Review

Histones and histone modifications in perinuclear chromatin anchoring: from yeast to man

Jennifer C Harr[1], Adriana Gonzalez-Sandoval[1,2] & Susan M Gasser[1,2,*]

Abstract

It is striking that within a eukaryotic nucleus, the genome can assume specific spatiotemporal distributions that correlate with the cell's functional states. Cell identity itself is determined by distinct sets of genes that are expressed at a given time. On the level of the individual gene, there is a strong correlation between transcriptional activity and associated histone modifications. Histone modifications act by influencing the recruitment of non-histone proteins and by determining the level of chromatin compaction, transcription factor binding, and transcription elongation. Accumulating evidence also shows that the subnuclear position of a gene or domain correlates with its expression status. Thus, the question arises whether this spatial organization results from or determines a gene's chromatin status. Although the association of a promoter with the inner nuclear membrane (INM) is neither necessary nor sufficient for repression, the perinuclear sequestration of heterochromatin is nonetheless conserved from yeast to man. How does subnuclear localization influence gene expression? Recent work argues that the common denominator between genome organization and gene expression is the modification of histones and in some cases of histone variants. This provides an important link between local chromatin structure and long-range genome organization in interphase cells. In this review, we will evaluate how histones contribute to the latter, and discuss how this might help to regulate genes crucial for cell differentiation.

See the Glossary for abbreviations used in this article.

Changes in heterochromatic histone modifications during cell type commitment

The radial distribution of chromatin changes significantly during cell differentiation (Fig 1). This can be observed during physiological events, for example, during mammalian hematopoiesis [1–4] or *Caenorhabditis elegans* development [5,6], and occurs during *in vitro* differentiation events, such as the induction of neuronal cell types from pluripotent embryonic stem cells (ESC) [7–9]. Whereas all cells of an organism contain the same DNA sequence, it is the expression of cell type-specific genes at the right time that determines cell fate and function. Tissue-specific changes in gene expression correlate with changes in histone modifications and *de novo* CpG methylation [10,11]. Morphologically, differentiation correlates with the appearance of dark-staining foci of heterochromatin. Consistently, compact chromatin domains at the INM or around the nucleolus are less apparent in undifferentiated ES cells than in their differentiated counterparts [3,12,13]. Given that gene activity depends not only on transcription factor availability, but on histone modification and local chromatin structure (reviewed in [14,15]), the sequestration of promoters at the INM may well impact one of these steps, or impose an additional layer of regulation that reinforces stable cell type commitment.

At the heart of the matter are histone modifications, which are known to control local chromatin structure. Indeed, in almost all commonly studied eukaryotic species except budding yeast, heterochromatin is composed of DNA and associated histones bearing specific post-translational modifications. In budding yeast, the absence of histone tail modifications, and explicitly the active removal of histone H4 lysine 16 acetylation (H4K16ac), enables the formation of a heritably silent state characterized by the binding of Sir3 to nucleosomes [16]. In mammals, flies [17–19], and *C. elegans* [20–22], heterochromatic regions are enriched for histone H3K9 di- and/or trimethylation (H3K9me2 and me3), while in fission yeast H3K9me2 appears to dominate [23]. There are two general classes of heterochromatin in mammalian cells: facultative (regulated) heterochromatin that can be enriched for H3K9me2/3 and H3K27me3, and constitutive heterochromatin that is enriched for H3K9me3 and/or H4K20me3. Whereas H4K20me1 is found in promoters and hyper-active genes, H4K20me3 colocalizes with H3K9me3 in large repeat domains (pericentromeric satellites in mouse) and colocalizes with Heterochromatin protein 1 isotypes (HP1α and HP1β) in differentiated cells [24–27]. In all cases, the resulting repressive chromatin domain restricts access for RNA polymerase or its regulatory factors and, as shown in fission yeast [28,29], may promote transcript degradation. In contrast, the promoters of stably expressed genes harbor histone tail acetylation on histones H3 and H4 and H3K4 trimethylation (H3K4me3).

The Polycomb repressive complex 2 (PRC2) mediates trimethylation of H3K27, which is a further histone modification found on

1 Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
2 Faculty of Natural Sciences, University of Basel, Basel, Switzerland
   *Corresponding author. Tel: +41 61 697 7255; Fax: +41 61 697 3976; E-mail: susan.gasser@fmi.ch

Glossary

BAF	barrier to autointegration factor
Bqt3	bouquet formation protein 3
CEC-4	*Caenorhabditis elegans* chromodomain protein-4
ChIP	chromatin immunoprecipitation
cKrox	Kruppel-related zinc finger protein cKrox
Clr4	histone H3 (Lys9) methyltransferase
Csi1	Cop9 signalosome interactor 1
CXXC2	CXXC-type zinc finger protein 2
DAPI	4′,6-diamidino-2-phenylindole
Dsh1	defect of the gene silencing at centromeric heterochromatin
Esc1	enhancer of silent chromatin 1
ESC	embryonic stem cell
ESET	ERG-associated protein with SET domain
Est1	elongator of short telomeres 1
EZH2	enhancer of zeste 2
FBXL10	F-box and leucine-rich repeat protein 10
Fft3	fission yeast fun-thirty
FISH	fluorescence *in situ* hybridization
G9a	histone-lysine 9N-methyltransferase
H3	histone H3
H4	histone H4
HDAC	histone deacetylase
Heh1	helix-extension-helix domain-containing protein 1
HLH-1	helix loop helix-1
HMT	histone methyltransferase
HP1	heterochromatin protein 1
HPL-2	HP1-like (heterochromatin protein) 2
Ima1	integral inner nuclear membrane protein Ima1
INM	inner nuclear membrane
iPS cells	induced pluripotent stem cells
JHDM1B	JmjC domain-containing histone demethylation protein 1B
KDM2B	lysine-specific demethylase 2B
LAD	lamina-associated domain
LAP2	lamina-associated polypeptide 2
LBR	lamin B receptor
LEM	LAP2, emerin, and MAN1
LIN-61	abnormal cell LINeage-61
LOCKS	large domains of chromatin bearing H3K9 modifications
MAN1	integral inner nuclear membrane protein Man1
MBT	malignant brain tumor repeats
me1	monomethyl
me2	dimethyl
me3	trimethyl
MEF	mouse embryonic fibroblast
MET-2	histone METhyltransferase-like-2
Mps1	MonoPolar Spindle protein 1
MSC	MAN1-SCR1 C-terminal
MyoD	myoblast determination protein 1
NDY1	not dead yet-1
PRC	Polycomb repressive complex (1 or 2)
PRR14	proline-rich 14
Rap1	repressor/activator site-binding protein
RNAi	RNA interference
Sad1	spindle pole body-associated protein
SAMS	S-adenosyl methionine synthetase
SET25	SET (trithorax/Polycomb) domain containing
SETDB1	SET domain, bifurcated 1
SHREC	Snf2/Hdac-containing Repressor Complex
SIR	silent information regulator
Snf2	Sucrose Non-Fermenting2
SPB	spindle pole body
Src1	spliced mRNA and cell cycle-regulated gene
SUN	Sad1 and UNC84 domain-containing 1
SUV39H1	suppressor of variegation 3–9 homolog 1
TAD	topology-associated domain
UNC-84	uncoordinated protein 84
vLAD	variable lamina-associated domain
Y2H	yeast two-hybrid
yKU	yeast KU protein
YY1	Ying-Yang-1
Zbtb7b	zinc finger and BTB domain-containing 7B

repressed chromatin in differentiated cells [30]. Intriguingly, during zebrafish development and in ESCs, H3K27me3 often coincides with H3K4me3 at poised but transcriptionally inactive promoters [31,32]. Loss of H3K27me3 at some of these promoters later in development correlates with their activation, even though the role of this bivalency in controlling developmental gene expression has been questioned [33,34] (discussed in another review in this series [35]). Indeed, a second Polycomb complex, PRC1, mediates histone H2A lysine 119 monoubiquitination prior to nucleation of an H3K27me3 domain, and FBXL10 (also known as KDM2B, NDY1, JHDM1B, and CXXC2), a component of a variant PRC1, wards off CpG methylation at Polycomb target sites [36]. Thus, PRC1 (or its variant forms) may be critical for keeping promoters silent, while ensuring that they are not irreversibly repressed by DNA methylation. Both pathways suggest that Polycomb-mediated repression silences genes in a manner that allows for either subsequent activation or subsequent repression [37,38].

In addition to the accumulation of H3K27me domains, the distribution of H3K9me2 and me3 changes with the establishment of differentiated cell states. The differentiation of hematopoietic progenitor cells, for instance, correlates with increased sequestration of H3K9me3-containing heterochromatin at the INM or around nucleoli, although in these cells global levels of H3K9 methylation seemed not to change [3,39]. Differentiation of stem cells was dependent on G9a, the H3K9 methyltransferase responsible for mono- and dimethylation [3]. Another study reported an increase in levels of H3K9me2 during ESC differentiation, and the concurrent formation of large domains of chromatin bearing H3K9-modifications (LOCKS), which covered one-third of the genome in 100-kb stretches, in differentiated tissues [40]. These different conclusions about H3K9 methylation levels may stem from different modes of quantitation, chromatin isolation [41,42], or from reported cross-reactivity between antibodies recognizing H3K9me2 and me3 [43,44]. Nonetheless, there is a consensus that de-methylation of H3K9 enhances the efficiency of differentiated cell reprogramming [45–48]. Furthermore, the subnuclear distribution of H3K9me3 clearly changes as cells differentiate, with H3K9me3-positive domains being sequestered at the INM. This has been particularly well documented during rodent development [49,50].

The major ligand of H3K9 methylation is a protein called heterochromatin protein 1 (HP1), which has at least three isoforms in mammalian cells and two in *C. elegans* and *Schizosaccharomyces pombe*. All HP1 proteins, including the three mammalian isoforms, HP1α, HP1β, and HP1γ, contain an N-terminal chromodomain and a C-terminal chromo-shadow domain. The chromodomain specifically recognizes both H3K9me2 and me3 [51,52], while the
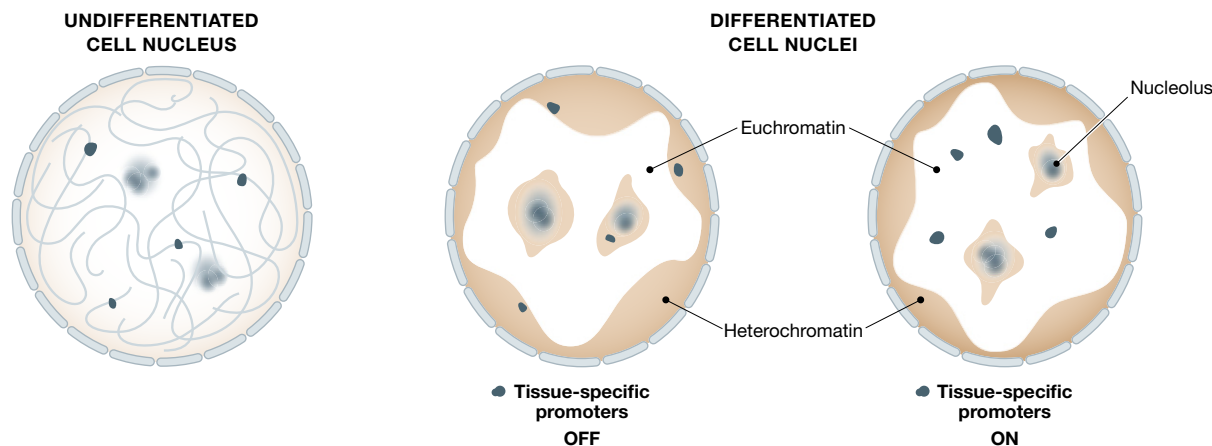
Figure 1. Chromatin distribution changes occur upon cell differentiation.
In differentiated cells, there are distinct domains of dark-staining heterochromatin at the nuclear periphery and around the nucleolus. Tissue-specific genes are found in heterochromatic zones when repressed and are in euchromatic zones when active. This is true in many species.

chromo-shadow domain mediates the interaction with other proteins. A spacer domain between them binds RNA. Intriguingly, the different HP1 variants have very distinct roles in the ESC-to-differentiated-cell transition, and not all HP1 binding correlates with heterochromatic gene repression. Indeed, the residence time of HP1 on chromatin is very short [53], and in *S. pombe*, its RNA binding functions are associated with the restricted spread of a silent domain [28,29].

Confirming this complex role for H3K9me ligands, a recent paper reported that the mammalian HP1β participates both in proper differentiation and in maintaining ESC pluripotency [54]. In ESCs, HP1β is diffusely distributed across the nucleus, unlike in differentiated cells, where it is highly enriched on pericentric heterochromatin. Loss of the HP1β gene in ESCs drove them toward differentiation, while in differentiated cells its loss had the opposite effect, increasing the efficiency with which they were reprogrammed into iPS cells, much like loss of H3K9 methylation [54]. Ablation of HP1α, which binds centromeric satellite sequences in all cells, did not have the same effect. Thus, H3K9 methylation and its ligands can act in diverse ways to control gene expression and cell fate, at least in *in vitro* systems.

During mammalian embryogenesis, early-stage nuclei are characterized by extensive chromatin remodeling, including dynamic changes in DNA methylation, histone variants, and histone modifications [55]. The core histones H2A, H3.1, and H3.2 exchange rapidly in and out of chromatin in murine pre-implantation embryos, yet this is lost as cells transition from undifferentiated to more determined states [56]. Unlike the case in most somatic cells, pericentromeric major satellite sequences in early embryos are initially localized around pre-nucleolar bodies and only later become clustered in chromocenters [57]. Nonetheless, in the maternal genome, these sequences are characterized by classical markers of constitutive heterochromatin, such as H3K9me3, H4K20me3, and HP1β [58,59], which exclude the binding of the Polycomb repressor complex PRC1 [60]. On the paternal genome, on the other hand, a transcriptionally repressed heterochromatic state requires PRC1 and PRC2 proteins, presumably as compensation for the lack of paternal transmission of H3K9me3-marked nucleosomes in sperm [59]. Early paternal heterochromatin is also characterized by high levels of the histone H3

variant H3.3, and overexpression of H3.3 bearing a K27 mutation impairs chromosome segregation and ultimately arrests development [61]. Thus, not only chromatin modifications, but also histone variants are important for heterochromatin formation. The nuclear organization of repetitive DNA is not only highly dynamic in early embryos, but also involves gamete-specific modes of repression.

Accompanying differentiation and the transition from dispersed chromatin to more defined heterochromatic and euchromatic patterns, there is a cell type-specific establishment of late-replicating domains. These coincide with the so-called topologically associating domains (TADs). TADs monitor DNA–DNA interaction probabilities, providing a measure of physical proximity between DNA sequences within the interphase nucleus [62,63]. Not surprisingly, differences in replication timing correlate with different transcription states, and at least a subset of late-replicating domains are associated with the nuclear lamina [63,64].

If we are to prove a causal link between subnuclear position and altered genome function, it will be necessary to show through genetic means that the mechanism that mediates positioning also leads to transcriptional repression and/or late replication. While this link is still elusive in mammalian systems, it has been provided by studies in *C. elegans*, where histone H3K9 methylation was shown to be essential both for silencing and for the positioning of chromatin at the nuclear periphery [22]. Furthermore, an INM-associated factor, CEC-4, was identified, which specifically recognizes H3K9-methylated chromatin and sequesters it at the nuclear periphery [65]. These studies provide a mechanism to coordinate subnuclear positioning and the establishment of gene repression, as discussed below.

LADs and chromatin marks that correlate with peripheral attachment

The nuclear periphery is composed of integral INM proteins and nuclear lamins, collectively called the lamina [66], which binds chromatin in a still poorly defined manner. As mentioned above, chromatin that is associated with the lamina (lamina-associated domains or LADs) is densely packed and enriched for repressive

histone modifications, most notably H3K9me2/me3 [67,68]. LADs, which monitor even transient association with the nuclear lamina, can cover up to 30% of the genome in mammalian cells, and domains range from 0.1 to 10 Mb in size [67]. While there is a correlation between LADs and LOCKs, the data are derived from disparate cell types and further studies are needed to determine whether LADs and LOCKs are interdependent or whether they can be uncoupled. LAD sequences tend to be gene poor, yet both the border regions of LADs and cell type-specific or variable LADs (vLADs) are significantly enriched for developmentally regulated, cell type-specific genes [9,69]. Such dynamic LAD regions have been previously described as facultative LADs [70]. Nematodes also show a striking correlation between lamin association and repressive histone modifications like H3K9 methylation (H3K9me1, me2, and me3) [20–22]. Thus, in both mammalian cells and worms, H3K9 methylation is enriched in chromatin that associates with the INM [20,67,70,71].

Transgenes that are integrated into the *C. elegans* genome as multicopy arrays (200–300 tandem copies) accumulate high levels of H3K9me3 and H3K27me3 in a copy number-dependent manner and are quantitatively associated with the INM [5,72]. Consistently, array-borne promoters of ubiquitously expressed genes are transcriptoinally repressed both in embryonic and in differentiated somatic cells. When integrated arrays carry tissue-specific promoters, on the other hand, tissue-specific gene activation shifts the domain away from the lamina. This illustrates a general correlation between nuclear position and differentiated cell type gene expression (Fig 1) [5].

A genome-wide RNAi screen was carried out using these large *C. elegans* gene arrays to identify the mechanisms that link heterochromatin to the INM. Whereas the loss of various chromatin modifiers led to a loss of transcriptional repression, only one RNAi target released the array from the nuclear periphery [22]. This was S-adenosyl methionine synthetase (SAMS), whose loss reduced global histone methylation [22]. The search for downstream effectors of SAMS focused on histone methyltransferases (HMTs), although there was no single HMT whose ablation led to array delocalization. By combining mutants, it could be shown that the elimination of two HMTs, MET-2 and SET-25, compromised INM binding and derepressed the array-borne promoter. MET-2 is an ESET/SetDB1 homolog that mediates H3K9me1 and me2, and SET-25 is a variant of Suv39h and G9a, which deposits H3K9me3. Elimination of the two enzymes completely eliminated H3K9 methylation in both embryos and larvae and blocked anchoring both of the array and of endogenous repeat-rich heterochromatin in embryos [22]. SET-25, the HMT that deposits the H3K9me3 mark that is necessary for array repression, colocalizes with the silent domain. This colocalization of the HMT, the histone mark, and its reader HPL-1 in perinuclear foci suggested a self-reinforcing mechanism for the establishment and propagation of heterochromatin.

Deposition of H3K9me3 by SET-25 was required for complete array silencing, yet active arrays bearing histone H3K9me1 and me2 were anchored as efficiently as the repressed array [22]. This illustrates a second general principle, that INM association is not sufficient to repress transcription. Moreover, not all transcription events trigger release [5,9,73]. Nonetheless, H3K9 methylation plays an essential role in both events. H3K9me1/me2 can mediate anchoring without silencing (at least in embryos). Anchoring then

facilitates trimethylation by SET-25, which remains bound to chromatin by binding H3K9me3. The binding of HP1 homologs and/or LIN-61, an MBT domain protein that also recognizes H3K9me2/me3, also represses transcription but does not anchor the silent domain [22,74]. Finally, we note that although H3K9 methylation is essential for anchoring in early *C. elegans* embryos, this pathway is not the only heterochromatin-anchoring mechanism that functions in worms. At later developmental stages, such as the first larval stage, sequences that were released from the INM in H3K9 methylation-deficient embryos become re-anchored, although H3K9 methylation remains absent [22]. Thus, alternative pathways for heterochromatin anchoring are induced during terminal differentiation.

A complementary study in mammalian cells has implicated H3K9me2/me3 in positioning chromatin at the nuclear periphery, along with the repressive histone modification H3K27me3 [69]. Harr *et al* focused on LAD borders, which are enriched for genes that are repressed in a cell type-dependent manner, that bear H3K9me2/3 and H3K27me3, and are critical for cell fate determination [9,69]. Whereas a number of mammalian cell studies have investigated the positioning of repetitive reporters, the system used by the Reddy laboratory instead scored for unique sequences that trigger relocation to the nuclear periphery. The targeting sequences were introduced adjacent to a repetitive *lacO* array, which was shown to carry H3K9me2/3, that was not sufficient for perinuclear localization in these differentiated murine fibroblasts. However, the integration of sequences derived from vLAD-specific DNA shifted the reporter to the INM [69,75]. Relocation was reduced upon knockdown of the H3K9 methylation-depositing HMT, SUV39H1, or by treatment with a G9a inhibitor. Similarly, Belmont and colleagues showed that the peripheral positioning of a randomly integrated β-globin locus was dependent on both Suv39H-mediated H3K9me3 and G9a-mediated H3K9me2 [76]. G9a inhibition had effects on endogenous sequences as well, reducing the association of LADs with the INM genome-wide [77]. In Harr *et al*, however, reducing levels of PRC2 to compromise H3K27 methylation and/or treatment with specific EZH2 inhibitors, led to similar reductions in the perinuclear positioning of vLADs [69].

The question arose as to what might recruit PRC2 to vLADs, and therefore, YY1, a transcription factor known to interact with PRC2 [78–81], was targeted to the reporter sequence. Targeted YY1 led to high levels of H3K27me3 on the tagged chromatin and enhanced its association with the INM [69]. Relocation was reduced upon inhibition of the PRC2 catalytic subunit, EZH2, implicating H3K27 methylation in the process. FISH studies confirmed that the localization of vLAD-associated cell type-specific genes at the INM was sensitive to EZH2 inhibition in fibroblasts [69]. In contrast, the removal of PRC2 components *mes-3* and *mes-6*, in *C. elegans*, did not release heterochromatic arrays from the INM, although it led to their derepression [22]. It should be noted that in worms and mammalian species, most PRC-2- or H3K27me3-positive foci are not found at the nuclear perimeter, suggesting that this mark is not sufficient for relocation [82].

We propose that the combinatorial presence of both H3K9me2/me3 and H3K27me3 may be required to shift locus position in mammalian cells (Fig 2). Consistently, both modifications are found at the borders of cell type-specific LADs [67,69]. Alternatively, a combination of histone marks and the binding of specific

transcription factors may regulate promoter position. Other studies have implicated the transcription factor-mediated recruitment of HDAC3, an INM-associated histone deacetylase, in both peripheral anchoring and repression [71,83,84]. These observations highlight the possibility that histone modifications and other factors could cooperate in a cell type-specific manner to target and maintain

heterochromatin at the INM in differentiated mammalian cells (Fig 2). Given that the nuclear envelope proteome is complex and includes many factors with the potential to bind chromatin, we expect that other anchoring factors will function in a cell type- and context-specific manner in differentiated mammalian cells [85–87].
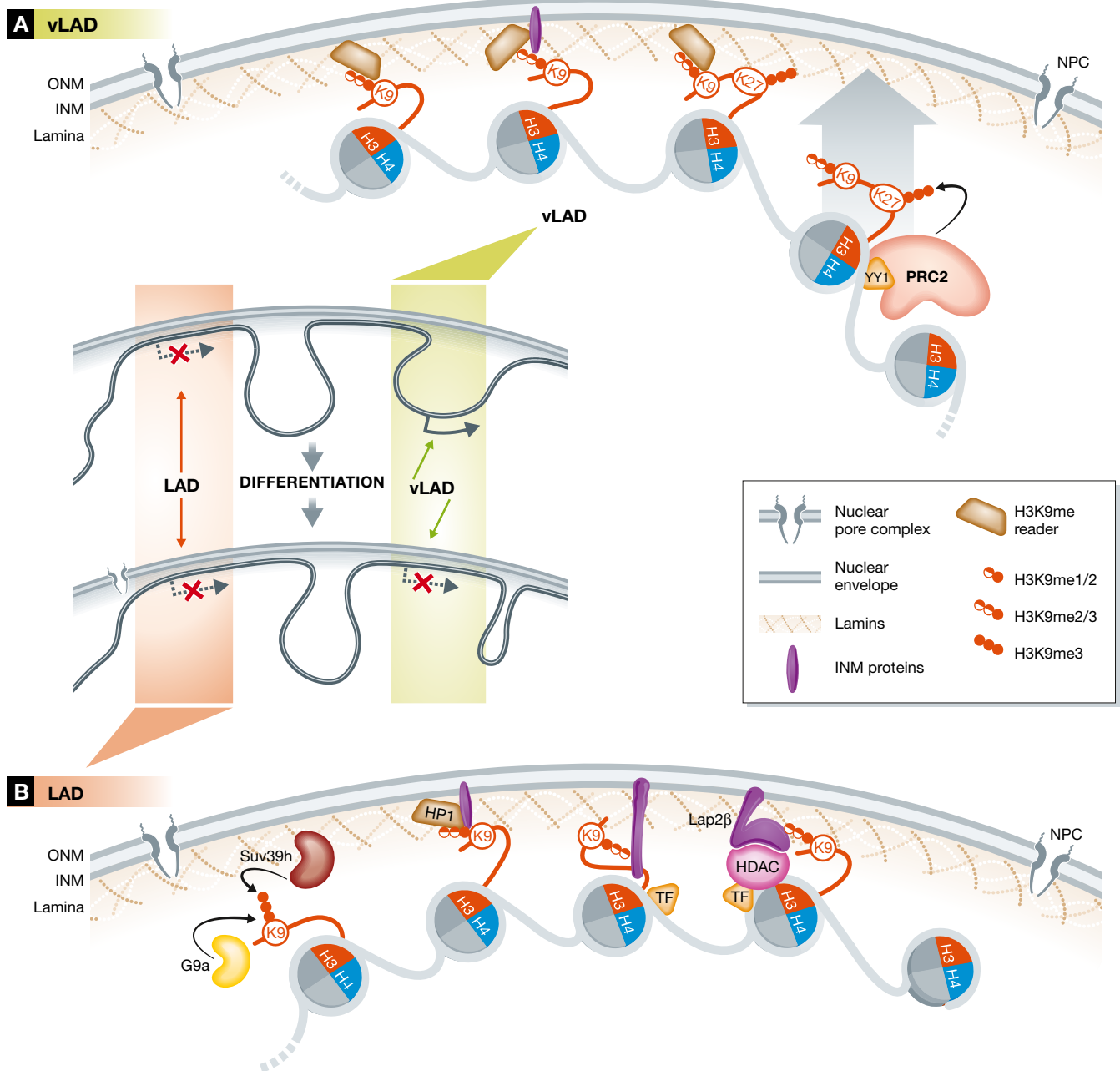

Figure 2. Anchoring chromatin to the nuclear periphery in mammalian cells.
As mammalian cells differentiate, additional domains become associated with the nuclear lamina, called variable LADs (vLADs). These changes between cell types are enriched in cell type-specific genes and are often found at the edges of LADs. (A) vLAD anchoring mechanisms. Borders of vLADs are enriched for both H3K9me2/3 and H3K27me3 and shift to the nuclear periphery in a manner dependent on PRC2 activity as well as on Suv39H1 and G9a. (B) Mechanisms implicated in the anchoring of constitutive/common LADs. They depend on H3K9 methylation deposited by G9a and Suv39h and involve ligands which may include HP1 and other unknown methylation readers. Transcription factor interactions with INM proteins, such as the cKrox (zbtb7b)/HDAC3/Lap2β bridge, may also be relevant for tissue-specific LADs.

Bridging from chromatin to nuclear envelope: anchors for heterochromatin

While genetic manipulations in rodents and human cells have implicated A-type lamins (lamins A and C) and LBR in maintaining heterochromatin at the INM, a direct link between these proteins and any unique marker of peripheral heterochromatin remains elusive. Early studies demonstrated *in vitro* affinity of LBR for HP1 [88] or for HP1–histone complexes [89], but neither HP1 nor histones are uniquely associated with INM-anchored chromatin domains. Moreover, missing from these early biochemical studies was evidence validating these interactions in living cells. Our laboratory carried out a screen in *C. elegans* to identify H3K9me ligands that anchor heterochromatin to the INM. We identified an uncharacterized chromodomain protein, CEC-4, which binds mono-, di-, and trimethylated H3K9 and is essential for array anchoring in *C. elegans* embryos. The loss of CEC-4 phenocopied the array release observed in cells lacking H3K9 methylation, but its loss did not reduce H3K9 methylation levels [65]. CEC-4 is localized to the INM independently of both H3K9 methylation and lamins, forming a perinuclear ring from the earliest stages of embryogenesis through adult tissue differentiation. CEC-4 is displaced from chromatin only when cells undergo mitosis, much like other nuclear lamina proteins [90]. Intriguingly, the localization of CEC-4 itself is not dependent on lamin nor on other known INM proteins, such as emerin, LEM-2, SUN-1, UNC-84, or the LEM domain ligand, BAF-1, and it localizes to the nuclear rim even when expressed in budding yeast [65].

Elimination of CEC-4 in worms released heterochromatin from the periphery, but did not derepress it. This is in contrast to other H3K9me ligands, including the HP1 homolog HPL-2, and LIN-61, whose ablation derepressed the array, but did not affect anchoring (Fig 3) [65]. Remarkably, embryonic gene expression is nearly unchanged despite the loss of CEC-4 under normal laboratory conditions, arguing that INM sequestration of H3K9me is not sufficient nor necessary for gene repression during development. Indeed, *cec-4*-deficient embryos, like the *set-25 met-2* double mutant, were able to differentiate into functional adult worms.

While this indicated that H3K9 methylation and its peripheral sequestration are not essential for carrying out an unperturbed program of development, there were profound differences in outcome when a master regulator of muscle differentiation was induced in synchronized embryos that lack CEC-4. Although *cec-4*-deficient embryos could induce muscle specification in response to HLH-1 (the MyoD homolog in worms), about 25% of these embryos were unable to restrict expression to the muscle-specific program, as occurs in the wild-type background. An L1 stage gut-specific reporter was expressed in *cec-4* mutant embryos, despite the ubiquitous expression of HLH-1, suggesting a further progression of the gut developmental program in *cec-4* deficient embryos [65]. It was concluded that perinuclear anchoring of chromatin during development may help to restrict cell differentiation programs by stabilizing the commitment to a specific cell fate. This may be relevant for development when the standard program is derailed, either by environmental or by endogenous perturbations.
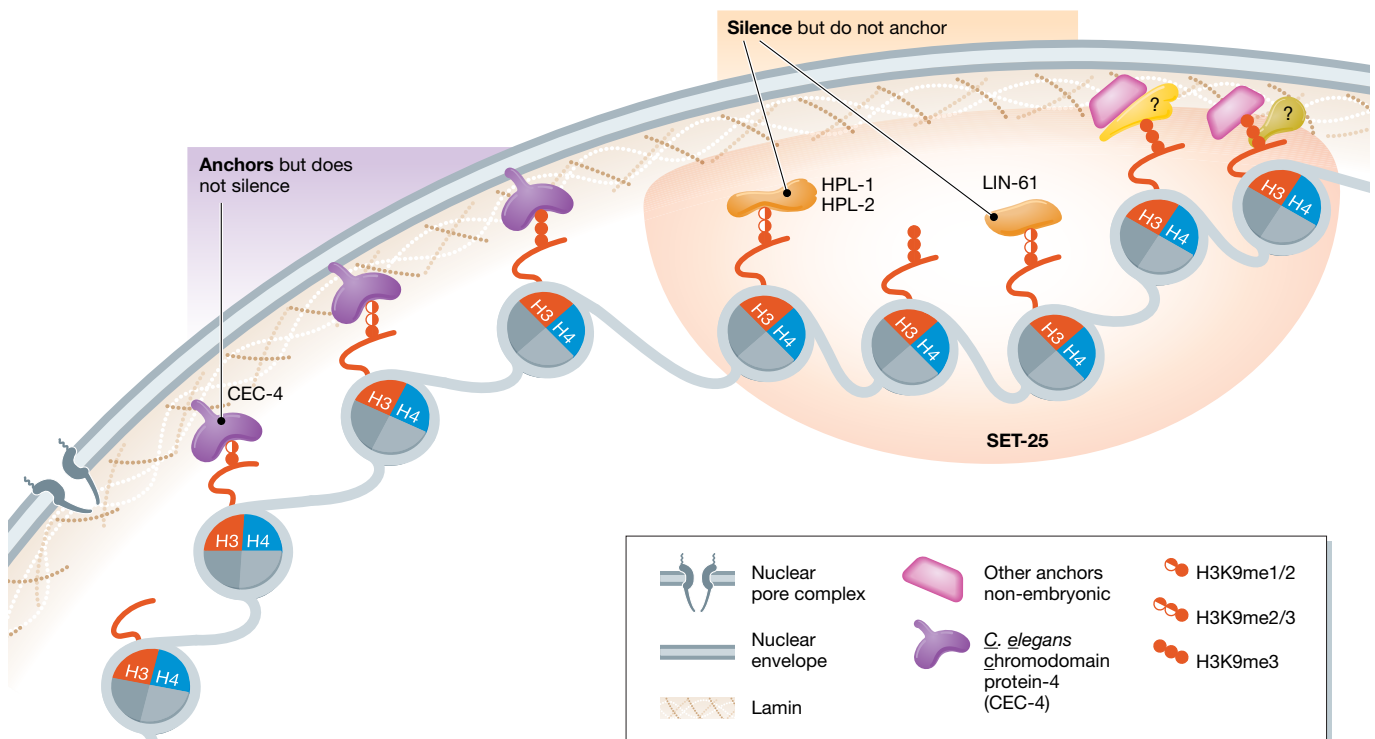

Figure 3. Anchoring chromatin to the nuclear periphery in *Caenorhabditis elegans*.
In *C. elegans* early embryos, CEC-4 is a H3K9me1, me2, or me3 ligand that mediates anchoring to the nuclear periphery, without necessarily repressing transcription. The H3K9me ligands, HPL1, HPL2, and LIN-61, mediate transcriptional repression by binding H3K9 methylation, but do not anchor chromatin. SET-25 recognizes the H3K9me3-containing chromatin that it creates and together with HP1 homologs and LIN-61 leads to repression. In differentiated cells, alternative anchors may be present.

Alternative pathways tether heterochromatin to the INM in termi-nally differentiated cells of *C. elegans* larvae and adults in a manner that is independent of H3K9 methylation and CEC-4. H3K27me3 also does not seem to be essential for this anchoring *per se*, although may be involved in a combinatorial fashion. In this context, it is useful to note that *C. elegans* does not have self-regenerating and committed stem cells, like those found in higher organisms. Its development and differentiation programs are thus inflexible at the single-cell level. We suspect that peripheral anchoring may be even more important for mammalian differentiation programs, given that committed but undifferentiated states are necessary to ensure tissue replenishment throughout the lifetime of the organism. Finally, although there is no obvious homolog of CEC-4 in mammalian genomes, it is possible that the functions of chromatin binding and perinuclear anchorage are shared among multiple polypeptides and genes in mammals, to allow for combinatorial flexibility in chro-matin anchoring.

Lamins, lamin-associated proteins, and other anchors conserved across species

Proteins resident at the INM are the most likely candidates to serve as heterochromatin anchors at the INM. A summary of the evidence supporting the role of such candidates is found in Table 1. In mammalian cells, the B- and A-type lamin proteins form a network of intermediate filaments that helps to stabilize the localization of other INM proteins including LAP2, emerin, and MAN1 (the so-called LEM proteins) [91]. In multicellular organisms, LEM domain proteins bind a small, highly dynamic chromatin-associated factor called BAF (barrier to autointegration), which helps mediate nuclear assembly, while lamin A/C binds to a range of transcription factors [92,93]. These lamin-associated factors may contribute to the binding of chromatin regions to the INM, although given that they also associate with active promoters, what it is that determines sequence specificity remains unclear [70,94]. The identification and knockdown of 23 INM proteins with transmembrane domains led to the mispositioning of whole chromosomes in mammalian cells [85]. However, each acted selectively on a different set of chromo-somes and in a tissue-specific manner [85,95]. Genetic studies also implicate A-type lamins in large-scale chromatin organization [49,96], but to rule out indirect effects, it will be necessary to find mutations that interfere with the recognition of a specific chromatin motif.

Whether acting directly or indirectly, it is clear that lamin A/C, LBR, and Lap2β contribute to the organization of sequences at the INM. Work from the Reddy laboratory showed that lamina-asso-ciating sequences are no longer maintained at the nuclear periphery in the absence of lamin A/C [69]. Similarly, in *C. elegans*, the loss of lamin in embryos, or the combined depletion of LEM-2 and MAN-1, led to a partial detachment of large transgene arrays from the INM and stochastically derepressed array-borne promoters [97]. In human cultured cells, it was shown that the factor PRR14 tethered heterochromatin to the nuclear periphery through its association with HP1 and most probably lamin A or C [98]. Whether it binds lamins directly or indirectly, however, is unclear. An extensive study of differentiated cell types by the Solovei laboratory showed that heterochromatin distribution in differentiated rodent tissues

depends to varying degrees on two different mechanisms that require LBR and lamin A/C, respectively [49]. Remarkably, in nocturnal rod photoreceptor cells of the retina, which lack both LBR and lamin A/C expression, chromatin has an inverted configuration, such that heterochromatin is found in the center of the nucleus and euchromatin at the periphery. Restoration of LBR, but not of lamin A/C, was sufficient to restore the conventional sequestration of heterochromatin at the INM, and the inverted chromatin configura-tion could be phenocopied in other differentiated tissues by ablating both LBR and lamin A/C. The authors propose a mechanism whereby LBR mediates peripheral chromatin localization during early development, while lamin A/C becomes more important as cells terminally differentiate [49]. Lamin A/C apparently requires additional proteins or factors to interact with chromatin, which could be factors such as PRR14, transcription factors, or RNA poly-merase II regulatory complexes.

LBR is a Tudor domain-containing protein, and through this region, it can interact directly with H4K20me2 *in vitro* [99,100]. It was also reported to bind HP1α and HP1γ [89,101], which are hall-mark proteins of heterochromatin [88]. While this sounds promis-ing, HP1α-containing chromocenters are not necessarily perinuclear and HP1γ is bound to many non-peripheral euchromatic loci [102]. Moreover, ablation of HP1α or HP1β in pluripotent or differentiated embryonic stem cells did not alter pericentric heterochromatin orga-nization [54]. Finally, H4K20me2, an *in vitro* ligand of LBR, is distributed broadly across the genome [27], suggesting that it is not significantly enriched in LADs. This does not exclude the possibility that in some situations, LBR-H4K20me2 or LBR-HP1 interactions support chromatin sequestration, but it is clear that this would need to depend on additional marks or interaction domains given the broad distribution of these ligands.

While there is little proof for specific lamin–chromatin recogni-tion, the existing data strongly suggest a role for lamin A/C in conjunction with other INM proteins as a backbone for chromatin sequestration. We note that Zullo *et al* [71] described a role for HDAC3, which deacetylates histones tails, and its interaction with Lap2β, as a tissue-specific chromatin tether (Fig 2). The deacetyla-tion may be a prelude to the deposition of repressive methylation marks. The dependence of peripheral chromatin targeting on histone methylation [22,69,76] and the involvement of INM and lamina proteins suggest that perinuclear anchors that recognize a signature of histone marks and bridge to structural proteins of the INM remain to be discovered in mammals. The characterization of the *C. elegans* anchoring factor, CEC-4, provides an important proof of principle that INM proteins can directly recognize and sequester chromatin bearing specific histone modifications.

Solving the problem in yeast(s)

One should not forget that nature has solved the problem of segre-gating heterochromatic from euchromatic domains multiple times. In budding yeast, telomeres and silent mating-type loci are sequestered at the INM through the interaction of Esc1, a membrane-associated protein, with Sir4, a core component of the repressive SIR complex. There is a second, redundant anchoring pathway that depends on the interaction of Sir4 with yKU, a heterodimer that interacts with the membrane-spanning SUN domain protein Mps3 through a

Table 1. Protein associations implicated in nuclear architecture through direct (d) or indirect (i) interactions.

Protein	Interaction/ association	Method	Interaction	Species/cell type	Effect	References
H3K9me2 and me3	Heterochromatin formation and differentiation	Microscopy, IF, EM, immunohistochemistry ChIP	i/d	*Mammalian* (mouse *ex vivo* expanded MEFs, mouse germ cells, ESC and hematopoietic and retinal cells, cultured mouse fibroblasts), *Caenorhabditis elegans*, *Schizosaccharomyces pombe, Drosophila*	Many differentiated cell types show changes in H3K9me2/3 distribution and increased sequestration of H3K9me3-containing heterochromatin at the INM or around nucleoli	[3,17–23,40,50]
H3K9me2 and me3	Subnuclear positioning and repression	Microscopy, IF, immuno-FISH, Dam-ID, shRNA, RNAi, siRNA, drug treatments	d/i	*C. elegans* embryos, *mammalian* (cultured mouse NIH3T3 and C57BL/6 fibroblasts)	Essential both for silencing and for perinuclear positioning of chromatin in *C. elegans* embryos. Contributes to perinuclear anchoring of reporters (*mammalian*)	[22,69,76]
H4K20me3	H3K9me2/3 and large repeat-rich heterochromatic regions	ChIP, microscopy, IF, and metaphase spreads	i	*Mammalian* (mouse MEL-F4N cells, erythroid G1E and MEF cells, human HeLa cells, and *ex vivo* expanded MEFs and mouse trophoblast stem cells)	H4K20me3 colocalizes with H3K9me3 in large repeat domains (pericentromeric), colocalizes with HP1β, in DAPI-dense regions	[25–27]
H3K27me3	Repressed chromatin in differentiated cells and poised inactivation in stem cells	ChIP-seq, sequential ChIP, GMAT (genome-wide mapping technique)	i	*Mammalian* (human CD4^{+} T cells, mouse; ES cells, primary lung fibroblasts, immortalized embryonic fibroblasts, C2C12 myoblasts, neuro2a neuroblastoma cells) and *zebrafish* embryos	PRC2 trimethylates H3K27, which represses developmentally important genes. In ES cells, H3K27me3 can coincide with H3K4me3 at poised but transcriptionally inactive promoters	[30–32]
HP1(N-terminal chromodomain)	H3K9me2 and me3	Binding assays, NMR, *in vitro* pull-down assays microscopy, IF, co-IP, ChIP-seq, siRNA	d/i	*Mammalian* (mouse ESC and MEFs, human cancerous HeLa cells, Chinese hamster ovary cells (CHO), mouse oocytes, and mouse sperm)	Roles depend on isoform. The chromodomain recognizes H3K9me2 and me3, the chromo-shadow domain binds proteins. In mammals HP1 variants have distinct roles during ESC differentiation. HP1 binding does not necessarily correlate with repression	[51–54,58,59]

Table 1. (continued)

Protein	Interaction/ association	Method	Interaction	Species/cell type	Effect	References
Swi6 (HP1 homolog)	RNA as well as H3K9me2 and me3	ChIP-seq, microscopy (FRAP)	i	*S. pombe*	Necessary for silent chromatin to which it binds, but also restricts the spread of silent chromatin	[28,29]
HPL-2 (HP1 homologous H3K9me2/3 binding factor)	H3K9me3 HIS-24 (H1)	Microscopy, relocation assay, RNAi	d/i	*C. elegans*	Works with histone H1 to regulate transcription. Loss depresses but does not delocalize heterochromatic array	[22,65,118]
HPL-1 (HP1 homologous H3K9me2/3 binding factor)	H3K9me2/3	Microscopy, relocation assay, RNAi	d/i	*C. elegans*	Represses transcription, does not anchor, yet localizes to heterochromatic arrays. RNAi did not derepress test array	[22,65]
Lamin (LMN-1)	Perinuclear large transgene arrays	Relocation assays, RNAi	i	*C. elegans*	Depletion of the single lamin led to partial detachment of large transgene arrays through development	[97]
A-type lamins (LMN A and C)	Peripherally associating test regions and some developmental-specific genes	Microscopy, relocation assays, shRNA	i	*Mammalian* (mouse C57BL/6 fibroblast cells and primary MEFs)	Reduction in A-type lamins (A and C) released anchored test regions and cell type-specific genes	[69]
LEM-2	Perinuclear chromatin association	ChIP	i	*C. elegans*	H3K9me2 and me3 are enriched in chromatin recovered by LEM-2 ChIP	[22]
Lem2	Perinuclear chromatin association	ChIP, genetic screen with mutant forms	d/i	*S. pombe*	Anchors telomeric and centromeric heterochromatin at the INM, and partially ablates repression. Non-overlapping C-terminal MSC and N-terminal LEM domain mediate silencing and anchoring, respectively	[109]
LEM-2 and Man-1	Perinuclear large transgenes arrays	Relocation assays, RNAi	i	*C. elegans* embryos	Combined depletion of LEM-2 and MAN-1 leads to partial detachment of large transgene arrays.	[97]
HMTs: MET-2 and SET-25	H3K9me involvement INM binding and repression	RNAi and microscopy relocation assays	i/d	*C. elegans* embryos	MET-2, a SETDB1 homolog, mediates mono- and dimethylation, and SET-25 deposits H3K9me3. Elimination of both	[22]

Table 1. (continued)

Protein	Interaction/ association	Method	Interaction	Species/cell type	Effect	References
					eliminated H3K9 methylation in embryos and larvae and blocked anchoring of arrays and of endogenous repeat-rich domains	
HMTs: G9a and Suv39h	H3K9me involvement in perinuclear association	shRNA, siRNA, RNAi, inhibitors, microscopy, IF, immuno-FISH, Dam-ID, ChIP, ^{m6}A-tracer microscopy/ FISH	d/i	*Mammalian* (mouse C57BL/6 fibroblast cells, mouse NIH 3T3, human Tig3ET cells, BJ–human telomerase reverse transcriptase (hTERT) cells, and HTC75 a human fibrosarcoma cell line)	G9a mediates H3K9 dimethylation and Suv39h mediates H3K9 trimethylation. Peripheral association of test regions, selected endogenous genes and LADs were reduced by reduction in Suv39h and G9a	[69,76,77]
CEC-4	H3K9me1/2/3 and perinuclear association	Microscopy, relocation assays, RNAi	d/i	*C. elegans* embryos	CEC-4 binds mono-, di-, and trimethylated H3K9 and is essential for array anchoring at the nuclear periphery in embryos. Its loss triggers array release as in cells lacking H3K9 methylation, but did not reduce H3K9 methylation levels	[65]
LIN-61	H3K9me2/3	Microscopy, relocation assay, RNAi	d/i	*C. elegans* embryos	Represses transcription, does not anchor to the periphery	[22,65,74]
YY1	PRC2- and H3K27me3-dependent perinuclear association	Microscopy, relocation assay/ChIP, drug inhibition, and shRNA	i	*Mammalian* (mouse C57BL/6 fibroblast cells)	YY1 when tethered to chromatin leads to high levels of PRC2-dependent H3K27me3 and peripheral localization. Inhibition of the PRC2 catalytic subunit, EZH2, reduced perinuclear association	[69]
PRR14	Tethers heterochromatin to the nuclear periphery through HP1	Microscopy, IF, colocalization siRNA, shRNA	i	*Mammalian* (human HeLa cells and euploid human retinal pigment epithelial 1 [RPE1] cells)	PRR14 tethers heterochromatin to the nuclear periphery in interphase cells binding both HP1 and lamin A/C	[98]
Emerin	HDAC3	*In vitro* binding assays, *in vivo*, co-IPs	d	*Mammalian* (mouse C2C12 myoblasts and emerin$^{-/-}$ mouse myogenic progenitors)	INM protein, emerin recruits HDAC3 to the nuclear periphery and stimulates its catalytic activity	[83]

Table 1.   (continued)

Protein	Interaction/ association	Method	Interaction	Species/cell type	Effect	References
Lap2β	HDAC3	Y2H (*in vitro*), co-IP (*in vivo*)	d	*Mammalian* (human osteosarcoma U2OS cells and human lung carcinoma H1299 cells)	Lap2β and HDAC3 interact and lead to the induction of H4 deacetylase.	[84]
Zbtb7b (cKrox)	HDAC3/Lap2β	EMSA, co-IP IF colocalization, shRNA	d	*Mammalian* (mouse NIH 3T3 fibroblasts)	Zbtb7b interacts with lamina-associated sequences (LASs) and is predicted to recruit HDAC3 which in turn interacts with Lap2B. Reduction in Zbtb7b and HDAC3 dissociates LASs from the nuclear periphery	[71]
LBR and lamin A/C	heterochromatin localization	Microscopy, immuno-FISH, IF	i	*Mammalian* (mouse photoreceptor red cells and photoreceptor cells from 39 other mammalian species, mouse ganglion cells and mouse fibroblasts and mouse tissue and skin samples from mice)	Cells lacking LBR and lamin A/C have an inverted chromatin configuration, with heterochromatin in the center of the nucleus and euchromatin at the periphery. Restoration of LBR, but not of lamin A/C, restored normal configuration.	[49,50]
LBR	H4K20me2	*In vitro* binding assays, co-IP, immunostaining, protein structural analysis	d	Turkey erythrocytes and *E. coli* (BL21 [DE3]), *mammalian* (human cultured cancer cells (HeLa), human pancreatic carcinoma, epithelial-like cell line (PanC1), and transformed human embryonic kidney cells (HEK293t))	The Tudor domain of LBR directly binds H4K20me2. ChIP and co-IPs confirm the interaction of these *in vivo*, and immunostaining confirms colocalization at the periphery	[99,100]
LBR	HP1 variants and core histones	*In vitro* Y2H and co-IP	d	*Mammalian* (human cultured cancer cells (HeLa) and transformed human embryonic kidney cells (HEK293t))	LBR Tudor domain binds HP1α and HP1γ, which may help tether chromatin to the INM. HP1 forms a quaternary complex with LBR and a subset of core histones *in vitro*	[88,89]
Esc1	Sir4	*In vitro, in vivo*, Y2H, targeted relocation	d	*S. cerevisiae*	Telomeres and silent mating-type loci are sequestered at the INM through Sir4 binding to Esc1, an INM protein, but also through yKu and Mps3	[103–105]

Table 1. (continued)

Protein	Interaction/ association	Method	Interaction	Species/cell type	Effect	References
Sir4	Sir3/Sir2/ nucleosomes/ Esc1/Mps3, and possibly Nup170	*In vitro, in vivo*, Y2H, targeted interaction, and silencing assays	d	*S. cerevisiae*	Establishment of heritable silent chromatin. Sir4 binds chromatin and DNA non-specifically, and tightly binds Sir3, which recognizes unacetylated histone H4 tails with high specificity. Sir4 mediates interaction with INM anchors Esc1 and Mps3, and Nup170	[16,116,117,119,120]
Src1/Heh1 (Lem2 homolog)	rDNA	Genetic evidence, localization studies	d/i	*S. cerevisiae*	Tethers rDNA to the INM to stabilize repeated domains, although Src1/Heh1 is dispensable for anchoring SIR-repressed domains	[113]

telomerase cofactor called Est1 (reviewed in [103]). Sir4 binds chromatin and DNA non-specifically, but associates tightly with Sir3, which binds the unacetylated histone H4 tail with high specificity. Both interactions are essential for transcriptional silencing (Fig 4A). Thus, in budding yeast, it is the absence of histone marks that promote both repression and anchoring, the former reflecting Sir3 binding to histones and the latter, Sir4's interaction with INM anchorage sites [16]. Whereas Sir3 and Sir4 function together to silence transcription, Sir4's anchoring can occur in the absence of Sir3-mediated repression (Fig 4A). That is possible because Sir4 can also be recruited to telomeres by the TG-repeat binding protein Rap1, or by the end-binding protein yKU, to anchor telomeres in the absence of Sir3 [104,105]. Thus, mutations in budding yeast have allowed silencing to be functionally separated from perinuclear tethering, although Sir proteins are involved in both functions (Fig 4A). Without INM tethering, silencing at telomeres is partially compromised, but mating-type repression persists due to redundant mechanisms for SIR complex nucleation [106]. Intriguingly, the most pronounced defects incurred upon the loss of heterochromatin anchoring in budding yeast were altered telomere length regulation, and increased promiscuity in recombination, possibly due to reduced end protection [106].

In fission yeast, telomeric and centromeric heterochromatin domains are also anchored at the INM, again through multiple mechanisms (Fig 4B). One contributor to this anchorage is Lem2 [107], a member of the conserved family of lamin-associated proteins, that contains both an N-terminal LEM motif, two predicted transmembrane domains as well as an MSC domain [108]. Given that there is only a partial defect in chromatin repression upon the loss of anchoring in *S. pombe*, Braun and colleagues screened for locus-specific defects in silencing to identify interacting factors, and then, they further analyzed a role for these hits in localization using double and triple mutants [109]. Notably, they found that LEM2 cooperates with the RNAi machinery assembly factor Dsh1 to anchor telomeres and with the centromere factor Csi1 to cluster

centromeric heterochromatin at the spindle pole body (SPB, an integral INM structure). The SPB itself is anchored through the SUN domain protein Sad1 (Fig 4B). Two other LAP homologs, Man1 and Ima1, did not show synergistic phenotypes with Dsh1 or Csi1, suggesting that Lem2 is the only LAP homolog involved in silencing in fission yeast [109]. Intriguingly, at *S. pombe* centromeres, the functions of silencing and anchoring mapped to non-overlapping domains of Lem2. The N-terminal LEM domain promoted centromere tethering, while silencing required the MSC domain [109]. On a mechanistic level, silencing by Lem2 involves the recruitment of the repressor complex SHREC (a Snf2/HDAC repressor complex) to heterochromatin, which contributes not only to repression but also to perinuclear sequestration [110]. Intriguingly, loss of Clr4, the HMT that methylates H3K9, also displaces silent mating-type loci [111].

At telomeres, *S. pombe* Lem2 contributes to both repression and tethering, and the functions are not entirely separable [109] (Fig 4B). Recently, Steglich *et al* [112] demonstrated that the association of telomeres with the INM is reduced in the absence of the nucleosome remodeler Fft3. While single *fft3* mutants did not show defects in telomere anchoring, the double mutant *bqt3 fft3* showed increased delocalization, which was accompanied by the upregulation of subtelomeric genes. Such defects, however, were mild in comparison with those observed in *lem2Δ*. Remarkably, the budding yeast homolog of Lem2 (known as Src1 or Heh1) is implicated in the tethering of rDNA to the INM to stabilize the repeated domain, although Src1/Heh1 is dispensable for anchoring SIR-repressed chromatin [113].

Summary

Histone H3K9 methylation is essential for the sequestration of chromatin at the nuclear envelope in *C. elegans*, and most likely this extends to other species, given the conserved role of H3K9 HMTs in heterochromatin formation [114]. The sequestration of
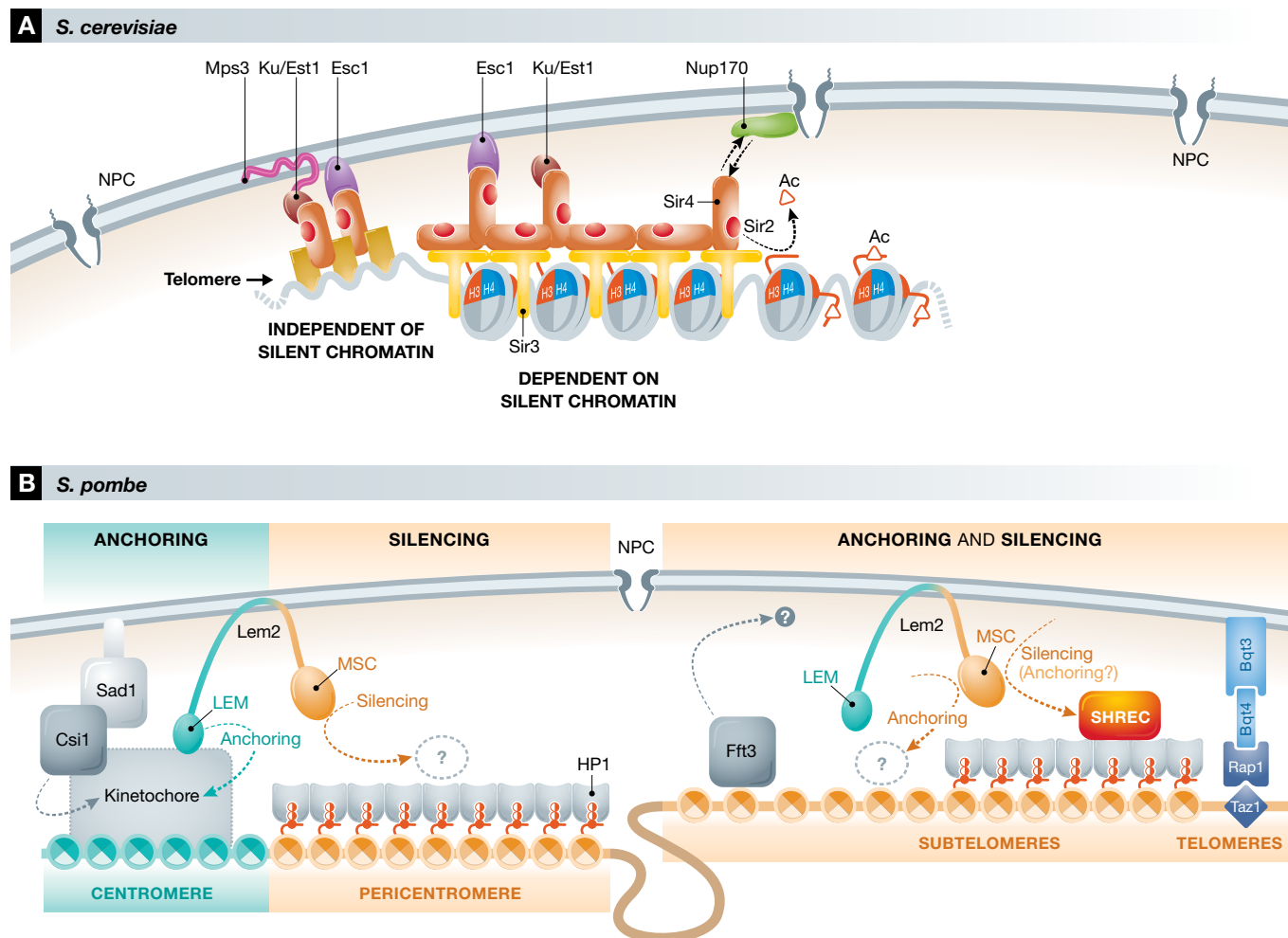
Figure 4. Anchoring heterochromatin in budding and fission yeast.
(A) In budding yeast, telomere anchoring occurs in both a silencing-dependent and a silencing-independent manner. At telomeric repeats, Sir4 binds Rap1 and/or yKU to mediate interaction with Esc1, an INM protein. At subtelomeric nucleosomes, Sir4 binds as part of the repressive SIR2-3-4 complex to silence chromatin. Sir2 deacetylates to allow for Sir3 binding. Sir3 and Sir4 interact. This mediates interaction with Esc1. Interaction of Sir4 with yKU can mediate interaction with a SUN domain protein, Mps3 (reviewed in [116]). Interaction with Nup170 has also been reported [117]. (B) In fission yeast, Lem2 has distinct N-terminal LEM and C-terminal MSC domains [108]. LEM2 cooperates with the RNAi machinery assembly factor Dsh1 to anchor telomeres (not shown), and with the centromere factor Csi1 to cluster centromeric heterochromatin at the SPB, which is anchored through the SUN domain protein Sad1. The MSC domain contributes to pericentric heterochromatin through still unclear mechanisms, but silencing and anchoring can be separated. At telomeres, anchoring and silencing are not separated by mutation of either LEM or MSC domains [109]. Silencing acts through the SHREC complex. Telomeres have alternative pathways of anchoring which include Fft3 and telomeric repeat binding factors Taz1 (see text). (B) is derived from a model in [109].

heterochromatin at the INM contributes to the overall organization of the genome into distinct domains called LADs, which are a subset of late-replicating domains and TADs. The cell type-specific roles of various INM components in all species, and the embryo-specific role of worm CEC-4, a chromodomain protein of the INM, suggest that there are differentiation- and developmental state-specific mechanisms that contribute to nuclear organization, rather than one universally conserved mechanism. Given the complexity of the INM proteome and the juxtaposition of tissue-specific transcription factors with the INM and lamin (for further review, see [85,86,115]), we expect that combinatorial mechanisms will anchor heterochromatin in differentiated cells. Functional screens in partially compromised backgrounds will be necessary to dissect the mechanisms that

spatially segregate active and inactive genomic domains. Histone modifications are likely to be at the heart of these potentially redundant mechanisms.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Kosak ST, Skok JA, Medina KL, Riblet R, Le Beau MM, Fisher AG, Singh H (2002) Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. *Science* 296: 158–162

2. Brown KE, Amoils S, Horn JM, Buckle VJ, Higgs DR, Merkenschlager M, Fisher AG (2001) Expression of alpha- and beta-globin genes occurs within different nuclear domains in haemopoietic cells. *Nat Cell Biol* 3: 602–606

3. Ugarte F, Sousae R, Cinquin B, Martin EW, Krietsch J, Sanchez G, Inman M, Tsang H, Warr M, Passegué E *et al* (2015) Progressive chromatin condensation and H3K9 methylation regulate the differentiation of embryonic and hematopoietic stem cells. *Stem Cell Rep* 5: 728–740

4. Hübner B, Lomiento M, Mammoli F, Illner D, Markaki Y, Ferrari S, Cremer M, Cremer T (2015) Remodeling of nuclear landscapes during human myelopoietic cell differentiation maintains co-aligned active and inactive nuclear compartments. *Epigenetics Chromatin* 8: 47

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

6. Fakhouri THI, Stevenson J, Chisholm AD, Mango SE (2010) Dynamic chromatin organization during foregut development mediated by the organ selector gene PHA-4/FoxA. *PLoS Genet* 6: e1001060

7. Dixon JR, Jung I, Selvaraj S, Shen Y, Antosiewicz-Bourget JE, Lee AY, Ye Z, Kim A, Rajagopal N, Xie W *et al* (2015) Chromatin architecture reorganization during stem cell differentiation. *Nature* 518: 331–336

8. Williams RRE, Azuara V, Perry P, Sauer S, Dvorkina M, Jørgensen H, Roix J, McQueen P, Misteli T, Merkenschlager M *et al* (2006) Neural induction promotes large-scale chromatin reorganisation of the Mash1 locus. *J Cell Sci* 119: 132–140

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

10. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim T-K, Koche RP *et al* (2007) Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 448: 553–560

11. Mohn F, Weber M, Rebhan M, Roloff TC, Richter J, Stadler MB, Bibel M, Schübeler D (2008) Lineage-specific polycomb targets and *de novo* DNA methylation define restriction and potential of neuronal progenitors. *Mol Cell* 30: 755–766

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

13. Fussner E, Djuric U, Strauss M, Hotta A, Perez-Iratxeta C, Lanner F, Dilworth FJ, Ellis J, Bazett-Jones DP (2011) Constitutive heterochromatin reorganization during somatic cell reprogramming. *EMBO J* 30: 1778–1789

14. Chen T, Dent SYR (2014) Chromatin modifiers and remodellers: regulators of cellular differentiation. *Nat Rev Genet* 15: 93–106

15. Gómez-Díaz E, Corces VG (2014) Architectural proteins: regulators of 3D genome organization in cell fate. *Trends Cell Biol* 24: 703–711

16. Oppikofer M, Kueng S, Gasser SM (2013) SIR-nucleosome interactions: structure-function relationships in yeast silent chromatin. *Gene* 527: 10–25

17. Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, Schöfer C, Weipoltshammer K, Pagani M, Lachner M, Kohlmaier A *et al* (2001) Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* 107: 323–337

18. Ebert A, Schotta G, Lein S, Kubicek S, Krauss V, Jenuwein T, Reuter G (2004) Su(var) genes regulate the balance between euchromatin and heterochromatin in Drosophila. *Genes Dev* 18: 2973–2983

19. Fodor BD, Shukeir N, Reuter G, Jenuwein T (2010) Mammalian Su(var) genes in chromatin control. *Annu Rev Cell Dev Biol* 26: 471–501

20. 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

21. Liu T, Rechtsteiner A, Egelhofer TA, Vielle A, Latorre I, Cheung M-S, Ercan S, Ikegami K, Jensen M, Kolasinska-Zwierz P *et al* (2011) Broad chromosomal domains of histone modification patterns in *C. elegans*. *Genome Res* 21: 227–236

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

23. Al-Sady B, Madhani HD, Narlikar GJ (2013) Division of labor between the chromodomains of HP1 and Suv39 methylase enables coordination of heterochromatin spread. *Mol Cell* 51: 80–91

24. Talasz H, Lindner HH, Sarg B, Helliger W (2005) Histone H4-lysine 20 monomethylation is increased in promoter and coding regions of active genes and correlates with hyperacetylation. *J Biol Chem* 280: 38814–38822

25. Vakoc CR, Mandat SA, Olenchock BA, Blobel GA (2005) Histone H3 lysine 9 methylation and HP1gamma are associated with transcription elongation through mammalian chromatin. *Mol Cell* 19: 381–391

26. Martens JHA, O'Sullivan RJ, Braunschweig U, Opravil S, Radolf M, Steinlein P, Jenuwein T (2005) The profile of repeat-associated histone lysine methylation states in the mouse epigenome. *EMBO J* 24: 800–812

27. Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, Reinberg D, Jenuwein T (2004) A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev* 18: 1251–1262

28. Stunnenberg R, Kulasegaran-Shylini R, Keller C, Kirschmann MA, Gelman L, Bühler M (2015) H3K9 methylation extends across natural

boundaries of heterochromatin in the absence of an HP1 protein. *EMBO J* 34: 2789–2803

29. Keller C, Kulasegaran-Shylini R, Shimada Y, Hotz H-R, Bühler M (2013) Noncoding RNAs prevent spreading of a repressive histone mark. *Nat Struct Mol Biol* 20: 994–1000

30. Barski A, Cuddapah S, Cui K, Roh T-Y, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K (2007) High-resolution profiling of histone methylations in the human genome. *Cell* 129: 823–837

31. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K *et al* (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 125: 315–326

32. Vastenhouw NL, Zhang Y, Woods IG, Imam F, Regev A, Liu XS, Rinn J, Schier AF (2010) Chromatin signature of embryonic pluripotency is established during genome activation. *Nature* 464: 922–926

33. Denissov S, Hofemeister H, Marks H, Kranz A, Ciotta G, Singh S, Anastassiadis K, Stunnenberg HG, Stewart AF (2014) Mll2 is required for H3K4 trimethylation on bivalent promoters in embryonic stem cells, whereas Mll1 is redundant. *Development* 141: 526–537

34. Voigt P, Tee W-W, Reinberg D (2013) A double take on bivalent promoters. *Genes Dev* 27: 1318–1338

35. Harikumar A, Meshorer E (2015) Chromatin remodeling and bivalent histone modifications in embryonic stem cells. *EMBO Rep* 16: 1609–1619

36. Boulard M, Edwards JR, Bestor TH (2015) FBXL10 protects Polycomb-bound genes from hypermethylation. *Nat Genet* 47: 479–485

37. Blackledge NP, Farcas AM, Kondo T, King HW, McGouran JF, Hanssen LLP, Ito S, Cooper S, Kondo K, Koseki Y *et al* (2014) Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. *Cell* 157: 1445–1459

38. Wu X, Johansen JV, Helin K (2013) Fbxl10/Kdm2b recruits polycomb repressive complex 1 to CpG islands and regulates H2A ubiquitylation. *Mol Cell* 49: 1134–1146

39. Lienert F, Mohn F, Tiwari VK, Baubec T, Roloff TC, Gaidatzis D, Stadler MB, Schübeler D (2011) Genomic prevalence of heterochromatic H3K9me2 and transcription do not discriminate pluripotent from terminally differentiated cells. *PLoS Genet* 7: e1002090

40. Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP (2009) Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nat Genet* 41: 246–250

41. Hu S, Cheng L, Wen B (2012) Large chromatin domains in pluripotent and differentiated cells. *Acta Biochim Biophys Sin* 44: 48–53

42. Filion GJ, van Steensel B (2010) Reassessing the abundance of H3K9me2 chromatin domains in embryonic stem cells. *Nat Genet* 42: 4; author reply 5–6

43. Egelhofer TA, Minoda A, Klugman S, Lee K, Kolasinska-Zwierz P, Alekseyenko AA, Cheung M-S, Day DS, Gadel S, Gorchakov AA *et al* (2011) An assessment of histone-modification antibody quality. *Nat Struct Mol Biol* 18: 91–93

44. Peach SE, Rudomin EL, Udeshi ND, Carr SA, Jaffe JD (2012) Quantitative assessment of chromatin immunoprecipitation grade antibodies directed against histone modifications reveals patterns of co-occurring marks on histone protein molecules. *Mol Cell Proteomics* 11: 128–137

45. Baxter J, Sauer S, Peters A, John R, Williams R, Caparros M-L, Arney K, Otte A, Jenuwein T, Merkenschlager M *et al* (2004) Histone hypomethylation is an indicator of epigenetic plasticity in quiescent lymphocytes. *EMBO J* 23: 4462–4472

46. Bao X, Wu H, Zhu X, Guo X, Hutchins AP, Luo Z, Song H, Chen Y, Lai K, Yin M *et al* (2015) The p53-induced lincRNA-p21 derails somatic cell reprogramming by sustaining H3K9me3 and CpG methylation at pluripotency gene promoters. *Cell Res* 25: 80–92

47. Sridharan R, Gonzales-Cope M, Chronis C, Bonora G, McKee R, Huang C, Patel S, Lopez D, Mishra N, Pellegrini M *et al* (2013) Proteomic and genomic approaches reveal critical functions of H3K9 methylation and heterochromatin protein-1γ in reprogramming to pluripotency. *Nat Cell Biol* 15: 872–882

48. Chen J, Liu H, Liu J, Qi J, Wei B, Yang J, Liang H, Chen Y, Chen J, Wu Y *et al* (2013) H3K9 methylation is a barrier during somatic cell reprogramming into iPSCs. *Nat Genet* 45: 34–42

49. Solovei I, Wang AS, Thanisch K, Schmidt CS, Krebs S, Zwerger M, Cohen TV, Devys D, Foisner R, Peichl L *et al* (2013) LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. *Cell* 152: 584–598

50. Solovei I, Kreysing M, Lanctôt C, Kösem S, Peichl L, Cremer T, Guck J, Joffe B (2009) Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution. *Cell* 137: 356–368

51. Nielsen PR, Nietlispach D, Mott HR, Callaghan J, Bannister A, Kouzarides T, Murzin AG, Murzina NV, Laue ED (2002) Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. *Nature* 416: 103–107

52. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 410: 116–120

53. Cheutin T, McNairn AJ, Jenuwein T, Gilbert DM, Singh PB, Misteli T (2003) Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science* 299: 721–725

54. Mattout A, Aaronson Y, Sailaja BS, Raghu Ram EV, Harikumar A, Mallm J-P, Sim KH, Nissim-Rafinia M, Supper E, Singh PB *et al* (2015) Heterochromatin Protein 1β (HP1β) has distinct functions and distinct nuclear distribution in pluripotent versus differentiated cells. *Genome Biol* 16: 213

55. Fadloun A, Eid A, Torres-Padilla M-E (2013) Mechanisms and dynamics of heterochromatin formation during mammalian development: closed paths and open questions. *Curr Top Dev Biol* 104: 1–45

56. Bošković A, Eid A, Pontabry J, Ishiuchi T, Spiegelhalter C, Raghu Ram EVS, Meshorer E, Torres-Padilla M-E (2014) Higher chromatin mobility supports totipotency and precedes pluripotency *in vivo*. *Genes Dev* 28: 1042–1047

57. Martin C, Brochard V, Migné C, Zink D, Debey P, Beaujean N (2006) Architectural reorganization of the nuclei upon transfer into oocytes accompanies genome reprogramming. *Mol Reprod Dev* 73: 1102–1111

58. Santos F, Peters AH, Otte AP, Reik W, Dean W (2005) Dynamic chromatin modifications characterise the first cell cycle in mouse embryos. *Dev Biol* 280: 225–236

59. Puschendorf M, Terranova R, Boutsma E, Mao X, Isono K, Brykczynska U, Kolb C, Otte AP, Koseki H, Orkin SH *et al* (2008) PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nat Genet* 40: 411–420

60. Tardat M, Albert M, Kunzmann R, Liu Z, Kaustov L, Thierry R, Duan S, Brykczynska U, Arrowsmith CH, Peters AHFM (2015) Cbx2 targets PRC1 to constitutive heterochromatin in mouse zygotes in a parent-of-origin-dependent manner. *Mol Cell* 58: 157–171

61. Santenard A, Ziegler-Birling C, Koch M, Tora L, Bannister AJ, Torres-Padilla M-E (2010) Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3. *Nat Cell Biol* 12: 853–862

62. Dileep V, Ay F, Sima J, Vera DL, Noble WS, Gilbert DM (2015) Topologi-
     cally associating domains and their long-range contacts are established
     during early G1 coincident with the establishment of the replication-
     timing program. *Genome Res* 25: 1104–1113

63. Pope BD, Ryba T, Dileep V, Yue F, Wu W, Denas O, Vera DL, Wang Y,
     Hansen RS, Canfield TK *et al* (2014) Topologically associating domains
     are stable units of replication-timing regulation. *Nature* 515: 402–405

64. Hiratani I, Ryba T, Itoh M, Rathjen J, Kulik M, Papp B, Fussner E,
     Bazett-Jones DP, Plath K, Dalton S *et al* (2010) Genome-wide dynamics
     of replication timing revealed by *in vitro* models of mouse embryogen-
     esis. *Genome Res* 20: 155–169

65. Gonzalez-Sandoval A, Towbin BD, Kalck V, Cabianca DS, Gaidatzis D,
     Hauer MH, Geng L, Wang L, Yang T, Wang X *et al* (2015) Perinuclear
     anchoring of H3K9-methylated chromatin stabilizes induced cell fate in
     C. elegans embryos. *Cell* 163: 1333–1347

66. Wilson KL, Berk JM (2010) The nuclear envelope at a glance. *J Cell Sci*
     123: 1973–1978

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

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

69. Harr JC, Luperchio TR, Wong X, Cohen E, Wheelan SJ, Reddy KL (2015)
     Directed targeting of chromatin to the nuclear lamina is mediated by
     chromatin state and A-type lamins. *J Cell Biol* 208: 33–52

70. Meuleman W, Peric-Hupkes D, Kind J, Beaudry J-B, Pagie L, Kellis M,
     Reinders M, Wessels L, van Steensel B (2013) Constitutive nuclear
     lamina-genome interactions are highly conserved and associated with
     A/T-rich sequence. *Genome Res* 23: 270–280

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

72. 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

73. Rohner S, Kalck V, Wang X, Ikegami K, Lieb JD, Gasser SM, Meister P (2013)
     Promoter- and RNA polymerase II-dependent hsp-16 gene association
     with nuclear pores in *Caenorhabditis elegans*. *J Cell Biol* 200: 589–604

74. Koester-Eiserfunke N, Fischle W (2011) H3K9me2/3 binding of the MBT
     domain protein LIN-61 is essential for *Caenorhabditis elegans* vulva
     development. *PLoS Genet* 7: e1002017

75. Harr JC, Reddy KL (2015) Tagged chromosomal insertion site system: a
     method to study lamina-associated chromatin. *Methods in Enzymology*.

76. Bian Q, Khanna N, Alvikas J, Belmont AS (2013) β-Globin cis-elements
     determine differential nuclear targeting through epigenetic modifi-
     cations. *J Cell Biol* 203: 767–783

77. Kind J, Pagie L, Ortabozkoyun H, Boyle S, de Vries SS, Janssen H, Amen-
     dola M, Nolen LD, Bickmore WA, van Steensel B (2013) Single-cell
     dynamics of genome-nuclear lamina interactions. *Cell* 153: 178–192

78. Wilkinson F, Pratt H, Atchison ML (2010) PcG recruitment by the YY1
     REPO domain can be mediated by Yaf2. *J Cell Biochem* 109: 478–486

79. Satijn DP, Hamer KM, den Blaauwen J, Otte AP (2001) The polycomb
     group protein EED interacts with YY1, and both proteins induce neural
     tissue in *Xenopus* embryos. *Mol Cell Biol* 21: 1360–1369

80. Basu A, Wilkinson FH, Colavita K, Fennelly C, Atchison ML (2014) YY1
     DNA binding and interaction with YAF2 is essential for Polycomb
     recruitment. *Nucleic Acids Res* 42: 2208–2223

81. Atchison L, Ghias A, Wilkinson F, Bonini N, Atchison ML (2003) Transcrip-
     tion factor YY1 functions as a PcG protein *in vivo*. *EMBO J* 22: 1347–1358

82. Eberhart A, Feodorova Y, Song C, Wanner G, Kiseleva E, Furukawa T,
     Kimura H, Schotta G, Leonhardt H, Joffe B *et al* (2013) Epigenetics of
     eu- and heterochromatin in inverted and conventional nuclei from
     mouse retina. *Chromosome Res* 21: 535–554

83. Demmerle J, Koch AJ, Holaska JM (2012) The nuclear envelope protein
     emerin binds directly to histone deacetylase 3 (HDAC3) and activates
     HDAC3 activity. *J Biol Chem* 287: 22080–22088

84. Somech R, Shaklai S, Geller O, Amariglio N, Simon AJ, Rechavi G, Gal-
     Yam EN (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

85. Zuleger N, Boyle S, Kelly DA, de Las Heras JI, Lazou V, Korfali N,
     Batrakou DG, Randles KN, Morris GE, Harrison DJ *et al* (2013) Specific
     nuclear envelope transmembrane proteins can promote the location of
     chromosomes to and from the nuclear periphery. *Genome Biol* 14: R14

86. Malik P, Korfali N, Srsen V, Lazou V, Batrakou DG, Zuleger N, Kavanagh
     DM, Wilkie GS, Goldberg MW, Schirmer EC (2010) Cell-specific and
     lamin-dependent targeting of novel transmembrane proteins in the
     nuclear envelope. *Cell Mol Life Sci* 67: 1353–1369

87. Korfali N, Wilkie GS, Swanson SK, Srsen V, de Las Heras J, Batrakou
     DG, Malik P, Zuleger N, Kerr ARW, Florens L *et al* (2012) The nuclear
     envelope proteome differs notably between tissues. *Nucleus* 3:
     552–564

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

89. Polioudaki H, Kourmouli N, Drosou V, Bakou A, Theodoropoulos PA,
     Singh PB, Giannakouros T, Georgatos SD (2001) Histones H3/H4 form a
     tight complex with the inner nuclear membrane protein LBR and hete-
     rochromatin protein 1. *EMBO Rep* 2: 920–925

90. Foisner R (2003) Cell cycle dynamics of the nuclear envelope. *Scien-
     tificWorldJournal* 3: 1–20

91. Brachner A, Foisner R (2011) Evolvement of LEM proteins as
     chromatin tethers at the nuclear periphery. *Biochem Soc Trans* 39:
     1735–1741

92. Wilson KL, Foisner R (2010) Lamin-binding proteins. *Cold Spring Harb
     Perspect Biol* 2: a000554

93. Margalit A, Brachner A, Gotzmann J, Foisner R, Gruenbaum Y (2007)
     Barrier-to-autointegration factor–a BAFfling little protein. *Trends Cell
     Biol* 17: 202–208

94. Kubben N, Adriaens M, Meuleman W, Voncken JW, van Steensel B,
     Misteli T (2012) Mapping of lamin A- and progerin-interacting genome
     regions. *Chromosoma* 121: 447–464

95. de Las Heras JI, Meinke P, Batrakou DG, Srsen V, Zuleger N, Kerr AR,
     Schirmer EC (2014) Tissue specificity in the nuclear envelope supports
     its functional complexity. *Nucleus* 4: 460–477

96. McCord RP, Nazario-Toole A, Zhang H, Chines PS, Zhan Y, Erdos MR,
     Collins FS, Dekker J, Cao K (2013) Correlated alterations in genome
     organization, histone methylation, and DNA-lamin A/C interactions
     in Hutchinson-Gilford progeria syndrome. *Genome Res* 23:
     260–269

97. Mattout A, Pike BL, Towbin BD, Bank EM, Gonzalez-Sandoval A, Stadler
     MB, Meister P, Gruenbaum Y, Gasser SM (2011) An EDMD mutation in

C. elegans lamin blocks muscle-specific gene relocation and compromises muscle integrity. *Curr Biol* 21: 1603–1614

98. Poleshko A, Mansfield KM, Burlingame CC, Andrake MD, Shah NR, Katz RA (2013) The human protein PRR14 tethers heterochromatin to the nuclear lamina during interphase and mitotic exit. *Cell Rep* 5: 292–301

99. Liokatis S, Edlich C, Soupsana K, Giannios I, Panagiotidou P, Tripsianes K, Sattler M, Georgatos SD, Politou AS (2012) Solution structure and molecular interactions of lamin B receptor Tudor domain. *J Biol Chem* 287: 1032–1042

100. Hirano Y, Hizume K, Kimura H, Takeyasu K, Haraguchi T, Hiraoka Y (2012) Lamin B receptor recognizes specific modifications of histone H4 in heterochromatin formation. *J Biol Chem* 287: 42654–42663

101. Ye Q, Callebaut I, Pezhman A, Courvalin JC, Worman HJ (1997) Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR. *J Biol Chem* 272: 14983–14989

102. Minc E, Allory Y, Worman HJ, Courvalin J-C, Buendia B (1999) Localization and phosphorylation of HP1 proteins during the cell cycle in mammalian cells. *Chromosoma* 108: 220–234

103. Taddei A, Schober H, Gasser SM (2010) The budding yeast nucleus. *Cold Spring Harb Perspect Biol* 2: a000612

104. Hediger F, Neumann FR, Van Houwe G, Dubrana K, Gasser SM (2002) Live imaging of telomeres. *Curr Biol* 12: 2076–2089

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

106. Taddei A, Van Houwe G, Nagai S, Erb I, van Nimwegen E, Gasser SM (2009) The functional importance of telomere clustering: global changes in gene expression result from SIR factor dispersion. *Genome Res* 19: 611–625

107. Gonzalez Y, Saito A, Sazer S (2012) Fission yeast Lem2 and Man1 perform fundamental functions of the animal cell nuclear lamina. *Nucleus* 3: 60–76

108. Brachner A, Reipert S, Foisner R, Gotzmann J (2005) LEM2 is a novel MAN1-related inner nuclear membrane protein associated with A-type lamins. *J Cell Sci* 118: 5797–5810

109. Barrales RR, Forn M, Georgescu PR, Sarkadi Z, Braun S (2016) Control of heterochromatin localization and silencing by the nuclear membrane protein Lem2. *Genes Dev* 30: 133–148

110. Alfredsson-Timmins J, Kristell C, Henningson F, Lyckman S, Bjerling P (2009) Reorganization of chromatin is an early response to nitrogen starvation in *Schizosaccharomyces pombe*. *Chromosoma* 118: 99–112

111. Alfredsson-Timmins J, Henningson F, Bjerling P (2007) The Clr4 methyltransferase determines the subnuclear localization of the mating-type region in fission yeast. *J Cell Sci* 120: 1935–1943

112. Steglich B, Strålfors A, Khorosjutina O, Persson J, Smialowska A, Javerzat J-P, Ekwall K (2015) The Fun30 chromatin remodeler Fft3 controls nuclear organization and chromatin structure of insulators and subtelomeres in fission yeast. *PLoS Genet* 11: e1005101

113. Mekhail K, Seebacher J, Gygi SP, Moazed D (2008) Role for perinuclear chromosome tethering in maintenance of genome stability. *Nature* 456: 667–670

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

115. Wong X, Luperchio TR, Reddy KL (2014) NET gains and losses: the role of changing nuclear envelope proteomes in genome regulation. *Curr Opin Cell Biol* 28C: 105–120

116. Kueng S, Oppikofer M, Gasser SM (2013) SIR proteins and the assembly of silent chromatin in budding yeast. *Annu Rev Genet* 47: 275–306

117. Van de Vosse DW, Wan Y, Lapetina DL, Chen W-M, Chiang J-H, Aitchison JD, Wozniak RW (2013) A role for the nucleoporin Nup170p in chromatin structure and gene silencing. *Cell* 152: 969–983

118. Studencka M, Wesołowski R, Opitz L, Salinas-Riester G, Wisniewski JR, Jedrusik-Bode M (2012) Transcriptional repression of Hox genes by *C. elegans* HP1/HPL and H1/HIS-24. *PLoS Genet* 8: e1002940

119. Kueng S, Tsai-Pflugfelder M, Oppikofer M, Ferreira HC, Roberts E, Tsai C, Roloff T-C, Sack R, Gasser SM (2012) Regulating repression: roles for the sir4 N-terminus in linker DNA protection and stabilization of epigenetic states. *PLoS Genet* 8: e1002727

120. Martino F, Kueng S, Robinson P, Tsai-Pflugfelder M, van Leeuwen F, Ziegler M, Cubizolles F, Cockell MM, Rhodes D, Gasser SM (2009) Reconstitution of yeast silent chromatin: multiple contact sites and O-AADPR binding load SIR complexes onto nucleosomes *in vitro*. *Mol Cell* 33: 323–334