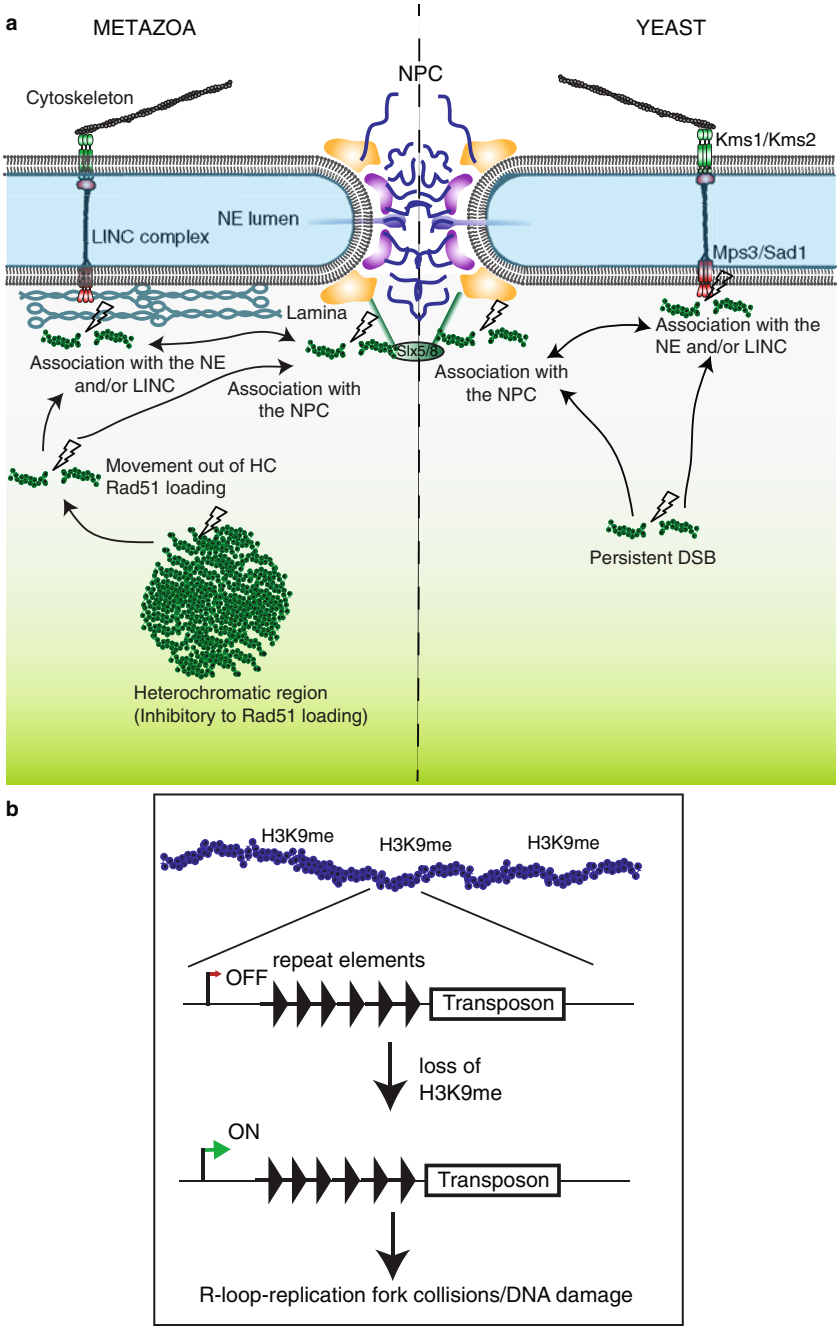

Fig. 2.3 Subnuclear compartments and epigenetic modifications influence genome integrity. (a) Studies in yeast first revealed that persistent DNA double-strand breaks (DSBs) that initially reside in the nuclear interior move to the nuclear periphery to associate with the nuclear pore complex (Continued)

domain family; (King et al. 2006)) and its yeast-specific binding partner Nur1 (Mekhail et al. 2008). Remarkably, deletion of Heh1 leads to a loss of nucleolar structure and an increase in copy number changes within the rDNA repeats, suggesting heightened homologous recombination, which could drive repeat expansion or contraction (Mekhail et al. 2008). Indeed, it was previously established that loading of recombination factors such as Rad52 onto a lesion within the rDNA occurs only once it moves out of the peripheral nucleolar compartment (Torres-Rosell et al. 2007), first suggesting the concept that nuclear compartments can influence DNA repair mechanisms. In addition, recent studies suggest that such regulation is conserved in higher eukaryotes, as the H3K9me-binding protein HP-1 inhibits Rad51 loading onto heterochromatic DNA double strand breaks (DSB) in *Drosophila* (Chiolo et al. 2011) (Fig. 2.3a) while tethering of a site-specific DSB to the nuclear periphery also abrogates loading of homologous recombination factors in human cells (Lemaître et al. 2014).

In addition to influencing repair mechanisms specifically in repetitive regions of the genome, there is ample evidence that the nuclear periphery influences both mechanisms that drive DNA DSBs and the pathways that repair such lesions (Seeber and Gasser 2016) (Fig. 2.3a). Returning to *C. elegans*, the DNA damage observed in the absence of H3K9me was traced to an increase in RNA-DNA hybrids or "R-loops," which can lead to collisions with replication forks to drive fork collapse (Zeller et al. 2016) (Fig. 2.3b). In this way, aberrant transcription could still be the root cause of the genome integrity defects in worms lacking H3K9me. Interestingly, an earlier study linked topological stress at highly transcribed genes associated with the NPC to DNA damage, which was suggested to be normally attenuated through phosphorylation events orchestrated by the Mec1/ATR pathway (Bermejo et al. 2011). In this context, controlled release of these genomic regions from the periphery preserves genome integrity, and could be avoided in the absence of this pathway through deletion of proteins necessary for NPC basket formation (Bermejo et al. 2011). The possibility for DNA damage driven by topological constraints imposed by chromatin-NE tethers remains to be fully characterized, but presents an important area of future research, particularly as several recent reports suggest site-specific DNA damage programs that play critical roles in development (Madabhushi et al. 2015) – a tantalizing clue that such mechanisms might be functionally important and not just unintended by-products of genome organization.

◀ (NPC) or the SUN proteins (Mps3/Sad1). In fission yeast, persistent DSBs that associate with the SUN protein Sad1 form LINC complexes that interact with cytoplasmic microtubules. More recently, studies in Metazoa reveal that irradiation-induced DSBs within internal heterochromatin (HC) do not load Rad51, while movement of these DSBs into the euchromatic environment facilitates Rad51 loading. These DSBs can also go on to associate with either LINC complexes or the NPC at the nuclear periphery. In both yeast and Metazoa, peripheral association is promoted by post-translational modification (SUMOylation) of repair factors. (b) Loss of H3K9me in *C. elegans* leads to derepression of transposable elements. The resulting transcript, in the form of an R-loop, leads to collisions with the replication machinery, driving formation of DSBs.

Considering that DNA damage might occur in specific regions of the genome, are there hotspots that become fragile in the absence of H3K9me? Here, again, the advantages of the *C. elegans* system come into play. While over half of the human genome consists of repetitive elements (REs: SINES, LINES, retrotransposons, TY elements, etc.), most of these cannot be uniquely mapped from next-generation sequencing data due to the lack of unique sequence features. By contrast, while the *C. elegans* genome houses many REs, 80% of these are uniquely mappable, allowing the authors to demonstrate that R-loops accumulate specifically within REs, concomitant with loss of H3K9me marks (Zeller et al. 2016) (Fig. 2.3b). Moreover, large indels in these regions, often adjacent to transposon sequences, suggests that aberrant transcription of transposons might drive the loss of genome stability. In this context, it is the act of transcription rather than the transposon up-regulation itself that drives genome instability, suggesting that transposons need not "jump" to drive losses in genome integrity. Importantly, loss of the H3K9me-binding protein HP-1 leads to loading of the recombination factor Rad51 inside heterochromatic domains in *Drosophila* (Chiolo et al. 2011), suggesting a possible mechanism by which abrogation of H3K9me compromises genome integrity due to illegitimate recombination.

2.12 Inputs of Nuclear Compartmentalization on DNA Repair Mechanisms

Beyond the ability of H3K9me/HP-1 to inhibit Rad51 loading, what else is known about how distinct nuclear compartments influence DNA repair mechanisms when a DNA lesion does occur? Pioneering studies in yeast over the past ten years have unearthed a great deal of insights into this question. While much of this work has been extensively reviewed elsewhere (Seeber and Gasser 2016), here it is important to highlight that both the NPC and LINC complexes (or their constituent parts, such as the factors associated with the NPC basket or the SUN protein Mps3 in budding yeast) have been shown to be repositories for persistent DNA DSBs, each with unique contexts and consequences ((Nagai et al. 2008; Kalocsay et al. 2009; Oza et al. 2009; Swartz et al. 2014; Horigome et al. 2014); Fig. 2.3a).

Combining systems to tag genomic loci with heterologous operator arrays recognized by fluorescent protein fusions of their cognate binding proteins (for example, lacO/lacI or tetO/tetR) with inducible, site-specific DSB induction systems has allowed investigators to monitor the compartmentalization of single DSBs within the nuclear volume; in haploid yeast, such DSBs are irreparable but are recruited to the nuclear periphery, where they colocalize with NPCs (Nagai et al. 2008). The nuclear aspect of the NPC is linked to a SUMO-targeted ubiquitin ligases or STUbL – the Slx5/Slx8 heterodimer; driving association of a DSB with the nup, Nup84, or Slx8 is sufficient to increase rates of homologous recombination through gene conversion, but also promotes errant repair mechanisms such as break-induced replication and alternative non-homologous end joining ((Nagai et al. 2008);

Fig. 2.3a). Extensive further work has revealed that SUMOylation through the ligases Siz2 and Mms21 occurs upstream of peripheral DSB recruitment and Slx5/8, although the key substrates remain enigmatic (Horigome et al. 2016).

Through a molecularly distinct pathway, persistent DSBs are recruited to the SUN protein Mps3 (in budding yeast (Kalocsay et al. 2009; Oza et al. 2009)) or Sad1 (in fission yeast (Swartz et al. 2014); Fig. 2.3a). This pathway is active specifically during S and/or G2, lies downstream of initial processing events that commit the DSB to repair by homologous recombination, and has specific requirements for the Ino80 chromatin remodeling complex and the histone variant H2AZ (Kalocsay et al. 2009; Horigome et al. 2014). Association with Mps3/Sad1 is thought to both inhibit (perhaps non-allelic) DSB repair or errant repair of deprotected telomeres by homologous recombination, and/or to promote repair from alternative homologous templates (Oza et al. 2009; Swartz et al. 2014; Horigome et al. 2014); this pathway may be related to the role that LINC complexes play in promoting proper homologous chromosome pairing in meiosis (Hiraoka and Dernburg 2009).

For some time, the broad conservation of the mechanistic roles for the nuclear periphery in DSB repair had been questioned, derived primarily from the irreparable nature of the DSB models used in many yeast studies and the relatively smaller nuclear volume compared to mammalian cells. However, several recent studies highlight that the same pathways first identified in yeast are active in multicellular eukaryotes. Indeed, the observation that Rad51 loading occurs only after irradiation-induced DNA lesions move out of heterochromatic compartments in *Drosophila* (Chiolo et al. 2011) mirrors the earlier work of Torres-Rossel, who made the same observation for Rad52 loading onto the rDNA (Torres-Rosell et al. 2007). Moreover, it was recently demonstrated that SUMO ligases are necessary for the movement of DSBs out of heterochromatic compartments (Ryu et al. 2015); a subset of these DSBs then move to the nuclear periphery to associate with the NPC and/or LINC complex components in a pathway dependent on STUbLs (Ryu et al. 2015), again similar to results observed in yeast (Horigome et al. 2016). One critical question that remains to be fully investigated is how the chromatin mobility necessary for a DSB to move from an internal heterochromatic compartment to the nuclear periphery is achieved, particularly given the observation that chromatin loci are highly constrained in mammalian cells, as suggested by the inability of genic loci to be effectively tethered to the NE without passage of cells through mitosis (Kumaran and Spector 2008; Reddy et al. 2008; Zullo et al. 2012; Kind et al. 2013).

Again, seminal work in yeast suggests that formation of a DSB significantly increases its mobility within the nucleus, concomitant with a global increase in chromatin mobility (Miné-Hattab and Rothstein 2012; Seeber et al. 2013); these mechanisms have both common and specific genetic requirements, but likely involve chromatin remodeling complexes (such as Ino80 in budding yeast); their conservation remains to be tested. Roles for the LINC complex and the cytoskeleton in mediating an increase in the mobility of DNA lesions also appear to be conserved, as cytoplasmic microtubules act to promote DSB (or critically short

telomere) mobility, supporting interhomologue or ectopic homologous recombination in fission yeast (Swartz et al. 2014) or deleterious end joining reactions in mammalian cells (Lottersberger et al. 2015) (Fig. 2.3a). The LINC complex may play additional roles in regulating repair mechanism choice by suppressing NHEJ at lesions caused by cisplatin treatment in *C. elegans* (Lawrence et al. 2016). Thus, increased mobility of DSBs may facilitate encounters that allow repair to occur, but whether this promotes faithful repair or reactions that drive genome stability may depend on the context (i.e. how many lesions there are in a single nucleus). One open question is whether the LINC complex is associated with DNA lesions (as in fission yeast (Swartz et al. 2014)) or acts "at a distance" (Lottersberger et al. 2015). A recent study suggests that the LINC complex may do both, including roles for regulating repair factor localization and/or function through the nucleoplasmic domain of SUN proteins (Lawrence et al. 2016). It is worth noting that roles for direct physical association of DNA lesions with the NPC or LINC complex could be particularly important in repetitive, heterochromatic regions, as genetic ablation of DSB-nuclear periphery interactions leads to fusions and aneuploidies specifically in regions of the genome rich in H3K9me (Ryu et al. 2015). Taken together, these observations provide an additional rationale for why repetitive, H3K9me regions of the genome are found associated with the nuclear periphery: to poise them for regulation of DNA repair through NPCs or the LINC complex without the need for dramatic chromatin mobility.

2.13 Outlook

The advent of new molecular approaches to investigate the interface between the nuclear periphery and the genome has reinforced the decades-old concept that the INM maintains a tight association with transcriptionally silent chromatin. Surprisingly, however, recent studies suggest that transcription per se has limited impact on this association. Indeed, our understanding of the functional impacts that chromatin tethering to the INM has on its emergent biology is currently in flux. This revolution is being driven by pioneering work in multiple model systems; an emerging theme is that the nuclear periphery plays a critical role in maintaining genome stability. As the number of repetitive elements, many of which are derived from transposons and retroviruses, have infiltrated our genome, it is perhaps not surprising that we have developed effective means to silence these factors (Gasser 2016). How the periphery contributes to silencing repetitive elements is just beginning to come to light, but what is most exciting is that their de-repression in the germline might be deliberate to promote adaptation to environmental stress (Gangaraju et al. 2011). In this context, the nuclear periphery may be most critical during differentiation and when cell fate decisions must be established and maintained. This ongoing reconceptualization of the functions that the nuclear periphery supports provides an essential foundation to further understand the ever growing list of genetic connections between defects in the nuclear lamina and disease.

References

Aebi U, Cohn J, Buhle L et al (1986) The nuclear lamina is a meshwork of intermediate-type filaments. Nature 323:560–564. https://doi.org/10.1038/323560a0

Ahmed S, Brickner DG, Light WH et al (2010) DNA zip codes control an ancient mechanism for gene targeting to the nuclear periphery. Nat Cell Biol 12:111–118. https://doi.org/10.1038/ncb2011

Al-Haboubi T, Shumaker DK, Köser J et al (2011) Distinct association of the nuclear pore protein Nup153 with A- and B-type lamins. Nucleus 2:500–509. https://doi.org/10.4161/nucl.2.5.17913

Alber F, Dokudovskaya S, Veenhoff LM et al (2007) The molecular architecture of the nuclear pore complex. Nature 450:695–701. https://doi.org/10.1038/nature06405

Antonin W, Ungricht R, Kutay U (2011) Traversing the NPC along the pore membrane: targeting of membrane proteins to the INM. Nucleus 2:87–91. https://doi.org/10.4161/nucl.2.2.14637

Appen vonA, Kosinski J, Sparks L et al (2015) In situ structural analysis of the human nuclear pore complex. Nature 526:140–143. https://doi.org/10.1038/nature15381

Barton LJ, Soshnev AA, Geyer PK (2015) Networking in the nucleus: a spotlight on LEM-domain proteins. Curr Opin Cell Biol 34:1–8. https://doi.org/10.1016/j.ceb.2015.03.005

Bermejo R, Capra T, Jossen R et al (2011) The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores. Cell 146:233–246. https://doi.org/10.1016/j.cell.2011.06.033

Bian Q, Khanna N, Alvikas J et al (2013) β-Globin cis-elements determine differential nuclear targeting through epigenetic modifications. J Cell Biol 203:767–783. https://doi.org/10.1083/jcb.201305027

Blobel G (1985) Gene gating: a hypothesis. Proc Natl Acad Sci 82:8527–8529

Blobel G (2010) Three-dimensional organization of chromatids by nuclear envelope-associated structures. Cold Spring Harb Symp Quant Biol 75:545–554. https://doi.org/10.1101/sqb.2010.75.004

Boban M, Pantazopoulou M, Schick A et al (2014) A nuclear ubiquitin-proteasome pathway targets the inner nuclear membrane protein Asi2 for degradation. J Cell Sci 127:3603–3613. https://doi.org/10.1242/jcs.153163

Boni A, Politi AZ, Strnad P et al (2015) Live imaging and modeling of inner nuclear membrane targeting reveals its molecular requirements in mammalian cells. J Cell Biol 209:705–720. https://doi.org/10.1083/jcb.201409133

Boveri T (1909) Die Blastomerenkerne von A*scaris megalocephala* und die Theorie der Chromosomenindividualität. Arch Zellforsch 3:181–268

Brachner A, Foisner R (2011) Evolvement of LEM proteins as chromatin tethers at the nuclear periphery. Biochem Soc Trans 39:1735–1741. https://doi.org/10.1042/BST20110724

Brickner DG, Ahmed S, Meldi L et al (2012) Transcription factor binding to a DNA zip code controls interchromosomal clustering at the nuclear periphery. Dev Cell 22:1234–1246. https://doi.org/10.1016/j.devcel.2012.03.012

Bui KH, Appen von A, DiGuilio AL et al (2013) Integrated structural analysis of the human nuclear pore complex scaffold. Cell 155:1233–1243. https://doi.org/10.1016/j.cell.2013.10.055

Burke B, Stewart CL (2013) The nuclear lamins: flexibility in function. Nat Rev Mol Cell Biol 14:13–24. https://doi.org/10.1038/nrm3488

Burke B, Stewart CL (2014) Functional architecture of the cell's nucleus in development, aging, and disease. Curr Top Dev Biol 109:1–52. https://doi.org/10.1016/B978-0-12-397920-9.00006-8

Chiolo I, Minoda A, Colmenares SU et al (2011) Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. Cell 144:732–744. https://doi.org/10.1016/j.cell.2011.02.012

Cremer T, Cremer M (2010) Chromosome territories. Cold Spring Harb Perspect Biol 2: a003889–a003889. https://doi.org/10.1101/cshperspect.a003889

Cronshaw JM, Krutchinsky AN, Zhang W et al (2002) Proteomic analysis of the mammalian nuclear pore complex. J Cell Biol 158:915–927. https://doi.org/10.1083/jcb.200206106

Davidson PM, Lammerding J (2014) Broken nuclei–lamins, nuclear mechanics, and disease. Trends Cell Biol 24:247–256. https://doi.org/10.1016/j.tcb.2013.11.004

Davies BSJ, Fong LG, Yang SH et al (2009) The posttranslational processing of prelamin A and disease. Annu Rev Genomics Hum Genet 10:153–174. https://doi.org/10.1146/annurev-genom-082908-150150

Denais CM, Gilbert RM, Isermann P et al (2016) Nuclear envelope rupture and repair during cancer cell migration. Science 352:353–358. https://doi.org/10.1126/science.aad7297

Deng M, Hochstrasser M (2006) Spatially regulated ubiquitin ligation by an ER/nuclear membrane ligase. Nature 443:827–831. https://doi.org/10.1038/nature05170

De Vos WH, Houben F, Kamps M et al (2011) Repetitive disruptions of the nuclear envelope invoke temporary loss of cellular compartmentalization in laminopathies. Hum Mol Genet 20:4175–4186. https://doi.org/10.1093/hmg/ddr344

Dixon JR, Gorkin DU, Ren B (2016) Chromatin Domains: The Unit of Chromosome Organization. Mol Cell 62:668–680. https://doi.org/10.1016/j.molcel.2016.05.018

Dixon JR, Selvaraj S, Yue F et al (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485:376–380. https://doi.org/10.1038/nature11082

Doucet CM, Talamas JA, Hetzer MW (2010) Cell cycle-dependent differences in nuclear pore complex assembly in metazoa. Cell 141:1030–1041. https://doi.org/10.1016/j.cell.2010.04.036

Ellenberg J, Siggia ED, Moreira JE et al (1997) Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis. J Cell Biol 138:1193–1206

Fanara P, Hodel MR, Corbett AH, Hodel AE (2000) Quantitative analysis of nuclear localization signal (NLS)-importin alpha interaction through fluorescence depolarization. Evidence for auto-inhibitory regulation of NLS binding. J Biol Chem 275:21218–21223. https://doi.org/10.1074/jbc.M002217200

Finlan LE, Sproul D, Thomson I et al (2008) Recruitment to the nuclear periphery can alter expression of genes in human cells. PLoS Genet 4:e1000039. https://doi.org/10.1371/journal.pgen.1000039

Fong LG, Ng JK, Lammerding J et al (2006) Prelamin A and lamin A appear to be dispensable in the nuclear lamina. J Clin Invest 116:743–752. https://doi.org/10.1172/JCI27125

Foresti O, Rodriguez-Vaello V, Funaya C et al (2014) Quality control of inner nuclear membrane proteins by the Asi complex. Science 346:751–755. https://doi.org/10.1126/science.1255638

Fudenberg G, Imakaev M, Lu C et al (2016) Formation of Chromosomal Domains by Loop Extrusion. Cell Rep 15:2038–2049. https://doi.org/10.1016/j.celrep.2016.04.085

Funakoshi T, Clever M, Watanabe A et al (2011) Localization of Pom121 to the inner nuclear membrane is required for an early step of interphase nuclear pore complex assembly. Mol Biol Cell 22:1058–1069. https://doi.org/10.1091/mbc.E10-07-0641

Furusawa T, Rochman M, Taher L et al (2015) Chromatin decompaction by the nucleosomal binding protein HMGN5 impairs nuclear sturdiness. Nat Commun 6:6138. https://doi.org/10.1038/ncomms7138

Gangaraju VK, Yin H, Weiner MM et al (2011) Drosophila Piwi functions in Hsp90-mediated suppression of phenotypic variation. Nat Genet 43:153–158. https://doi.org/10.1038/ng.743

Gasser SM (2016) Selfish DNA and Epigenetic Repression Revisited. Genetics 204:837–839. https://doi.org/10.1534/genetics.116.196287

Gonzalez-Sandoval A, Towbin BD, Kalck V et al (2015) Perinuclear anchoring of H3K9-methylated chromatin stabilizes induced cell fate in C. elegans embryos. Cell 163:1333–1347. https://doi.org/10.1016/j.cell.2015.10.066

Grossman E, Dahan I, Stick R et al (2012) Filaments assembly of ectopically expressed Caenorhabditis elegans lamin within Xenopus oocytes. J Struct Biol 177:113–118. https://doi.org/10.1016/j.jsb.2011.11.002

Gruenbaum Y, Foisner R (2015) Lamins: nuclear intermediate filament proteins with fundamental functions in nuclear mechanics and genome regulation. Annu Rev Biochem 84:15030609 3657004. https://doi.org/10.1146/annurev-biochem-060614-034115

Gruenbaum Y, Lee KK, Liu J et al (2002) The expression, lamin-dependent localization and RNAi depletion phenotype for emerin in C. elegans. J Cell Sci 115:923–929

Guelen L, Pagie L, Brasset E et al (2008) Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature 453:948–951. https://doi.org/10.1038/nature06947

Guilluy C, Osborne LD, Van Landeghem L et al (2014) Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. Nat Cell Biol 16:376–381. https://doi.org/10.1038/ncb2927

Harr JC, Luperchio TR, Wong X et al (2015) Directed targeting of chromatin to the nuclear lamina is mediated by chromatin state and A-type lamins. J Cell Biol 208:33–52. https://doi.org/10.1083/jcb.201405110

Hatch EM, Fischer AH, Deerinck TJ, Hetzer MW (2013) Catastrophic nuclear envelope collapse in cancer cell micronuclei. Cell 154:47–60. https://doi.org/10.1016/j.cell.2013.06.007

Hawryluk-Gara LA, Shibuya EK, Wozniak RW (2005) Vertebrate Nup53 interacts with the nuclear lamina and is required for the assembly of a Nup93-containing complex. Mol Biol Cell 16:2382–2394. https://doi.org/10.1091/mbc.E04-10-0857

Hirano Y, Hizume K, Kimura H et al (2012) Lamin B receptor recognizes specific modifications of histone H4 in heterochromatin formation. J Biol Chem 287:42654–42663. https://doi.org/10.1074/jbc.M112.397950

Hiraoka Y, Dernburg AF (2009) The SUN rises on meiotic chromosome dynamics. Dev Cell 17:598–605. https://doi.org/10.1016/j.devcel.2009.10.014

Horigome C, Bustard DE, Marcomini I et al (2016) PolySUMOylation by Siz2 and Mms21 triggers relocation of DNA breaks to nuclear pores through the Slx5/Slx8 STUbL. Genes Dev 30:931–945. https://doi.org/10.1101/gad.277665.116

Horigome C, Oma Y, Konishi T et al (2014) SWR1 and INO80 chromatin remodelers contribute to DNA double-strand break perinuclear anchorage site choice. Mol Cell 55:626–639. https://doi.org/10.1016/j.molcel.2014.06.027

Ikegami K, Egelhofer TA, Strome S, Lieb JD (2010) Caenorhabditis elegans chromosome arms are anchored to the nuclear membrane via discontinuous association with LEM-2. Genome Biol 11:R120. https://doi.org/10.1186/gb-2010-11-12-r120

Jin F, Li Y, Dixon JR et al (2013) A high-resolution map of the three-dimensional chromatin interactome in human cells. Nature 503:290–294. https://doi.org/10.1038/nature12644

Kalocsay M, Hiller NJ, Jentsch S (2009) Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. Mol Cell 33:335–343. https://doi.org/10.1016/j.molcel.2009.01.016

Katta SS, Smoyer CJ, Jaspersen SL (2014) Destination: inner nuclear membrane. Trends Cell Biol 24:221–229. https://doi.org/10.1016/j.tcb.2013.10.006

Khmelinskii A, Blaszczak E, Pantazopoulou M et al (2014) Protein quality control at the inner nuclear membrane. Nature 516:410–413. https://doi.org/10.1038/nature14096

Kind J, Pagie L, de Vries SS et al (2015) Genome-wide maps of nuclear lamina interactions in single human cells. Cell 163:134–147. https://doi.org/10.1016/j.cell.2015.08.040

Kind J, Pagie L, Ortabozkoyun H et al (2013) Single-cell dynamics of genome-nuclear lamina interactions. Cell 153:178–192. https://doi.org/10.1016/j.cell.2013.02.028

King MC, Drivas TG, Blobel G (2008) A network of nuclear envelope membrane proteins linking centromeres to microtubules. Cell 134:427–438. https://doi.org/10.1016/j.cell.2008.06.022

King MC, Lusk CP, Blobel G (2006) Karyopherin-mediated import of integral inner nuclear membrane proteins. Nature 442:1003–1007. https://doi.org/10.1038/nature05075

Kolb T, Maass K, Hergt M et al (2011) Lamin A and lamin C form homodimers and coexist in higher complex forms both in the nucleoplasmic fraction and in the lamina of cultured human cells. Nucleus 2:425–433. https://doi.org/10.4161/nucl.2.5.17765

Korfali N, Florens L, Schirmer EC (2016) Isolation, Proteomic Analysis, and Microscopy Confirmation of the Liver Nuclear Envelope Proteome. Methods Mol Biol 1411:3–44. https://doi.org/10.1007/978-1-4939-3530-7_1

Korfali N, Wilkie GS, Swanson SK et al (2010) The leukocyte nuclear envelope proteome varies with cell activation and contains novel transmembrane proteins that affect genome architecture. Mol Cell Proteomics 9:2571–2585. https://doi.org/10.1074/mcp.M110.002915

Korfali N, Wilkie GS, Swanson SK et al (2012) The nuclear envelope proteome differs notably between tissues. Nucleus 3:552–564. https://doi.org/10.4161/nucl.22257

Kosak ST, Skok JA, Medina KL et al (2002) Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. Science 296:158–162. https://doi.org/10.1126/science.1068768

Kralt A, Jagalur NB, van den Boom V et al (2015) Conservation of inner nuclear membrane targeting sequences in mammalian Pom121 and yeast Heh2 membrane proteins. Mol Biol Cell 26:3301–3312. https://doi.org/10.1091/mbc.E15-03-0184

Kubben N, Adriaens M, Meuleman W et al (2012) Mapping of lamin A- and progerin-interacting genome regions. Chromosoma 121:447–464. https://doi.org/10.1007/s00412-012-0376-7

Kumaran RI, Spector DL (2008) A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence. J Cell Biol 180:51–65. https://doi.org/10.1083/jcb.200706060

Laba JK, Steen A, Veenhoff LM (2014) Traffic to the inner membrane of the nuclear envelope. Curr Opin Cell Biol 28:36–45. https://doi.org/10.1016/j.ceb.2014.01.006

Lawrence KS, Tapley EC, Cruz VE et al (2016) LINC complexes promote homologous recombination in part through inhibition of nonhomologous end joining. J Cell Biol 215:801–821. https://doi.org/10.1083/jcb.201604112

Lemaître C, Grabarz A, Tsouroula K et al (2014) Nuclear position dictates DNA repair pathway choice. Genes Dev 28:2450–2463. https://doi.org/10.1101/gad.248369.114

Lokareddy RK, Hapsari RA, van Rheenen M et al (2015) Distinctive Properties of the Nuclear Localization Signals of Inner Nuclear Membrane Proteins Heh1 and Heh2. Structure 23:1305–1316. https://doi.org/10.1016/j.str.2015.04.017

Lombardi ML, Lammerding J (2011) Keeping the LINC: the importance of nucleocytoskeletal coupling in intracellular force transmission and cellular function. Biochem Soc Trans 39:1729–1734. https://doi.org/10.1042/BST20110686

Lottersberger F, Karssemeijer RA, Dimitrova N, de Lange T (2015) 53BP1 and the LINC Complex Promote Microtubule-Dependent DSB Mobility and DNA Repair. Cell 163:880–893. https://doi.org/10.1016/j.cell.2015.09.057

Lusk CP, Blobel G, King MC (2007) Highway to the inner nuclear membrane: rules for the road. Nat Rev Mol Cell Biol 8:414–420. https://doi.org/10.1038/nrm2165

Maciejowski J, Li Y, Bosco N et al (2015) Chromothripsis and Kataegis Induced by Telomere Crisis. Cell 163:1641–1654. https://doi.org/10.1016/j.cell.2015.11.054

Madabhushi R, Gao F, Pfenning AR et al (2015) Activity-induced DNA breaks govern the expression of neuronal early-response genes. Cell 161:1592–1605. https://doi.org/10.1016/j.cell.2015.05.032

Meinema AC, Laba JK, Hapsari RA et al (2011) Long unfolded linkers facilitate membrane protein import through the nuclear pore complex. Science 333:90–93. https://doi.org/10.1126/science.1205741

Meister P, Towbin BD, Pike BL et al (2010) The spatial dynamics of tissue-specific promoters during C. elegans development. Genes Dev 24:766–782. https://doi.org/10.1101/gad.559610

Mekhail K, Seebacher J, Gygi SP, Moazed D (2008) Role for perinuclear chromosome tethering in maintenance of genome stability. Nature 456:667–670. https://doi.org/10.1038/nature07460

Miné-Hattab J, Rothstein R (2012) Increased chromosome mobility facilitates homology search during recombination. Nat Cell Biol 14:510–517. https://doi.org/10.1038/ncb2472

Mitchell JM, Mansfeld J, Capitanio J et al (2010) Pom121 links two essential subcomplexes of the nuclear pore complex core to the membrane. J Cell Biol 191:505–521. https://doi.org/10.1083/jcb.201007098

Moir RD, Yoon M, Khuon S et al (2000) Nuclear lamins A and B1: different pathways of assembly during nuclear envelope formation in living cells. J Cell Biol 151:1155–1168

Mudumbi KC, Schirmer EC, Yang W (2016) Single-point single-molecule FRAP distinguishes inner and outer nuclear membrane protein distribution. Nat Commun 7:12562. https://doi.org/10.1038/ncomms12562

Nagai S, Dubrana K, Tsai-Pflugfelder M et al (2008) Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science 322:597–602. https://doi.org/10.1126/science.1162790

Nora EP, Lajoie BR, Schulz EG et al (2012) Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 485:381–385. https://doi.org/10.1038/nature11049

Ohba T, Schirmer EC, Nishimoto T et al (2004) Energy- and temperature-dependent transport of integral proteins to the inner nuclear membrane via the nuclear pore. J Cell Biol 167:1051–1062. https://doi.org/10.1083/jcb.200409149

Ostlund C, Ellenberg J, Hallberg E et al (1999) Intracellular trafficking of emerin, the Emery-Dreifuss muscular dystrophy protein. J Cell Sci 112(Pt 11):1709–1719

Oza P, Jaspersen SL, Miele A et al (2009) Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. Genes Dev 23:912–927. https://doi.org/10.1101/gad.1782209

Peric-Hupkes D, Meuleman W, Pagie L et al (2010) Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. Mol Cell 38:603–613. https://doi.org/10.1016/j.molcel.2010.03.016

Pickersgill H, Kalverda B, de Wit E et al (2006) Characterization of the Drosophila melanogaster genome at the nuclear lamina. Nat Genet 38:1005–1014. https://doi.org/10.1038/ng1852

Powell L, Burke B (1990) Internuclear exchange of an inner nuclear membrane protein (p55) in heterokaryons: in vivo evidence for the interaction of p55 with the nuclear lamina. J Cell Biol 111:2225–2234

Raab M, Gentili M, de Belly H et al (2016) ESCRT III repairs nuclear envelope ruptures during cell migration to limit DNA damage and cell death. Science 352:359–362. https://doi.org/10.1126/science.aad7611

Rabl C (1885) Über Zelltheilung. Morph Jb 10:214–330

Ragoczy T, Bender MA, Telling A et al (2006) The locus control region is required for association of the murine beta-globin locus with engaged transcription factories during erythroid maturation. Genes Dev 20:1447–1457. https://doi.org/10.1101/gad.1419506

Reddy KL, Zullo JM, Bertolino E, Singh H (2008) Transcriptional repression mediated by repositioning of genes to the nuclear lamina. Nature 452:243–247. https://doi.org/10.1038/nature06727

Rexach M, Blobel G (1995) Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. Cell 83:683–692

Robson MI, Las Heras de JI, Czapiewski R et al (2016) Tissue-Specific Gene Repositioning by Muscle Nuclear Membrane Proteins Enhances Repression of Critical Developmental Genes during Myogenesis. Mol Cell 62:834–847. https://doi.org/10.1016/j.molcel.2016.04.035

Rout MP, Aitchison JD, Suprapto A et al (2000) The yeast nuclear pore complex: composition, architecture, and transport mechanism. J Cell Biol 148:635–651

Ryu T, Spatola B, Delabaere L et al (2015) Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. Nat Cell Biol 17:1401–1411. https://doi.org/10.1038/ncb3258

Schirmer EC, Florens L, Guan T et al (2003) Nuclear membrane proteins with potential disease links found by subtractive proteomics. Science 301:1380–1382. https://doi.org/10.1126/science.1088176

Schreiner SM, Koo PK, Zhao Y et al (2015) The tethering of chromatin to the nuclear envelope supports nuclear mechanics. Nat Commun 6:7159. https://doi.org/10.1038/ncomms8159

Seeber A, Dion V, Gasser SM (2013) Checkpoint kinases and the INO80 nucleosome remodeling complex enhance global chromatin mobility in response to DNA damage. Genes Dev 27:1999–2008. https://doi.org/10.1101/gad.222992.113

Seeber A, Gasser SM (2016) Chromatin organization and dynamics in double-strand break repair. Curr Opin Genet Dev 43:9–16. https://doi.org/10.1016/j.gde.2016.10.005

Shimi T, Kittisopikul M, Tran J et al (2015) Structural organization of nuclear lamins A, C, B1, and B2 revealed by superresolution microscopy. Mol Biol Cell 26:4075–4086. https://doi.org/10.1091/mbc.E15-07-0461

Shimi T, Pfleghaar K, Kojima S-I et al (2008) The A- and B-type nuclear lamin networks: microdomains involved in chromatin organization and transcription. Genes Dev 22:3409–3421. https://doi.org/10.1101/gad.1735208

Smith S, Blobel G (1993) The first membrane spanning region of the lamin B receptor is sufficient for sorting to the inner nuclear membrane. J Cell Biol 120:631–637

Smoyer CJ, Katta SS, Gardner JM et al (2016) Analysis of membrane proteins localizing to the inner nuclear envelope in living cells. J Cell Biol 215:575–590. https://doi.org/10.1083/jcb.201607043

Smythe C, Jenkins HE, Hutchison CJ (2000) Incorporation of the nuclear pore basket protein nup153 into nuclear pore structures is dependent upon lamina assembly: evidence from cell-free extracts of Xenopus eggs. EMBO J 19:3918–3931. https://doi.org/10.1093/emboj/19.15.3918

Solovei I, Kreysing M, Lanctôt C et al (2009) Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution. Cell 137:356–368. https://doi.org/10.1016/j.cell.2009.01.052

Solovei I, Wang AS, Thanisch K et al (2013) LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. Cell 152:584–598. https://doi.org/10.1016/j.cell.2013.01.009

Somech R, Shaklai S, Geller O et al (2005) The nuclear-envelope protein and transcriptional repressor LAP2beta interacts with HDAC3 at the nuclear periphery, and induces histone H4 deacetylation. J Cell Sci 118:4017–4025. https://doi.org/10.1242/jcs.02521

Soullam B, Worman HJ (1993) The amino-terminal domain of the lamin B receptor is a nuclear envelope targeting signal. J Cell Biol 120:1093–1100

Soullam B, Worman HJ (1995) Signals and structural features involved in integral membrane protein targeting to the inner nuclear membrane. J Cell Biol 130:15–27

Stephens AD, Banigan EJ, Adam SA, et al (2017) Chromatin and lamin A determine two different mechanical response regimes of the cell nucleus. Mol Biol Cell. doi: https://doi.org/10.1091/mbc.E16-09-0653

Stewart RM, Zubek AE, Rosowski KA et al (2015) Nuclear-cytoskeletal linkages facilitate cross talk between the nucleus and intercellular adhesions. J Cell Biol 209:403–418. https://doi.org/10.1083/jcb.201502024

Sullivan T, Escalante-Alcalde D, Bhatt H et al (1999) Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. J Cell Biol 147:913–920

Swartz RK, Rodriguez EC, King MC (2014) A role for nuclear envelope-bridging complexes in homology-directed repair. Mol Biol Cell 25:2461–2471. https://doi.org/10.1091/mbc.E13-10-0569

Swift J, Ivanovska IL, Buxboim A et al (2013) Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. Science 341:1240104–1240104. https://doi.org/10.1126/science.1240104

Taddei A, Gasser SM (2012) Structure and function in the budding yeast nucleus. Genetics 192:107–129. https://doi.org/10.1534/genetics.112.140608

Taimen P, Pfleghaar K, Shimi T et al (2009) A progeria mutation reveals functions for lamin A in nuclear assembly, architecture, and chromosome organization. Proc Natl Acad Sci 106:20788–20793. https://doi.org/10.1073/pnas.0911895106

Thakar K, May CK, Rogers A, Carroll CW (2017) Opposing roles for distinct LINC complexes in regulation of the small GTPase RhoA. Mol Biol Cell 28:182–191. https://doi.org/10.1091/mbc.E16-06-0467

Theerthagiri G, Eisenhardt N, Schwarz H et al (2010) The nucleoporin Nup188 controls passage of membrane proteins across the nuclear pore complex. J Cell Biol 189:1129–1142. https://doi.org/10.1083/jcb.200912045

Therizols P, Illingworth RS, Courilleau C et al (2014) Chromatin decondensation is sufficient to alter nuclear organization in embryonic stem cells. Science 346:1238–1242. https://doi.org/10.1126/science.1259587

Torres-Rosell J, Sunjevaric I, De Piccoli G et al (2007) The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. Nat Cell Biol 9:923–931. https://doi.org/10.1038/ncb1619

Torrisi MR, Cirone M, Pavan A et al (1989) Localization of Epstein-Barr virus envelope glycoproteins on the inner nuclear membrane of virus-producing cells. J Virol 63:828–832

Torrisi MR, Lotti LV, Pavan A et al (1987) Free diffusion to and from the inner nuclear membrane of newly synthesized plasma membrane glycoproteins. J Cell Biol 104:733–737

Towbin BD, González-Aguilera C, Sack R et al (2012) Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. Cell 150:934–947. https://doi.org/10.1016/j.cell.2012.06.051

Towbin BD, Meister P, Pike BL, Gasser SM (2010) Repetitive transgenes in C. elegans accumulate heterochromatic marks and are sequestered at the nuclear envelope in a copy-number- and lamin-dependent manner. Cold Spring Harb Symp Quant Biol 75:555–565. https://doi.org/10.1101/sqb.2010.75.041

Turgay Y, Eibauer M, Goldman AE et al (2017) The molecular architecture of lamins in somatic cells. Nature 543:261–264. https://doi.org/10.1038/nature21382

Turner EM, Schlieker C (2016) Pelger-Huët anomaly and Greenberg skeletal dysplasia: LBR-associated diseases of cholesterol metabolism. Rare Dis 4:e1241363. https://doi.org/10.1080/21675511.2016.1241363

Ungricht R, Klann M, Horvath P et al (2015) Diffusion and retention are major determinants of protein targeting to the inner nuclear membrane. J Cell Biol 209:687–703. https://doi.org/10.1083/jcb.201409127

Vargas JD, Hatch EM, Anderson DJ et al (2012) Transient nuclear envelope rupturing during interphase in human cancer cells. Nucleus 3:88–100. https://doi.org/10.4161/nucl.18954

Vaughan A, Alvarez-Reyes M, Bridger JM et al (2001) Both emerin and lamin C depend on lamin A for localization at the nuclear envelope. J Cell Sci 114:2577–2590

Vogel MJ, Peric-Hupkes D, van Steensel B (2007) Detection of in vivo protein-DNA interactions using DamID in mammalian cells. Nat Protoc 2:1467–1478. https://doi.org/10.1038/nprot.2007.148

Webster BM, Colombi P, Jäger J et al (2014) Surveillance of nuclear pore complex assembly by ESCRT-III/Vps4. Cell 159:388–401. https://doi.org/10.1016/j.cell.2014.09.012

Webster BM, Lusk CP (2016) Border safety: quality control at the nuclear envelope. Trends Cell Biol 26:29–39. https://doi.org/10.1016/j.tcb.2015.08.002

Wilkie GS, Korfali N, Swanson SK et al (2011) Several novel nuclear envelope transmembrane proteins identified in skeletal muscle have cytoskeletal associations. Mol Cell Proteomics 10:M110.003129–M110.003129. https://doi.org/10.1074/mcp.M110.003129

Wu F, Yao J (2013) Spatial compartmentalization at the nuclear periphery characterized by genome-wide mapping. BMC Genomics 14:591. https://doi.org/10.1186/1471-2164-14-591

Wu TP, Wang T, Seetin MG et al (2016) DNA methylation on N(6)-adenine in mammalian embryonic stem cells. Nature 532:329–333. https://doi.org/10.1038/nature17640

Xie W, Chojnowski A, Boudier T et al (2016) A-type lamins form distinct filamentous networks with differential nuclear pore complex associations. Curr Biol 26:2651–2658. https://doi.org/10.1016/j.cub.2016.07.049

Yang Q, Rout MP, Akey CW (1998) Three-dimensional architecture of the isolated yeast nuclear pore complex: functional and evolutionary implications. Mol Cell 1:223–234

Yao J, Fetter RD, Hu P et al (2011) Subnuclear segregation of genes and core promoter factors in myogenesis. Genes Dev 25:569–580. https://doi.org/10.1101/gad.2021411

Zeller P, Padeken J, van Schendel R et al (2016) Histone H3K9 methylation is dispensable for Caenorhabditis elegans development but suppresses RNA:DNA hybrid-associated repeat instability. Nat Genet 48:1385–1395. https://doi.org/10.1038/ng.3672

Zink D, Amaral MD, Englmann A et al (2004) Transcription-dependent spatial arrangements of CFTR and adjacent genes in human cell nuclei. J Cell Biol 166:815–825. https://doi.org/10.1083/jcb.200404107

Zuleger N, Boyle S, Kelly DA et al (2013) Specific nuclear envelope transmembrane proteins can promote the location of chromosomes to and from the nuclear periphery. Genome Biol 14:R14. https://doi.org/10.1186/gb-2013-14-2-r14

Zuleger N, Kelly DA, Richardson AC et al (2011) System analysis shows distinct mechanisms and common principles of nuclear envelope protein dynamics. J Cell Biol 193:109–123. https://doi.org/10.1083/jcb.201009068

Zullo JM, Demarco IA, Piqué-Regi R et al (2012) DNA sequence-dependent compartmentalization and silencing of chromatin at the nuclear lamina. Cell 149:1474–1487. https://doi.org/10.1016/j.cell.2012.04.035