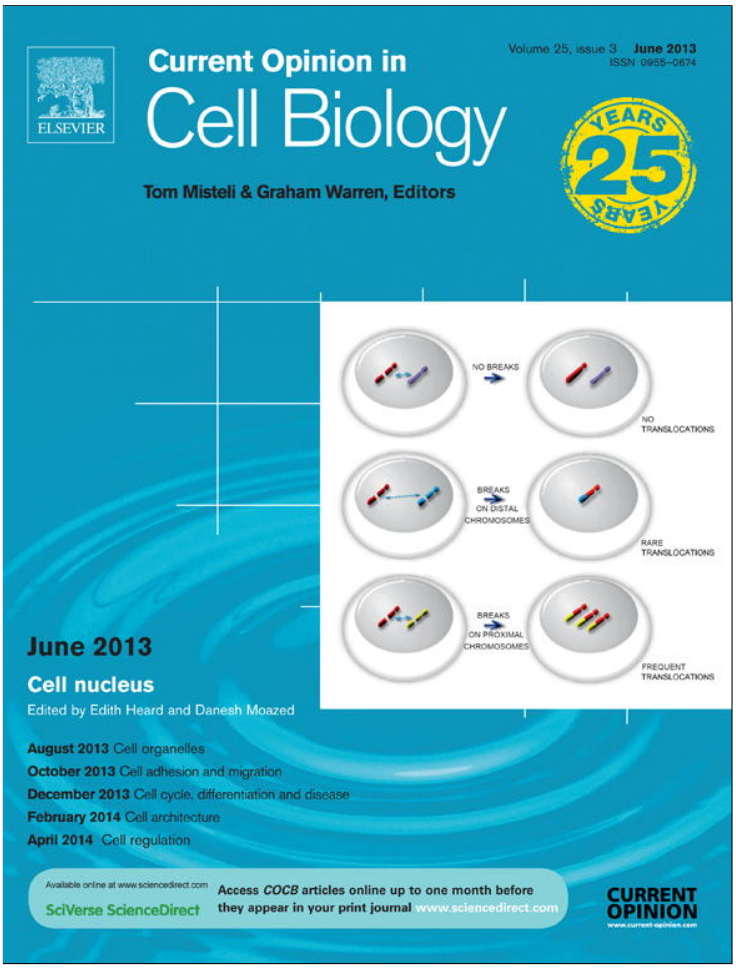

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Nuclear organization in the nematode *C. elegans*

Rahul Sharma[1,2] and Peter Meister[1]

With its invariant cell lineage, easy genetics and small genome, the nematode *Caenorhabditis elegans* has emerged as one of the prime models in developmental biology over the last 50 years. Surprisingly however, until a decade ago very little was known about nuclear organization in worms, even though it is an ideal model system to explore the link between nuclear organization and cell fate determination. Here, we review the latest findings that exploit the repertoire of genetic tools developed in worms, leading to the identification of important sequences and signals governing the changes in chromatin tridimensional architecture. We also highlight parallels and differences to other model systems.

Addresses
[1] Cell Fate and Nuclear Organization, Institute of Cell Biology, University of Bern, CH-3012 Bern, Switzerland
[2] Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

Corresponding author: Meister, Peter (peter.meister@izb.unibe.ch)

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Introduction

Largely inspired by early electron microscopy images, chromatin was classically split into transcriptionally silent heterochromatin either at the nuclear rim or close to the nucleolus and more active euchromatin located internally. Genome-wide techniques coupled with computational modeling approaches have revolutionized the way we envision chromatin distribution inside the nucleus, its determinants and the function of nuclear organization [1]. However, the key question in the field still remains whether gene position inside the nucleus is a cause or consequence of its expression [2]?

To address this chicken or egg question, it is essential to understand how genomes, small and large, organize themselves? Comparisons between computational models of chromatin fiber as a polymer and *in vivo* chromosome conformation capture studies have shown that chromosome conformations are not deterministic [3–5]. They result from the physical behavior of the fiber coupled to anchoring either at the nuclear periphery, between different loci or in the nuclear interior. Understanding how a chromosome folds, hence, necessitates uncovering sequences that drive localization and the signals that direct subnuclear addressing [6]. Does this inherent organization influence expression? In developing mammals and *Drosophila*, the interrelationship between a gene's physical position inside the nucleus and its transcriptional status has been the focus of a number of studies [7,8]. The emerging picture depicts silent genes either at the nuclear envelope (NE) or internal to their chromosome territory, which is the nuclear space occupied by a given chromosome. Active genes appear located internally, away from the nuclear rim and rather on the edge of their respective chromosome territory. However, the limitations of cellular systems in terms of genome engineering impaired the determination of 'acting' sequences directing gene positioning and to study the function of the location.

The nematode genome was the first fully sequenced metazoan genome and is only 97 megabases in size, split into five autosomes and a single X chromosome [9]. *Caenorhabditis elegans* chromosomes are holocentric: during mitosis, microtubules bind along the entire length of the chromosomes, rather than only at a localized centromere. Large repetitive regions are rare in the worm genome as compared to mammalian ones [9]. The chromosomal organization of about 20 000 genes is peculiar: chromosome centers are enriched for essential, conserved and more highly/broadly expressed genes, whereas chromosome arms show less genes, lower expression level, and less sequence evolutionary conservation as well as increased transposon frequency and repeat elements. The ease and variety of genetic manipulations, as well as easy microscopy makes the worm a perfect model system to study nuclear organization. Current data suggest that nuclear organization of the nematode genome obeys rules, which can be split into 'constitutive' and 'developmental' nuclear organization.

Constitutive nuclear organization: tethers at the nuclear envelope

In budding yeast, various models were built based on polymer physics, to understand chromosome folding, *in silico*. Models were most similar to the *in vivo* situation when assuming that telomeres and centromeres are tethered to the nuclear periphery, thereby suggesting the importance of these anchoring points in nuclear organization [10–12]. Similarly, in *C. elegans* nuclei, a number of attachment sites are likely to create the base layer of nuclear organization.

The major difference between yeast and metazoans is chromosome size: yeast chromosomes can intermingle because of their small size (230 kb–1.5 Mb), whereas larger chromosomes cannot mix due to their physical properties, restricting themselves in 'territories' [13,14]. Nevertheless, like yeast telomeres, portions of the genome in both *Drosophila* and mammalian cells, are localized close to the nuclear lamina, a classical silent heterochromatin subnuclear domain [15–17]. These regions were named lamina-associated domains or LADs. Fly and human LADs show equal distribution among all chromosomes and chromosome parts. LADs are reported in *C. elegans* embryos (a mixture of cell types and developmental stages) [18••,19••] but they are enriched in the outer thirds of the autosomes (3.5–5.7 Mb at either ends of the 13.2–20.9 Mb chromosomes), while being almost absent from the central part (Figure 1a and b). Upon comparison, only the left end of the X chromosomes in hermaphrodites is peripherally located, suggesting a difference between autosomes and sex chromosome organization that might be a result of dosage compensation [18••,19••].

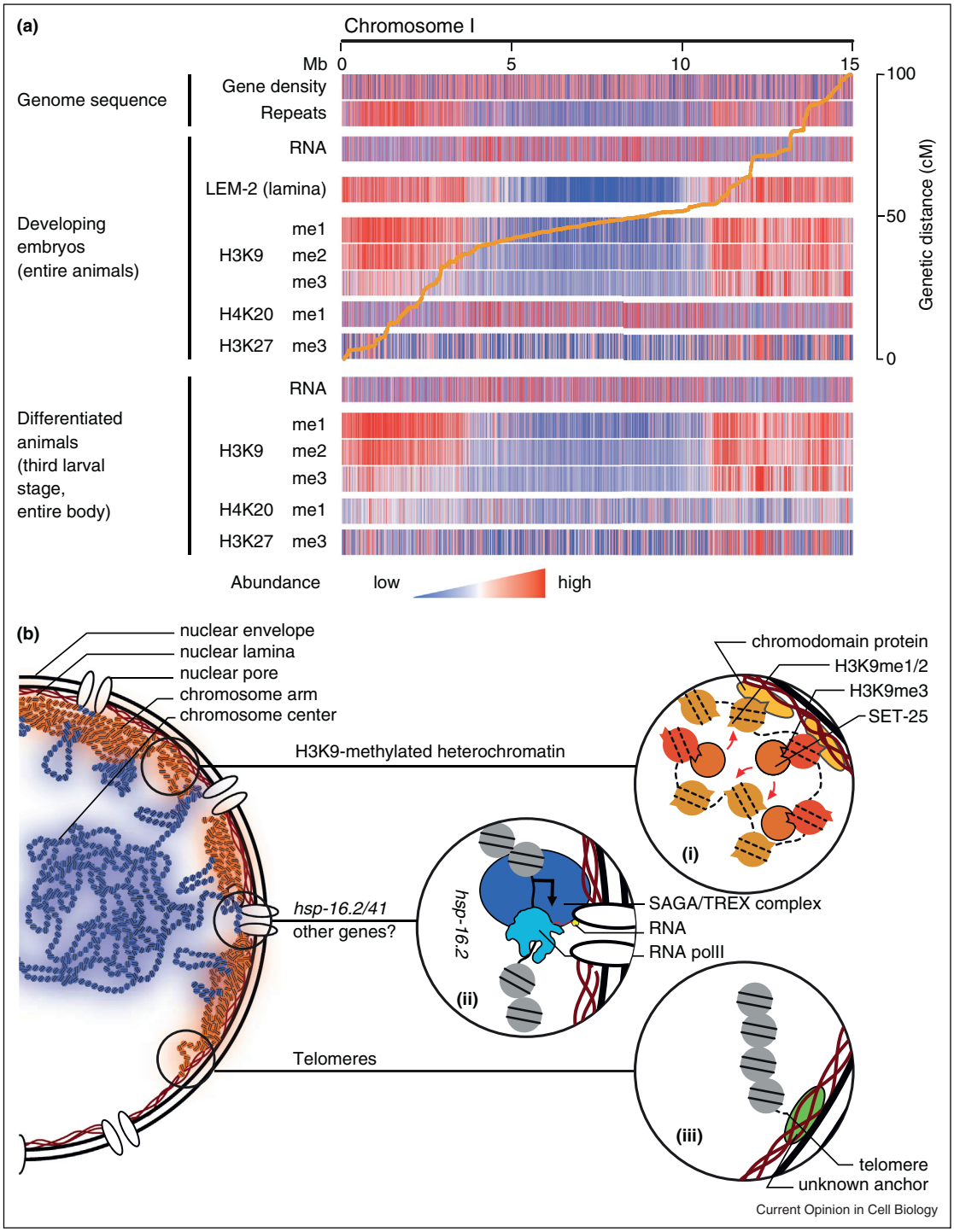
In mammals, LADs are mostly shared between cell types indicating that they are constitutive, despite drastic differences in cytological heterochromatin distributions between developmental stages or differentiated cells [16,20,21]. These constitutive LADs are characterized by their A/T sequence richness [16,22]. Similarly, sequence determinants cause perinuclear localization in worms: in chromosome fusions, internally translocated ends remain close to the nuclear periphery [18••] and AA/TT clusters are found to be less frequent in chromosome arms [23••]. Nematode LAD sequences also have a higher frequency of repeat elements, especially satellite repeats and helitrons, a rolling-circle type of transposon [18••]. Genes confined to embryonic LADs remain mostly repressed throughout development. Finally, it is unclear whether facultative LADs (LADs which detach or attach to the NE during development) are present in the worm genome, as there are no data on LADs in differentiated cells [18••].

LADs seem to share sequence similarities across species and one particular signal and a possible mechanism for perinuclear targeting emerged from the study of worm heterochromatin. Similar to fly and human cells, *C. elegans* LADs are enriched with silent chromatin marks H3K27me3, H3K9me2/3 as well as H4K20me1 [15,17,18••,23••,24••] (Figure 1a, data from [24••]). This correlation between histone methylation and nuclear localization is also found on artificial heterochromatic repetitive arrays, which undergo transcriptional silencing [25–27]. Using these arrays as a model, a genome-wide RNAi screen characterized genes that upon downregulation led to array derepression [19••]. Simultaneous mutations of *set-25* and *met-2*, two histone H3 lysine 9 methyl transferases (H3K9 HMTs) induced derepression and delocalization of heterochromatin (Figure 1b,i). These enzymes carry most, if not all, H3K9 methylation in *C. elegans* embryos and young larvae [19••]. In a *set-25/met-2* mutant, large portions of the chromosomes are released from the periphery, in particular the outer ends which exhibit high H3K9 methylation in wild-type worms (Figure 1a). H3K9 methylation therefore provides a signal for the attachment to the nuclear periphery. However, the attachment *per se* is not mediated by the enzymes depositing the mark: a perinuclear chromodomain protein binding methylated H3K9 has been shown to be the peripheral anchor of H3K9me chromatin (A. Gonzalez-Sandoval, B.D. Towbin, S.M. Gasser, pers. comm.). Chromosome arms and telomeres are however not completely released in a *set-25/met-2* mutant [19••], suggesting that other uncharacterized pathways tether telomeric chromatin at the NE (Figure 1b,iii).

If H3K9 methylation is one of the molecular signals for perinuclear targeting, the primary nucleation event that leads to methylated H3K9 chromatin packaging is not clearly understood, especially since MET-2 — as its mammalian homologs — is known to methylate histone H3 in the cytoplasm before they are incorporated into chromatin [19••,28,29]. Theoretically, mono-methylated and di-methylated H3K9 incorporation being restricted to repeats can be either due to removal from euchromatic regions and/or specific targeting to heterochromatic sequences. Non-exclusive arguments for both models exist. First, nematodes have a ubiquitously expressed JMJD-2, a Jumonji domain H3K9/36 demethylase which could demethylate H3K9 nucleosomes in euchromatic regions [30,31]. Demethylation in repetitive regions could be hindered by protection of methylated H3K9 by a specific binder. For instance, HPL-2, one of the HP1 homologs in worms and JMJD-2 have opposing effects in the germline and HPL-1, the other HP1 homolog localizes to SET-25 compartments [19••]. Secondly, feeding worms with dsRNA induces H3K9 trimethylation on the targeted gene, suggesting an RNA-based mechanism [32]. Silencing and methylation are transmitted to the next generation and this epigenetic inheritance requires *set-25* (the H3K9 trimethylase) and *hpl-2* [32,33]. Non-coding dsRNA arising from LADs transcription may be recognized by HP1 homologs as in fission yeast and mammals [34–38], which in turn would attract methylated H3 for chromatin packaging.

Although it was not formally demonstrated, the high frequency of repeated and transposable elements [39••] in LAD sequences suggests that the system emerged to silence dsRNA-generating elements like transposons and retroelements, likely in order to avoid potentially mutagenic transposition events. The biological function of H3K9 methylation and LADs in *C. elegans*, under laboratory growth conditions is however not clear: mutants

Figure 1



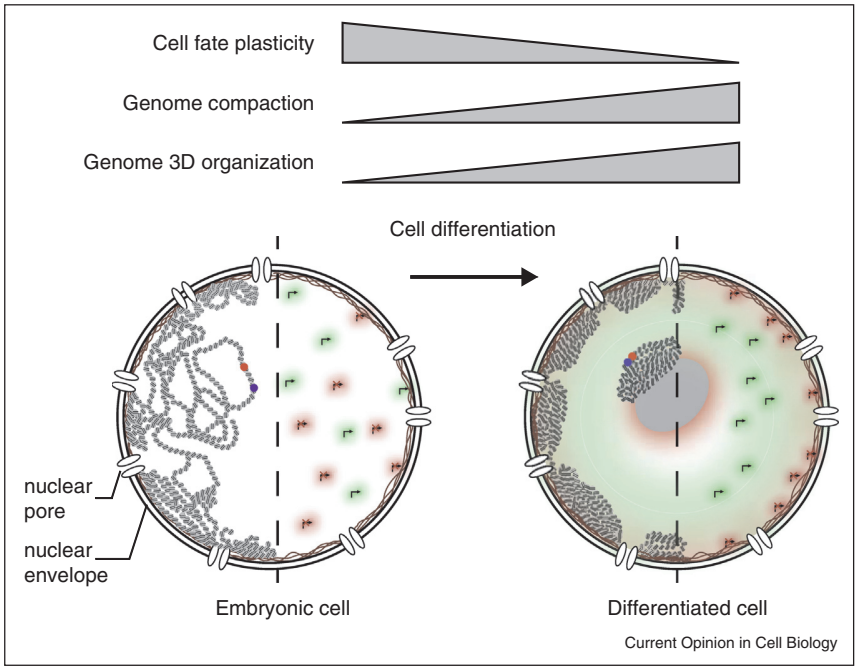
(a) Gene and repeats density, expression (RNA) and ChIP-chip profiles of the lamina-associated protein LEM-2 and silent histone marks for chromosome I. Profiles are shown for both embryos and third larval stage worms and are very similar for the other autosomes. Orange line depicts the recombination distances in centimorgan (cM) from the left telomere. Figure adapted from Liu *et al*. [24••] with permission of the authors. **(b)** Model depicting chromosomal organization and constitutive chromatin anchoring sites inside a nematode. Left: chromosome arms are associated with the lamina and rich in silent chromatin marks (orange); the more expressed central region, which shows no LEM-2 enrichment is shown in blue. Right: i. H3K9 methylated repetitive regions in the genome are sequestered to the nuclear periphery by SET-25 and thereby anchored to the NE by a chromodomain protein and trimethylated by SET-25, which binds to H3K9me3. ii. The heat shock promoter (*hsp-16.2*) interacts with the nuclear pore in a RNA polII dependent manner. iii. Telomeres appear anchored at the NE by an unknown mechanism, different from methylated H3K9 anchoring.

devoid of H3K9 methylation develop normally, and except a smaller brood size, neither obvious phenotype nor huge changes in gene expression could be detected [19••]. An additional, developmentally regulated layer of nuclear organization could compensate lack of H3K9 methylation: in double *set-25/met-2* mutants repetitive arrays relocate to the NE upon cell differentiation whereas they localize randomly in embryos [19••]. Moreover, H3K9 and H3K27 methylation are present in the same genomic regions contrary to the situation in mammalian cells (Figure 1a, [24••,26•]): the developmentally regulated H3K27 mark could compensate for the absence of H3K9 methylation (see below).

An interesting, although not fully understood correlation is observed between gene distribution, nuclear organization in embryonic somatic tissues and germline recombination rate: peripherally localized, H3K9/27 methylated, gene-poor and repeat rich chromosome arms have high recombination rate [18••,23••,24••,40–42]. On the other hand, gene-rich chromosome centers have low recombination rates and are not peripherally located in the soma. This suggests an evolutionary interplay between genome sequence, epigenetic marks and nuclear organization.

Other prominent chromatin anchoring sites are nuclear pores, where dense peripheral heterochromatin is interrupted, suggesting the presence of active genes at this site [43,44]. In yeast, some stress-induced genes tend to associate with pores upon activation [45–49]. In flies, the transcriptionally two-fold upregulated X chromosome in males as well as the *Hsp70* loci are seen peripherally located and interacting with nuclear pore components, in both sexes [50,51]. In *C. elegans*, ChIP by the modENCODE consortium with NPP-13 (www.modencode.org), an internal nuclear pore component did not identify large pore-interacting regions in contrast to the LADs. However, a gene-centered study showed that the heat-shock locus *hsp-16.2* is enriched at the nuclear periphery. The promoter alone is able to autonomously direct a randomly positioned locus close to nuclear pores [52•]. Upon transcriptional induction, the locus colocalizes with the nuclear pore and the open reading frame shows enrichment for NPP-13 by ChIP. Pore-proximal localization is correlated with maximal gene activation and requires active RNA polymerase II [52•] (Figure 1b,ii). Interestingly, the *hsp-16.2* locus is found on a chromosome arm, between two lamina-proximal domains. Genes located in these inter-domain loops show higher transcription levels [18••]. One hypothesis is that these genes are more likely to be located in a transcription-favorable environment close to nuclear pores as neighboring sequences interact with the nuclear lamina.

Figure 2



Changes in nuclear organization during development. Left halves: decrease in cellular plasticity during fate specification is associated with an overall increase in genome compaction level and progressive microscopic heterochromatin development. Right halves: nuclei become more organized during differentiation, with active developmentally regulated promoters (arrows with green halo) being internalized during differentiation, while inactive ones are segregated at the nuclear periphery (arrows with red halo).

In summary, three different types of sequences have been shown to constitutively organize the worm nucleus: silent repetitive DNA, anchored at the nuclear periphery in lamina-proximal repressive compartments via methylated H3K9, a chromatin modification; one 'stress-induced promoter' (and likely others) recruited to transcriptionally active compartments close to nuclear pores in a transcription-dependent mechanism and finally, telomeric regions attached to the nuclear periphery by unknown tethers.

Nuclear (re-)organization during development

The first clue that part of nuclear organization was developmentally regulated in worms, came from the observation that repetitive arrays carrying developmentally regulated promoters relocate from the NE in embryos to the nuclear center upon promoter activation in differentiated cells [26•]. To further understand the mechanisms directing gene localization during development, smaller *lacO*-tagged transgenes carrying developmentally regulated promoters were used to explore the link between cell differentiation, promoter activity and gene position. These small transgenes are usually neither silenced in the germline nor anchored at the nuclear rim [26•,53]. Early during development, transgenes do not show preferential localization, although their developmentally regulated promoters are not active. Upon differentiation however, these transgenes either shift towards the center of the nucleus in cells in which the promoters are activated or towards the nuclear periphery in the rest of the cells (Figure 2, right halves) [26•]. This differential positioning of transgenes is observed for some promoters quite early during development (about 200 cells stage) and also correlates with their respective transcriptional activation/repression [53]. Transgene positioning is maintained throughout development and reflects the behavior of endogenous loci [26•]. Here, the transcriptional state of developmentally regulated promoters clearly drives subnuclear localization. However, transcription itself is not sufficient: housekeeping promoters, even with high expression levels, are unable to induce array relocation away from the NE [26•] and additional uncharacterized factors are necessary for internal nuclear localization. Conversely, peripheral localization does not lead to complete repression either: peripherally located arrays can still be expressed, although to a reduced level [19••,26•]. Transcriptional repression at the periphery depends on the integrity of the nuclear lamina as downregulation of lamin-associated proteins leads to array derepression and decondensation [53,54•,55•].

Another developmentally influenced genome restructuring is the generation of the nucleolus, or nucleologenesis. Unlike other organisms in which rDNA repeats are dispersed on several chromosomes, nematodes have a single rDNA cluster on the right arm of chromosome I. The nucleolus itself disappears in the oocyte before fertilization and it reappears in the embryo at around eight cell stage. Precisely at that time, the zygotic genome is transcriptionally activated, thereby suggesting that transcriptional activation is coupled to nuclear organization [56,57].

Correlated with loss of cell fate plasticity, electron microscopy studies show an increase in dark-stained heterochromatin during differentiation of mammalian cells, suggesting increased compaction of the genome [20,21]. Similarly, serial sections of *C. elegans* embryos show that heterochromatin progressively appears during differentiation (Figure 2, left halves, [58], S. Rohner, C. Genoud and S.M. Gasser, pers. comm.). This change in cytological heterochromatin correlates with the potency state of the blastomeres: early blastomeres are pluripotent, since ectopic expression of cell-fate regulating transcription factors can induce a variety of cell-fates [59–62], reviewed in [63]. Plasticity loss occurs between the 24-cell and the 100-cell stage and is accompanied by an increase in chromatin compaction [64••]. Interestingly, one determinant of both chromatin compaction and cell plasticity is MES-2, the catalytic subunit of the polycomb complex protein (responsible for H3K27 methylation) and homolog of *Drosophila* Enhancer of Zeste (E(Z)). In *mes-2 mutant* animals, chromatin is less compact at the 100 cells stage and blastomeres retain their ability to respond to ectopic expression of cell-fate inducing transcription factors [64••]. Increased chromatin compaction moreover, appears to be a general phenomenon, independent of transcriptional state. In strains with developmentally regulated promoter arrays, the transgene relocates and then shrinks in volume in the nuclear center [26•]. Interestingly, decondensed chromatin is more accessible to transcription factors, suggesting a role for chromatin compaction state in transcriptional regulation, similar to mammalian cells [55•,65].

Interestingly, cell fate plasticity — and as an extension, nuclear organization — also appear to be under control of external cues: in the anterior part of the embryo, the Notch receptor homolog GLP-1 as well as two of its ligands drive plasticity loss during differentiation [66]. Epistasis analysis suggests that Notch and polycomb/MES-2 function in the same pathway. Interestingly, links between the Notch pathway and adult stem cells plasticity are well known in mammals and Notch activation was shown to be involved in premature aging [67,68]. The transcriptional network underlying fate plasticity is also conserved: *in vivo* reprogramming of an epidermal cell into a neuron depends on worm homologs of a transcription factor (Sox-2) and a chromatin modifier complex involved in the induction of pluripotent cells [69,70]. Together, this suggests remarkable parallels between *C. elegans* and other metazoans in cell plasticity regulation, correlated with changes in nuclear organization.

Finally, *C. elegans* has been used as a model for laminopathies, a family of diseases linked to mutations in lamin

genes and changes in nuclear structure. Most laminopathies affect a given tissue, although lamins are ubiquitously expressed. Changes in gene regulation were suggested to cause this wide variety of phenotypes. Many mutant lamins have been expressed in *C. elegans*, causing nuclear shape variations and/or mislocalization of the protein [71]. The expression of two mutations linked to a human muscular dystrophy leads to motility loss and altered muscle structure [54•,72]. In one particular mutant, mutant lamin expression also leads to muscle-specific retention of activated promoters at the NE and transcriptional repression [54•]. Studying these rare diseases in a genetically tractable entire organism might help understand their pathophysiology and the link to gene regulation.

Outlook

Although *C. elegans* is a new player in the field of chromatin biology, its unique combination of genetics and nuclear cell biology tools make it a potent model system offering a promising start towards exploring the links between cell fate, gene transcription and nuclear organization. The characterization of sequences and proteins involved in shaping the nuclear landscape paves the way for a functional analysis of nuclear structure and its implications.

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