Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Genetics & Development

Building silent compartments at the nuclear periphery: a recurrent theme

Peter Meister[1] and Angela Taddei[2,3]

In eukaryotes, the genetic material is stored in the nucleus, which is enclosed in a double lipid bilayer, the nuclear envelope (NE). It protects the genome from physical stress and separates it from the rest of the cell. On top of this physical function, growing evidence shows that the nuclear periphery contributes to the 3D organization of the genome. In turn, tridimensional organization of chromatin in the nuclear space influences genome expression. Here we review recent findings on the function of this physical barrier in gene repression and latest models on how silent subnuclear compartments at the NE are built in yeast as well as in the nematode *C. elegans* and mammalian cells; trying to draw parallels between the three systems.

Addresses
[1] Institute of Cell Biology, University of Bern, Baltzerstrasse 4, CH-3012 Bern, Switzerland
[2] Unité Mixte de Recherche 218, Centre National de la Recherche Scientifique, F-75248 Paris, Cedex 05, France
[3] Centre de Recherche, Institut Curie, F-75248 Paris, Cedex 05, France

Corresponding authors: Meister, Peter (peter.meister@izb.unibe.ch) and Taddei, Angela (angela.taddei@curie.fr)

Current Opinion in Genetics & Development 2013, **23**:xx–yy

This review comes from a themed issue on **Genome architecture and expression**

Edited by **Genevieve Almouzni** and **Frederick Alt**

0959-437X/$ – see front matter, © 2013 Elsevier Ltd. All rights reserved.

http://dx.doi.org/10.1016/j.gde.2012.12.001

The NE is one of the most obvious locations to characterize inside the nucleus; it has therefore very often been used as a stable landmark or reference point to quantify genes and chromosomes locations inside the nuclear lumen. The NE is pierced with pores; megadalton-size multiprotein complexes that regulate exchange between the cytoplasm and the nucleoplasm [1]. A growing body of evidence strongly argues that the NE has not only a structural role, but also contributes to the 3-dimensional organization of the genome and to its transcriptional regulation.

During mitosis, metazoan cells disassemble and reform their NE as well as the meshwork of intermediate filaments forming the nuclear lamina underlying the NE [2]. Both structures are crosslinked together by a number of proteins inserted in the nuclear membranes. The lamina provides rigidity to the NE and serves as a platform for the binding of a large number of lamin-associated proteins and specific silent epigenomic domains. Many mutations in lamins have severe consequences for the organism: they are responsible for a wide range of human diseases, most of them surprisingly tissue-specific although lamins are expressed ubiquitously. Altogether, this suggests a role of the lamina and NE in regulation of transcription [3]. Lamins and the nuclear lamina are not present in all organisms: budding yeast, like most organisms with a closed mitosis, lacks a lamina. Several proteins homologous to lamin-associated proteins in metazoan are however found inserted into the NE and contribute to chromatin anchoring [4]. Furthermore, the nuclear periphery is also associated with chromatin loci and shares similar transcriptional features from yeast to humans suggesting a functional parallel among eukaryotes.

Indeed, chromatin is non-homogenously distributed inside the nucleus, as shown by early microscopic observations using chromatin stains or later using electron microscopy [5,6]. In particular, most of the darkly stained chromatin (heterochromatin) is found around the nucleoli or at the nuclear periphery interrupted with euchromatin at nuclear pores. As a first approximation, heterochromatin is rather compact and transcriptionally silent, whereas the more open euchromatin contains transcriptionally active genes. Although no condensed chromatin is observed in yeast using EM, perinuclear clusters of silent chromatin are present, making peripheral heterochromatin localization a conserved feature of nuclear organization across budding yeast, nematodes, flies and mammalian cells [7]. This conservation raises the question of whether NE association of heterochromatin is a cause or consequence of its silent state.

This chicken or egg question was directly addressed by artificially relocating genes toward the nuclear rim first in yeast and later in mammalian cells [8–12]. In budding yeast, it was clearly shown that perinuclear tethering favors the silencing of genes flanked by specific *cis*-acting elements [8]. In mammalian cells, the results are somehow variable because of the different experimental systems, but similar conclusions were drawn: the nuclear periphery is not refractory to gene transcription, but can modulate the activity of certain genes [13,14]. Hence, parts of the nuclear periphery constitute a specific environment, a nuclear subcompartment repressive for transcription. The repressive nature of the nuclear periphery could be either owing to repressive activities such

Please cite this article in press as: Meister P, Taddei A. Building silent compartments at the nuclear periphery: a recurrent theme, Curr Opin Genet Dev (2013), http://dx.doi.org/10.1016/j.gde.2012.12.001

as histone modifiers anchored in the NE or arise from the juxtaposition of NE-anchored heterochromatin, leading to the local concentration of silencing factors. Hereafter we explore these two models in different experimental systems and try to derive common functional principles. While yeast telomeres provide a well-characterized example of a functional compartment, more recent studies have now started to uncover the molecular machinery involved in targeting and tethering heterochromatin to the nuclear lamina in metazoans.

Lessons from budding yeast: perinuclear anchoring and heterochromatin clustering generate repressive compartments

In budding yeast, computational studies suggest that the higher-order organization of the genome is determined to a large extent by physical properties of the chromatin fiber [15–17]. In these studies, the yeast genome is modeled as 16 randomly configured flexible polymer chains confined in a sphere of two micrometers of diameter representing the nucleus. Although these models differ in many ways they all had to include the tethering of centromeres and telomeres at the nuclear periphery to account for the 3-D organization of the genome observed *in vivo* by microscopy or by chromosome conformation capture [18]. Thus, perinuclear anchoring is a key determinant of interphase chromosome architecture that requires specific interactions. In haploid cells, heterochromatin is composed of 32 telomeres and the two cryptic mating type loci (*HM* loci). Inside the nucleus, it clusters into 3 to 8 foci enriched for Silent Information Regulators (SIRs), the silent chromatin proteins of budding yeast [19]. These perinuclear telomeres generate a zone that favors SIR-mediated repression and prevents promiscuous effects on a distinct subset of promoters in the nuclear interior [8,20]. Indeed, artificial tethering of a reporter gene to the nuclear periphery favors the establishment of silent chromatin [8]. Importantly, this is strictly dependent on flanking *cis*-acting elements, capable of recruiting SIRs as well as on the presence of telomere clusters and SIR foci at the nuclear periphery [8,20,21]. Hence, transcriptional repression does not reflect position *per se*, but access to local high concentrations of SIRs. Consistent with these findings, impairing telomere anchoring releases SIRs from the NE, thereby enhancing repression at internal loci and derepressing telomere-proximal genes [20,22].

At the molecular level, silencing is nucleated at the subtelomeric TG1–3 repeats by the binding of the transcription factor Rap1 that contains binding sites for the silencing factors Sir3 and Sir4 at the C terminus end [23] (Figure 1a, Nucleation). At other sites, recruitment of the SIR complex is mediated by other DNA binding proteins, which like Rap1 have other functions in the cell such as Orc1 and Abf1, at *HM* loci [24] and Ume6 at the *PAU* subtelomeric genes [25]. Intriguingly, recent data indicate that proteins tightly bound to DNA could favor the recruitment of the SIR complex apparently owing to the difficulty of the replication machinery to progress through tight DNA protein complexes [26]. Although in this case, the molecular links leading to the recruitment of the silencing complex remain to be deciphered, it raises the interesting possibility that replication 'stress' contribute to the establishment of transcriptional silencing.

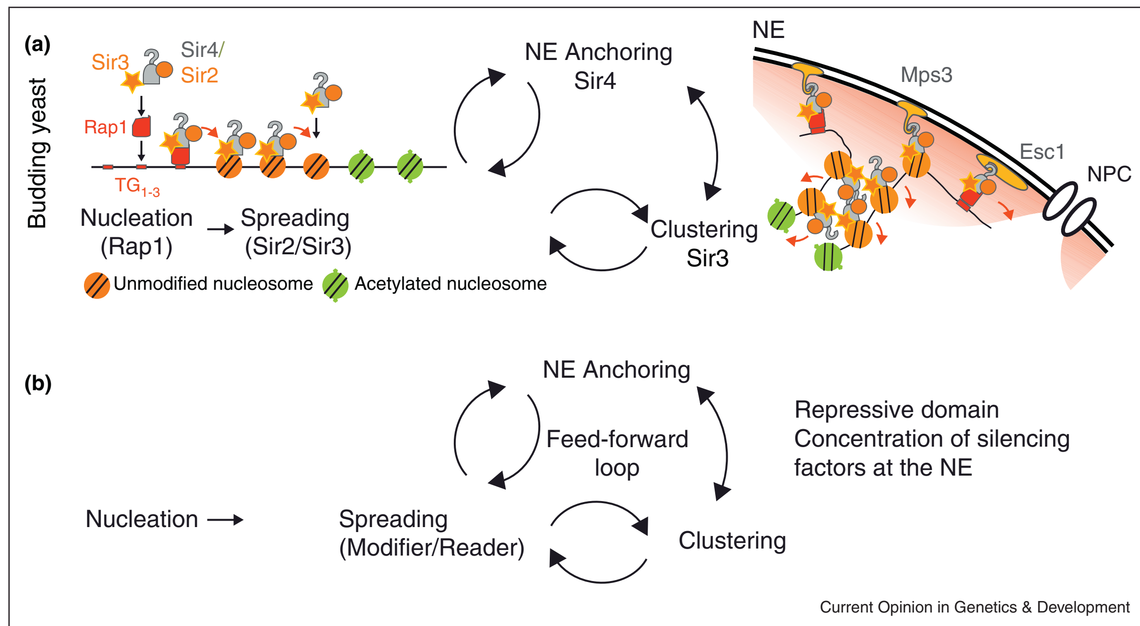
Once nucleated, heterochromatin enters a self-reinforcing feed-forward loop that constitutes spreading of the silencing complex along the chromatin fiber, its anchoring at the nuclear envelope as well as clustering mediated by *trans*-interaction that leads to the creation of a silent subnuclear compartment at the NE (Figure 1a).

Silent chromatin spreading involves the heterodimerization of Sir4 with Sir2, a NAD-dependent histone deacetylase that catalyzes the deacetylation of histone H4 tails, generating a preferred binding site for Sir3 [27]. This leads to the spreading of the SIR complex from sites of nucleation over a 2–3-kb subtelomeric region that results in the transcriptional repression of subtelomeric genes [4] (Figure 1a, Spreading). Anchoring to the NE is achieved and maintained via interaction between Sir4 and the yeast specific NE associated protein Esc1 or the SUN domain integral membrane protein Mps3 (Figure 1a, Anchoring) [28–30]. Similarly, other telomere-associated proteins are also able to anchor at the NE independently of Sir4 [4]. Clustering requires crosslinking factors that can bridge silent telomeres together to create a subnuclear compartment. Both Sir3 and Sir4 harbor a dimerization domain that could crosslink silenced telomeres together [31–36] and Sir3 was recently shown to be limiting for telomere clustering [37••] (Figure 1a, Clustering). Importantly the Sir3 ability to promote telomere clustering can be separated from the formation of heterochromatin. This argues that the clustering of heterochromatin regions does not arise from the aggregation of this type of chromatin but from specific protein–protein interactions.

Thus, the SIR2/3/4 complex has the ability to self-propagate on chromatin, anchor it to the nuclear periphery (through Sir4 and other telomeric factors) and mediate clustering of SIR bound chromatin (through Sir3). It is important to note that both clustering and perinuclear tethering of telomere can occur independently from the spreading of the SIR complex and thus from gene silencing [30,37••,38,39]. Reciprocally, silencing can be achieved at wild type *HM* loci in the absence of both clustering and anchoring [40]. However, in subtelomeric regions, SIR concentration is limiting for spreading and silencing cannot be separated from telomere clustering in this case. The formation of subnuclear compartments with locally high concentrations of silencing factors appears thus essential for silencing at these sites. The bifunctional roles of Sir3 and Sir4 in silencing and respectively clustering and

Please cite this article in press as: Meister P, Taddei A. Building silent compartments at the nuclear periphery: a recurrent theme, Curr Opin Genet Dev (2013), http://dx.doi.org/10.1016/j.gde.2012.12.001

Figure 1



Building silent compartments at the nuclear rim. **(a)** In budding yeast, the clustering of silent chromatin loci, including the 32 telomeres and silent mating type loci, generate repressive compartments mainly found close to the nuclear envelope (NE) in between the nuclear pore complexes (NPC). Repressed chromatin (in orange) is generated by the recruitment of the SIR complex comprising Sir2, Sir3, and Sir4. At telomeres, this nucleation event is achieved by the transcription factor Rap1 that binds the telomere TG repeats and interacts with Sir3 and Sir4. Sir4 heterodimerizes with the NAD+ dependent histone deacetylase Sir2, which deacetylates H4 histone tails from neighboring nucleosomes, thus generating binding sites for Sir3. The SIR complex thus spreads over a 2–3-kb subtelomeric region leading to the transcriptional repression of subtelomeric regions. Anchoring is achieved by Sir4 and other telomeric proteins that bind the NE associated proteins Esc1 and Mps3 (in yellow), while clustering is achieved by Sir3 that bridges telomeres probably through self-interaction in its C-terminus. **(b)** General principles leading to the formation of repressive compartments emerge from studies in yeast, nematodes and mouse. These involve the recruitment of repressive complexes combining three features: (1) the ability to anchor chromatin to the nuclear envelope through interaction with nuclear envelope associated proteins (anchoring in yellow); (2) the property to spread along the chromatin fiber through the association of a histone modifier) and a histone reader (orange) specific to the same mark (spreading); (3) the capacity to bridge chromatin fibers through protein–protein interactions (clustering). The combination of these three features creates a positive reciprocal loop (feed-forward loop) that leads to the concentration of silencing factors at the nuclear periphery: both anchoring and clustering can increase the local concentration of heterochromatin factors spreading along the chromatin fiber. This in turn, increases the number of anchoring and bridging factors associated with the chromatin fibers thus reinforcing their clustering and anchoring. Entry into this feed-forward loop requires a nucleation event that is not always identified but in specific cases is achieved by specific DNA binding protein (in red).

anchoring provide a mechanism for the self-establishment and propagation of a silent compartment. Given that SIR proteins are limiting for the spread of heterochromatin, both clustering and anchoring activities allow the increase in local concentration of SIR factors, which in turn, favors SIR complex spread into flanking chromatin, extending repression, and reinforcing clustering and anchoring. Such a mechanism (Figure 1b, feed-forward loop) may apply to other chromatin-based compartments with other functions and in other organisms. Indeed, some of these principles have been recently proposed to be at play in metazoans (see below).

Dynamics of chromatin association at the nuclear periphery and differentiation in metazoans

The use of genome-wide molecular techniques like chromatin immunoprecipitation or damID facilitated characterization of DNA sequences associated with the nuclear lamina (LADs = lamin associated domains), in various species [41–43,44••]. Lamina-proximal chromatin is indeed, on an average, transcriptionally less active and accordingly harbors somewhat more of the classical H3K9 and H3K27 silent histone methylation marks [41–43,44••]. During development, peripheral as well as centromeric heterochromatin significantly increases, as defined by EM; EM is not able to capture heterochromatin in Embryonic Stem Cells (ESCs), although pericentric regions show dense DAPI staining and Heterochromatin Protein 1 accumulation (HP1), suggesting a different heterochromatic state in ESCs [45–48]. Heterochromatin also progressively appears *in vitro* during differentiation of ESCs into neural progenitors and neuroblasts. Surprisingly, LADs quantified by damID appear remarkably stable upon *in vitro* differentiation of mouse ESCs or in fibroblasts with different heterochromatin distributions [49••]. Microscopy experiments suggested earlier that chromatin is globally in a

Please cite this article in press as: Meister P, Taddei A. Building silent compartments at the nuclear periphery: a recurrent theme, Curr Opin Genet Dev (2013), http://dx.doi.org/10.1016/j.gde.2012.12.001

more plastic state in ESCs than in differentiated cells [48,50]. The differential mobility of lamin-associated chromatin might thus explain the observed difference between EM and damID studies [49]. LADs conservation also suggests the existence of a backbone chromosome architecture, either sequence or activity dependent. This backbone structure is present in all cell types studied by damID, nevertheless, it can be modulated, as alternative organizations have been observed; for example in rod cells (cells channeling light in the eye to the photoreceptors) of nocturnal mammals, where heterochromatin accumulates toward the nuclear center as cells differentiate [51].

In nematodes, step-wise methylation of histone H3K9 targets repetitive heterochromatin at the nuclear envelope

To uncover factors involved in heterochromatin perinuclear anchoring, a genetic approach was used in *C. elegans* using a model of peripheral repetitive heterochromatin. In nematodes, which lack centromeres, all autosomes show enrichment for silent chromatin marks (H3K9 and H3K27 methylations) on the outer thirds of chromosomes (arms) relative to the inner third (center) [42,52]. These outer thirds are also located closer to the NE, thus are similar to mammalian LADs [44,53]. Addressing to the NE seems to be sequence-dependent since chromosome arm translocation to a chromosome center retained its association with the nuclear lamina [53]. However, many repetitive transgene arrays are packaged into heterochromatin that recapitulates perinuclear anchoring of endogenous heterochromatin arguing that high repeat density is the determinant of heterochromatin formation in nematodes [54,55]. It is noteworthy that this perinuclear heterochromatin is not completely refractory to transcription as a number of transgene arrays containing housekeeping promoters are expressed at the NE, although they are repressed to a certain point, especially in the germline [54,56].

A genetic screen revealed that histone H3 lysine 9 methylation marks target repetitive heterochromatin to the NE [44••]. Two enzymes are involved in H3K9 methylation in worms: MET-2, a mammalian SETDB1 homolog, is a cytoplasmic enzyme which monomethylates and dimethylates non-nucleosomal histones and SET-25, that contains a SET domain which is homologous to mammalian G9a, is a nuclear enzyme and primarily trimethylates nucleosomes formed with histones monomethylated and dimethylated by MET-2 (Figure 2a, Nucleation). This system is reminiscent of pericentric heterochromatin in mammalian cells, where non-nucleosomal histones are monomethylated by SETDB1 and Prdm3/16 [57–59], loaded through an unknown mechanism onto satellite repeats of the genome before additional methylation by SUV39H. Propagation of the methylation state of mammalian pericentric heterochromatin during replication was proposed to depend on SETDB1, the mammalian MET-2 homolog. SETDB1, in complex with monomethylated H3K9, HP1α and the histone chaperone CAF1, is found at pericentric heterochromatin during its replication. There, it could direct packaging of newly replicated pericentric repeats into monomethylated H3K9 nucleosomes before further methylation by the H3K9 methyltransferases SUV39H [58].

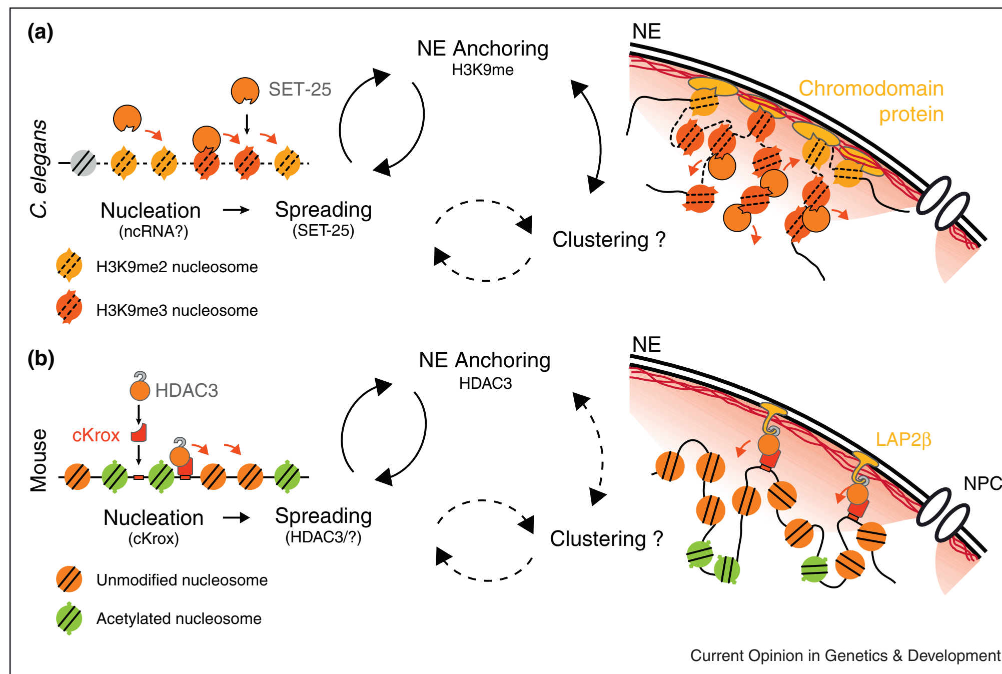
The nucleation event for heterochromatin formation in both worms and mammals is not clear, as methylated and non methylated histones are present in the non nucleosomal form: the methylated forms are either targeted to package repetitive DNA or loaded and demethylated/replaced in non-repetitive regions. In a similar fashion to *S. pombe* centromeres, a line of clues point toward the role of non-coding RNA in initiating heterochromatinization in *C. elegans*: genomic regions targeted by siRNA acquire H3K9 trimethylation [60••,61•]; *set-25* (the H3K9 trimethylase) and *hpl-2* (one of *C. elegans* HP1 homolog) have been implicated in heritable gene silencing [62•]. H3K9 methylation could be attracted by repetitions of transposons and retroelements that may produce ncRNA [53], which, in turn could be recognized by HP1, as in fission yeast and mammals [63–66].

Once primed for silencing, the feed-forward loop for heterochromatin silencing/anchoring has many similarities to the yeast SIR system. First, spreading of methylation is achieved by SET-25 which trimethylates H3K9me2 and localizes through its non-catalytic domain to H3K9me3, locally enriching for H3K9 trimethylation activity (Figure 2a, Spreading). Anchoring is mediated by H3K9 methylated chromatin which binds a peripherally located chromodomain protein (Figure 2a, Anchoring; A. Gonzalez-Sandoval, B.D. Towbin, S.M. Gasser, personal communication). The crosslinking factors involved in clustering heterochromatin are not known, although HPL-1, one of the two homologs of HP1 in worms, colocalizes with SET-25, but is not essential for SET-25 compartment formation [44••]. Notably in mammalian cells, absence of H3K9 monomethylation by Prdm3/16 also has profound effects on heterochromatin integrity, centromeric major satellite silencing as well as nuclear shape, suggesting that similar pathways are at play for perinuclear heterochromatin targeting [59•].

However, there exists a functional redundancy since other pathways seem to be involved in H3K9 methylation for perinuclear targeting. In double *set-25/met-2* mutant worms in which H3K9 methylation is below detection level, telomeres remain anchored at the nuclear rim [44••]. Moreover, repetitive chromatin, randomly located in these mutant embryos relocates to the NE once cells have differentiated [44••]. This suggests that redundant systems lead to peripheral relocation of heterochromatin. On the opposite, in wild-type worms, activation of developmentally regulated promoters leads to the relocation of repetitive arrays from the nuclear rim to the nuclear center [54]. Hence, for some promoters, transcriptional status can

Please cite this article in press as: Meister P, Taddei A. Building silent compartments at the nuclear periphery: a recurrent theme, Curr Opin Genet Dev (2013), http://dx.doi.org/10.1016/j.gde.2012.12.001

Figure 2



In metazoans, heterochromatin is usually found plastered against the inner face of the nuclear envelope (NE), which is cross-linked to a network of intermediate filaments, the nuclear lamina (in red) interrupted by nuclear pore complexes (NPCs). **(a)** In *C. elegans*, repetitive heterochromatin is enriched in the repressive histone mark H3K9me2 (orange nucleosomes) and H3K9me3 (red nucleosomes) through a yet unknown nucleation event. The spreading of H3K9me3 is achieved by SET-25 which trimethylates H3K9me2 and localizes to H3K9me3 through its non-catalytic domain, locally enriching for H3K9 trimethylation activity (Figure 2a, Spreading). Anchoring is mediated by H3K9 methylated chromatin [43], which binds a peripherally located chromodomain protein (in yellow). The crosslinking factors involved in clustering heterochromatin are not known. **(b)** In mouse, LADs, spanning the developmentally regulated IgH and Cyp3a loci contain multiple LAS (LAD associated sequences in red) enriched for a GAGA motif recognized by cKrox, which interacts with HDAC3 and the INM protein Lap2β (in yellow). HDAC3 is proposed to function both as an adaptor for lamina association as well as a transcriptional corepressor via its histone deacetylase activity [67••].

overcome 'default' targeting of repetitive sequence to the nuclear rim.

Lamina associated sequences mediate attachment to the nuclear envelope and transcriptional silencing in mouse cells

In mouse cells, an alternative approach to characterize factors involved in perinuclear heterochromatin formation was to dissect LAD sequences spanning the developmentally regulated *IgH* and *Cyp3a* loci. When ectopically inserted, these LADs are able to autonomously direct a nucleoplasmic locus to the periphery, as well as repress transcription of a reporter gene [67••]. Serial deletions of these sequences identified binding sites for the cKrox transcription factor as essential for the relocation activity, proposing it as nucleation sequence for heterochromatin silencing (Figure 2b, Nucleation by cKrox). Thus, as in budding yeast, a DNA binding protein with other transcriptional regulation activities in the cell can also nucleate heterochromatin. cKrox interacts with a protein complex composed of LAP2β (a lamin interacting protein) and HDAC3, a histone deacetylase, parts of a feed-forward loop similar to the ones observed in yeast and *C. elegans* [67••,68] (Figure 2b). Other redundant pathways are also likely at play given that not all LADs contain cKrox binding sites, and since even for this specific LAD, knock-down of HDAC3 or cKrox detached the LAD in at most 40% of the cells from the nuclear periphery [67••]. This suggests however a general mechanism of chromatin re-location to the NE and de-acetylation (Figure 2b). The mechanism of heterochromatin spreading beyond the immediate vicinity of the cKrox binding sites remains unknown. A prime candidate with spreading/anchoring features is here again heterochromatin protein 1: HP1 is able to spread via its interaction with SUV39H which trimethylates H3K9 and bind the product of the reaction, that is, trimethylated H3K9. Moreover, HP1 interacts with the Lamin B Receptor (LBR) located at the NE [69] and HP1 has a dimerization domain, which could be used for crosslinking and clustering heterochromatin [70]. Interestingly, a similar transcription factor dependent model of heterochromatin formation has been proposed for major satellite repeats in mouse cells [71].

Conclusions and perspectives

Building silent compartments at the nuclear rim seems to obey a similar logic in all experimental systems studied,

Please cite this article in press as: Meister P, Taddei A. Building silent compartments at the nuclear periphery: a recurrent theme, Curr Opin Genet Dev (2013), http://dx.doi.org/10.1016/j.gde.2012.12.001

although fine details vary. Heterochromatin formation starts with a nucleation event from which a spreading mechanism allows linear progression of heterochromatin along the chromatin fiber, thanks to the association of a histone modifier and a histone reader. Anchoring at the NE of silenced chromatin locally increases the concentration of silencing factors and maximizes probability of encounter with other silent genomic regions. Finally *trans* interactions created by bridging factors further strengthen silencing power by compaction and crosslinking. This will lead to the local concentration of silencing factors at the nuclear periphery making this area of the nucleus favorable for gene repression through a local trans-effect. Indeed, histone-modifiers attached to chromatin or to the NE can modify chromatin located nearby, 'spraying' in *trans* in addition to 'spreading' in *cis*. Although the general principles seem to emerge, the molecular details remain to be clarified in each specific system.

Interestingly, perinuclear silent compartments favor silencing within at the nuclear periphery and segregate silencing factors away from the rest of the (active) genome avoiding promiscuous silencing. Other compartments not associated with the NE could mechanistically behave similarly: genomic nucleolus-associated domains (NADs) [72,73], polycomb bodies grouping several Pc-regulated genes [74], centromeres [47]... However, among the nuclear landmarks, NE has the specificity to be at the interface with the cytoplasm. Understanding whether and how NE proteins bridging chromatin to cytoskeletal components convey mechanical force from the cytoplasm to perinuclear chromatin is an exciting challenge for the future.

Combining measurements of protein affinities and activities *in vivo* along with simulations of chromatin behavior based on polymer dynamics will allow further understanding of the biochemical and biophysical principles and prerequisites for the assembly of subnuclear compartments. This should allow designing gain of function experiments to test these models. Future challenges will be to decipher the regulation of the formation and dynamics of these compartments during cell cycle, upon differentiation or in response to environmental stimuli. Entry points into the feed-forward loops leading to the formation of these compartments also remain to be identified in many instances. Finally, the contribution of these compartments toward normal function and pathogenic states needs further investigation.

Acknowledgements

We thank Stephanie Kueng and Rahul Sharma for critical comments on the manuscript and Susan Gasser for communication of results before publication. The Meister laboratory is funded by the Swiss National Foundation (SNF assistant professor grant PP00P3_133744) and the University of Bern; the Taddei laboratory by CNRS, Institut Curie, ANR and an ERC starting grant [210508].

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Wente SR, Rout MP: **The nuclear pore complex and nuclear transport**. *Cold Spring Harb Perspect Biol* 2010, **2**:a000562.
2. Gomez-Cavazos JS, Hetzer MW: **Outfits for different occasions: tissue-specific roles of Nuclear Envelope proteins**. *Curr Opin Cell Biol* 2012, **24**:775-783.
3. Worman HJ: **Nuclear lamins and laminopathies**. *J Pathol* 2012, **226**:316-325.
4. Taddei A, Gasser SM: **Structure and function in the budding yeast nucleus**. *Genetics* 2012, **192**:107-129.
5. Flemming W: *Zell substanz, Kern und Zelltheilung*. Leipzig: Vogel; 1882.
6. Fawcett DW: **The nucleus**. In *An Atlas of Fine Structure: The Cell.* Edited by Saunders. W. B. Saunders Company; 1966.
7. Akhtar A, Gasser SM: **The nuclear envelope and transcriptional control**. *Nat Rev Genet* 2007, **8**:507-517.
8. Andrulis ED, Neiman AM, Zappulla DC, Sternglanz R: **Perinuclear localization of chromatin facilitates transcriptional silencing**. *Nature* 1998, **394**:592-595.
9. Finlan LE, Sproul D, Thomson I, Boyle S, Kerr E, Perry P, Ylstra B, Chubb JR, Bickmore WA: **Recruitment to the nuclear periphery can alter expression of genes in human cells**. *PLoS Genet* 2008, **4**:e1000039.
10. Kumaran RI, Spector DL: **A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence**. *J Cell Biol* 2008, **180**:51-65.
11. Reddy KL, Zullo JM, Bertolino E, Singh H: **Transcriptional repression mediated by repositioning of genes to the nuclear lamina**. *Nature* 2008, **452**:243-247.
12. Spector DL: **The dynamics of chromosome organization and gene regulation**. *Annu Rev Biochem* 2003, **72**:573-608.
13. Towbin BD, Meister P, Gasser SM: **The nuclear envelope—a scaffold for silencing?** *Curr Opin Genet Dev* 2009, **19**:180-186.
14. Ruault M, Dubarry M, Taddei A: **Re-positioning genes to the nuclear envelope in mammalian cells: impact on transcription**. *Trends Genet* 2008, **24**:574-581.
15. Tjong H, Gong K, Chen L, Alber F: **Physical tethering and volume exclusion determine higher-order genome organization in budding yeast**. *Genome Res* 2012, **22**:1295-1305.
16. Tokuda N, Terada TP, Sasai M: **Dynamical modeling of three-dimensional genome organization in interphase budding yeast**. *Biophys J* 2012, **102**:296-304.
17. Wong H, Marie-Nelly H, Herbert S, Carrivain P, Blanc H, Koszul R, Fabre E, Zimmer C: **A predictive computational model of the dynamic 3D interphase yeast nucleus**. *Curr Biol* 2012, **22**:1881-1890.
18. Duan Z, Andronescu M, Schutz K, McIlwain S, Kim YJ, Lee C, Shendure J, Fields S, Blau CA, Noble WS: **A three-dimensional model of the yeast genome**. *Nature* 2010, **465**:363-367.
19. Gotta M, Laroche T, Formenton A, Maillet L, Scherthan H, Gasser SM: **The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae***. *J Cell Biol* 1996, **134**:1349-1363.
20. Taddei A, Van Houwe G, Nagai S, Erb I, van Nimwegen E, Gasser SM: **The functional importance of telomere clustering: global changes in gene expression result from SIR factor dispersion**. *Genome Res* 2009, **19**:611-625.
21. Mondoux MA, Scaife JG, Zakian VA: **Differential nuclear localization does not determine the silencing status of**

Please cite this article in press as: Meister P, Taddei A. Building silent compartments at the nuclear periphery: a recurrent theme, Curr Opin Genet Dev (2013), http://dx.doi.org/10.1016/j.gde.2012.12.001

Saccharomyces cerevisiae telomeres. *Genetics* 2007, **177**:2019-2029.

22. Maillet L, Boscheron C, Gotta M, Marcand S, Gilson E, Gasser SM: **Evidence for silencing compartments within the yeast nucleus: a role for telomere proximity and Sir protein concentration in silencer-mediated repression**. *Genes Dev* 1996, **10**:1796-1811.

23. Rusche LN, Kirchmaier AL, Rine J: **The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae**. *Annu Rev Biochem* 2003, **72**:481-516.

24. Haber JE: **Mating-type genes and MAT switching in *Saccharomyces cerevisiae***. *Genetics* 2012, **191**:33-64.

25. Radman-Livaja M, Ruben G, Weiner A, Friedman N, Kamakaka R, Rando OJ: **Dynamics of Sir3 spreading in budding yeast: secondary recruitment sites and euchromatic localization**. *EMBO J* 2011, **30**:1012-1026.

26. Dubarry M, Loiodice I, Chen CL, Thermes C, Taddei A: **Tight protein–DNA interactions favor gene silencing**. *Genes Dev* 2011, **25**:1365-1370.

27. Oppikofer M, Kueng S, Martino F, Soeroes S, Hancock SM, Chin JW, Fischle W, Gasser SM: **A dual role of H4K16 acetylation in the establishment of yeast silent chromatin**. *EMBO J* 2011, **30**:2610-2621.

28. Andrulis ED, Zappulla DC, Ansari A, Perrod S, Laiosa CV, Gartenberg MR, Sternglanz R: **Esc1, a nuclear periphery protein required for Sir4-based plasmid anchoring and partitioning**. *Mol Cell Biol* 2002, **22**:8292-8301.

29. Bupp JM, Martin AE, Stensrud ES, Jaspersen SL: **Telomere anchoring at the nuclear periphery requires the budding yeast Sad1-UNC-84 domain protein Mps3**. *J Cell Biol* 2007, **179**:845-854.

30. Taddei A, Hediger F, Neumann FR, Bauer C, Gasser SM: **Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins**. *EMBO J* 2004, **23**:1301-1312.

31. Murphy GA, Spedale EJ, Powell ST, Pillus L, Schultz SC, Chen L: **The Sir4 C-terminal coiled coil is required for telomeric and mating type silencing in *Saccharomyces cerevisiae***. *J Mol Biol* 2003, **334**:769-780.

32. McBryant SJ, Krause C, Woodcock CL, Hansen JC: **The silent information regulator 3 protein, SIR3p, binds to chromatin fibers and assembles a hypercondensed chromatin architecture in the presence of salt**. *Mol Cell Biol* 2008, **28**:3563-3572.

33. Liou GG, Tanny JC, Kruger RG, Walz T, Moazed D: **Assembly of the SIR complex and its regulation by O-acetyl-ADP-ribose, a product of NAD-dependent histone deacetylation**. *Cell* 2005, **121**:515-527.

34. Liaw H, Lustig AJ: **Sir3 C-terminal domain involvement in the initiation and spreading of heterochromatin**. *Mol Cell Biol* 2006, **26**:7616-7631.

35. King DA, Hall BE, Iwamoto MA, Win KZ, Chang JF, Ellenberger T: **Domain structure and protein interactions of the silent information regulator Sir3 revealed by screening a nested deletion library of protein fragments**. *J Biol Chem* 2006, **281**:20107-20119.

36. Chang JF, Hall BE, Tanny JC, Moazed D, Filman D, Ellenberger T: **Structure of the coiled-coil dimerization motif of Sir4 and its interaction with Sir3**. *Structure* 2003, **11**:637-649.

37. •• Ruault M, De Meyer A, Loiodice I, Taddei A: **Clustering heterochromatin: Sir3 promotes telomere clustering independently of silencing in yeast**. *J Cell Biol* 2011, **192**:417-431.

Using a gain of function approach, the silencing factor Sir3 was show to be limiting for telomere clustering. Sir3 overexpression triggers the grouping of telomeric foci into larger foci that relocalize to the nuclear interior demonstrating that telomere clustering can occur independenty of perinuclear anchoring. Furthermore, Sir3's ability to mediate telomere clustering can be separated from its role in silencing. Indeed, nonacetylable Sir3, which is unable to spread into subtelomeric regions, can mediate telomere clustering independently of Sir2–Sir4 as long as it is targeted to telomeres by the Rap1 protein. Thus, arrays of Sir3 binding sites at telomeres appeared as the sole requirement to promote trans-interactions between telomeres.

38. Tham WH, Wyithe JS, Ko Ferrigno P, Silver PA, Zakian VA: **Localization of yeast telomeres to the nuclear periphery is separable from transcriptional repression and telomere stability functions**. *Mol Cell* 2001, **8**:189-199.

39. Hediger F, Neumann FR, Van Houwe G, Dubrana K, Gasser SM: **Live imaging of telomeres: yKu and Sir proteins define redundant telomere-anchoring pathways in yeast**. *Curr Biol* 2002, **12**:2076-2089.

40. Gartenberg MR, Neumann FR, Laroche T, Blaszczyk M, Gasser SM: **Sir-mediated repression can occur independently of chromosomal and subnuclear contexts**. *Cell* 2004, **119**:955-967.

41. Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, Eussen BH, de Klein A, Wessels L, de Laat W *et al.*: **Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions**. *Nature* 2008, **453**:948-951.

42. Gerstein MB, Lu ZJ, Van Nostrand EL, Cheng C, Arshinoff BI, Liu T, Yip KY, Robilotto R, Rechtsteiner A, Ikegami K *et al.*: **Integrative analysis of the *Caenorhabditis elegans* genome by the modENCODE project**. *Science* 2010, **330**:1775-1787.

43. Pickersgill H, Kalverda B, de Wit E, Talhout W, Fornerod M, van Steensel B: **Characterization of the Drosophila melanogaster genome at the nuclear lamina**. *Nat Genet* 2006, **38**:1005-1014.

44. •• Towbin BD, Gonzalez-Aguilera C, Sack R, Gaidatzis D, Kalck V, Meister P, Askjaer P, Gasser SM: **Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery**. *Cell* 2012, **150**:934-947.

Using a heterochromatic repetitive array and assuming that delocalization would lead to derepression, the authors use a genome-wide RNAi screen to uncover factors involved in perinuclear heterochromatin anchoring in *C. elegans*. Knock-down of components of the synthesis pathway of S-adenosylmethionine, the cellular methyl donor leads to derepression and delocalization of heterochromatin away from the NE. Assuming possible methylated target would be histones, they find that a combination of two H3K9 HMT phenocopies the RNAi phenotype. Moreover, a combination of microscopic and mass spectrometry analysis shows that one enzyme is cytoplasmic and catalyzes monomethylation and dimethylation of H3K9 while the other is nuclear, carries H3K9 trimethylation and interacts with trimethylated H3K9 chromatin, suggesting part of a feed-forward loop for the creation of silent compartments.

45. Ahmed K, Dehghani H, Rugg-Gunn P, Fussner E, Rossant J, Bazett-Jones DP: **Global chromatin architecture reflects pluripotency and lineage commitment in the early mouse embryo**. *PLoS ONE* 2010, **5**:e10531.

46. Efroni S, Duttagupta R, Cheng J, Dehghani H, Hoeppner DJ, Dash C, Bazett-Jones DP, Le Grice S, McKay RD, Buetow KH *et al.*: **Global transcription in pluripotent embryonic stem cells**. *Cell Stem Cell* 2008, **2**:437-447.

47. Probst AV, Almouzni G: **Heterochromatin establishment in the context of genome-wide epigenetic reprogramming**. *Trends Genet* 2011, **27**:177-185.

48. Meshorer E, Yellajoshula D, George E, Scambler PJ, Brown DT, Misteli T: **Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells**. *Dev Cell* 2006, **10**:105-116.

49. •• Peric-Hupkes D, Meuleman W, Pagie L, Bruggeman SW, Solovei I, Brugman W, Graf S, Flicek P, Kerkhoven RM, van Lohuizen M *et al.*: **Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation**. *Mol Cell* 2010, **38**:603-613.

Using the DamID technology based on a lamin-dam fusion during the induction of astrocyte differentiation from mouse ESCs, the authors define Lamin-Associated Domains (LADs). About 90% of these domains are stable during differentiation. However, LADs containing neuronal genes activated during differentiation are released from the nuclear periphery upon activation. Surprisingly a number of LADs are released before transcriptional activation of the genes ('unlocked').

50. Hiratani I, Ryba T, Itoh M, Yokochi T, Schwaiger M, Chang CW, Lyou Y, Townes TM, Schubeler D, Gilbert DM: **Global reorganization of replication domains during embryonic stem cell differentiation**. *PLoS Biol* 2008, **6**:e245.

Please cite this article in press as: Meister P, Taddei A. Building silent compartments at the nuclear periphery: a recurrent theme, Curr Opin Genet Dev (2013), http://dx.doi.org/10.1016/j.gde.2012.12.001

51. Solovei I, Kreysing M, Lanctot C, Kosem S, Peichl L, Cremer T, Guck J, Joffe B: **Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution**. *Cell* 2009, **137**:356-368.

52. Liu T, Rechtsteiner A, Egelhofer TA, Vielle A, Latorre I, Cheung MS, Ercan S, Ikegami K, Jensen M, Kolasinska-Zwierz P *et al.*: **Broad chromosomal domains of histone modification patterns in *C. elegans***. *Genome Res* 2010, **21**:227-236.

53. Ikegami K, Egelhofer T, Strome S, Lieb JD: ***Caenorhabditis elegans* chromosome arms are anchored to the nuclear membrane via discontinuous association with LEM-2**. *Genome Biol* 2010, **11**:R120.

54. Meister P, Towbin BD, Pike BL, Ponti A, Gasser SM: **The spatial dynamics of tissue-specific promoters during *C. elegans* development**. *Genes Dev* 2010, **24**:766-782.

55. Towbin BD, Meister P, Pike BL, Gasser SM: **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* 2010, **75**:555-565.

56. Hsieh J, Fire A: **Recognition and silencing of repeated DNA**. *Annu Rev Genet* 2000, **34**:187-204.

57. Loyola A, Bonaldi T, Roche D, Imhof A, Almouzni G: **PTMs on H3 variants before chromatin assembly potentiate their final epigenetic state**. *Mol Cell* 2006, **24**:309-316.

58. Loyola A, Tagami H, Bonaldi T, Roche D, Quivy JP, Imhof A, Nakatani Y, Dent SY, Almouzni G: **The HP1alpha-CAF1-SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin**. *EMBO Rep* 2009, **10**:769-775.

59. • Pinheiro I, Margueron R, Shukeir N, Eisold M, Fritzsch C, Richter FM, Mittler G, Genoud C, Goyama S, Kurokawa M *et al.*: **Prdm3 and Prdm16 are H3K9me1 methyltransferases required for mammalian heterochromatin integrity**. *Cell* 2012, **150**:948-960.

Using a biochemical approach to characterize enzymes involved in H3K9 monomethylation at heterochromatic centromeres, the authors isolate four SET-domain enzymes, ESET, SETD5, ASH1 and Prdm16. Based on the knock-down phenotypes and biochemical activity, Prdm16 and its homolog Prdm3 act as redundant H3K9 monomethyltransferases involved in centromeric heterochromatin formation. Both enzymes appear to act on soluble histones and are essential for centromere formation and clustering inside the nucleus as well as lamina organization

60. •• Gu SG, Pak J, Guang S, Maniar JM, Kennedy S, Fire A: **Amplification of siRNA in *Caenorhabditis elegans* generates a transgenerational sequence-targeted histone H3 lysine 9 methylation footprint**. *Nat Genet* 2012, **44**:157-164.

The authors observe that feeding dsRNA triggers deposition of H3K9me3 on the target gene, spreading about 10 kb around the region homologous to the dsRNA. They show that H3K9 trimethylation requires the nuclear but not the cytoplasmic RNAi pathway. Moreover, deposition of H3K9 methylation on the RNAi target gene led to a transgenerational epigenetic silencing of target genes, although triggering siRNAs are not detectable anymore in the progeny.

61. • Buckley BA, Burkhart KB, Gu SG, Spracklin G, Kershner A, Fritz H, Kimble J, Fire A, Kennedy S: **A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality**. *Nature* 2012, **489**:447-451.

Using a screening approach to isolate genes involved in heritable gene silencing, the authors isolate 4 alleles of the same gene, the nuclear argonaute *hrde-1*. *hrde-1* is necessary for deposition of methylated H3K9 on RNAi target genes, together with components of the nuclear RNAi pathway. Endogenous target genes of the nuclear and heritable gene silencing pathways appear to be germline genes, as HRDE-1 and co-precipitated a number of germline-gene small RNAs. Finally, deficiency of either pathway led to a 'mortal germline' phenotype where mutant worms are able to reproduce only a limited number of generations, suggesting heritable and nuclear epigenetic gene silencing are essential for germline immortality.

62. • Ashe A, Sapetschnig A, Weick EM, Mitchell J, Bagijn MP, Cording AC, Doebley AL, Goldstein LD, Lehrbach NJ, Le Pen J *et al.*: **piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans***. *Cell* 2012, **150**:88-99.

Using genetic screening approaches, the authors show that nuclear RNAi is essential for germline-targeted transgenerational epigenetic inheritance induced by environmental RNAi (RNAi by feeding) or germline-restricted piRNA (Piwi-interacting RNA). The tested genes include the nuclear and heritable gene silencing pathways, piRNA producing pathways as well as a number of chromatin proteins. Among seven SET-domain containing genes, only *set-25* and *set-32* mutations impaired silencing, as well as the HP1 homolog *hpl-2* deletion. This suggests a link between H3K9 methylation and a transgenerational germline gene silencing mechanism.

63. Muchardt C, Guilleme M, Seeler JS, Trouche D, Dejean A, Yaniv M: **Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1alpha**. *EMBO Rep* 2002, **3**:975-981.

64. Maison C, Bailly D, Peters AH, Quivy JP, Roche D, Taddei A, Lachner M, Jenuwein T, Almouzni G: **Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component**. *Nat Genet* 2002, **30**:329-334.

65. Keller C, Adaixo R, Stunnenberg R, Woolcock KJ, Hiller S, Buhler M: **HP1(Swi(6)) mediates the recognition and destruction of heterochromatic RNA transcripts**. *Mol Cell* 2012, **47**:215-227.

66. Maison C, Bailly D, Roche D, Montes de Oca R, Probst AV, Vassias I, Dingli F, Lombard B, Loew D, Quivy JP *et al.*: **SUMOylation promotes de novo targeting of HP1alpha to pericentric heterochromatin**. *Nat Genet* 2011, **43**:220-227.

67. •• Zullo JM, Demarco IA, Pique-Regi R, Gaffney DJ, Epstein CB, Spooner CJ, Luperchio TR, Bernstein BE, Pritchard JK, Reddy KL *et al.*: **DNA sequence-dependent compartmentalization and silencing of chromatin at the nuclear lamina**. *Cell* 2012, **149**:1474-1487.

Following up on damID studies which characterized LADs, the authors use two specific LADs from *Cyp3a* and *IgH*. When inserted by homologous recombination next to *lacO* sites, these LADs are able to address autonomously a nucleoplasmic locus toward the nuclear periphery as well as repress gene expression. Molecular dissection of the LADs sequences showed that lamina-targeting sequences are enriched for a GAGA motif, suggesting binding by the mammalian homolog of the *Drosophila* GAGA transcription factor homolog cKrox. Moreover, cKrox was shown to be in a complex with the histone deacetylase HDAC3 and the inner nuclear membrane protein LAB2β. GAGA motif repeats are sufficient for cKrox binding and recruitment to the nuclear lamina. This suggests a nucleation step mediated by cKrox binding, which recruits a histone modifier and a peripheral anchor.

68. Somech R, Shaklai S, Geller O, Amariglio N, Simon AJ, Rechavi G, Gal-Yam EN: **The nuclear-envelope protein and transcriptional repressor LAP2beta interacts with HDAC3 at the nuclear periphery, and induces histone H4 deacetylation**. *J Cell Sci* 2005, **118**:4017-4025.

69. Ye Q, Worman HJ: **Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to Drosophila HP1**. *J Biol Chem* 1996, **271**:14653-14656.

70. Canzio D, Chang EY, Shankar S, Kuchenbecker KM, Simon MD, Madhani HD, Narlikar GJ, Al-Sady B: **Chromodomain-mediated oligomerization of HP1 suggests a nucleosome-bridging mechanism for heterochromatin assembly**. *Mol Cell* 2011, **41**:67-81.

71. Bulut-Karslioglu A, Perrera V, Scaranaro M, de la Rosa-Velazquez IA, van de Nobelen S, Shukeir N, Popow J, Gerle B, Opravil S, Pagani M *et al.*: **A transcription factor-based mechanism for mouse heterochromatin formation**. *Nat Struct Mol Biol* 2012, **19**:1023-1030.

72. van Koningsbruggen S, Gierlinski M, Schofield P, Martin D, Barton GJ, Ariyurek Y, den Dunnen JT, Lamond AI: **High-resolution whole-genome sequencing reveals that specific chromatin domains from most human chromosomes associate with nucleoli**. *Mol Biol Cell* 2010, **21**:3735-3748.

73. Nemeth A, Conesa A, Santoyo-Lopez J, Medina I, Montaner D, Peterfia B, Solovei I, Cremer T, Dopazo J, Langst G: **Initial genomics of the human nucleolus**. *PLoS Genet* 2010, **6**:e1000889.

74. Bantignies F, Roure V, Comet I, Leblanc B, Schuettengruber B, Bonnet J, Tixier V, Mas A, Cavalli G: **Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila**. *Cell* 2011, **144**:214-226.

Please cite this article in press as: Meister P, Taddei A. Building silent compartments at the nuclear periphery: a recurrent theme, Curr Opin Genet Dev (2013), http://dx.doi.org/10.1016/j.gde.2012.12.001