The nuclear envelope and transcriptional control

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Abstract | Cells have evolved sophisticated multi-protein complexes that can regulate gene activity at various steps of the transcription process. Recent advances highlight the role of nuclear positioning in the control of gene expression and have put nuclear envelope components at centre stage. On the inner face of the nuclear envelope, active genes localize to nuclear-pore structures whereas silent chromatin localizes to non-pore sites. Nuclear-pore components seem to not only recruit the RNA-processing and RNA-export machinery, but contribute a level of regulation that might enhance gene expression in a heritable manner.

Nuclear periphery
A term that generally refers to the nuclear-membrane bilayer, its associated proteins and the embedded nuclear-pore complexes.

Dosage compensation
A phenomenon that ensures equalized gene expression of X-chromosomal genes between males and females. In *Drosophila melanogaster*, this results in approximately twofold higher levels of transcriptional activation in the single male X chromosome compared with the female X chromosomes.

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A glance at an electron micrograph of a mammalian cell nucleus reveals a special relationship between the nuclear envelope and chromatin. In differentiated nuclei, dense-staining, transcriptionally inactive 'heterochromatin' is usually plastered against the inner face of the nuclear membrane. These heterochromatic patches are interrupted by nuclear pores, which contain the light-staining nucleoplasm of 'open' or active domains (FIG. 1a,b). Similarly, in budding yeast, fluorescent markers for repressed chromatin and nuclear pores can be readily distinguished as distinct non-overlapping series of foci (FIG. 1c). Is this subnuclear compartmentation a side effect of differential chromatin folding, or does positional information actually contribute to gene regulation? Studies in budding yeast and *Drosophila melanogaster* suggest that perinuclear compartments do influence various nuclear activities, ranging from transcription and mRNA export to DNA repair and the stable transmission of a heritable state of repression. We expect equivalent functional effects to exist in mammalian systems.

A well-studied example of a functional perinuclear microenvironment is that which is created by telomere clustering at the nuclear envelope in yeast[1]. These foci sequester the silencing factors that promote and stabilize heterochromatin. Recent data suggest that the association of active, induced genes with nuclear pores creates a second functional compartment at the nuclear periphery. Genes can shift to pores in a transcription-dependent manner, and expression levels can drop if this relocation is impaired. Studies of dosage compensation in flies also indicate that pore-bound factors might help to upregulate gene expression in males. This Review examines new data suggesting that the positioning of chromatin at the nuclear envelope can contribute to gene regulation in both a positive and negative manner.

Epigenetic specification of cell identity

Transcriptional regulation is crucial for defining cell identity. This cannot be achieved soley by the orchestrated appearance and disappearance of transcription factors, but instead results from the complex interplay of sequence-specific DNA-binding factors and the epigenetic status of target sequences, which limit the fraction of the genome that is subject to sequence-specific regulatory control (reviewed in REF. 2). Combined with post-transcriptional steps that regulate the efficiency of mRNA processing, export and translation, these mechanisms lead to reproducible, cell-type-specific patterns of gene expression.

Eukaryotic genomes contain three classes of chromatin[2,3]. The first is open or actively transcribed chromatin, which contains genes with engaged RNA polymerases. The second is potentially active chromatin, which contains promoters that are poised to respond to activating signals, but from which stable transcripts are rare or nonexistent. These two states account for the vast majority of chromatin in yeast, yet comprise only a small fraction of a mammalian genome. The third type of chromatin encompasses a range of transcriptionally silent heterochromatic states, accounting for most of the DNA in differentiated somatic cells. Here genes are generally repressed in a heritable manner, and gene promoters tend to be inaccessible to transcription factors (reviewed in REF. 3). Although the existence of these characteristic chromatin domains is well established, the factors that create and regulate them are poorly characterized. One element that contributes to

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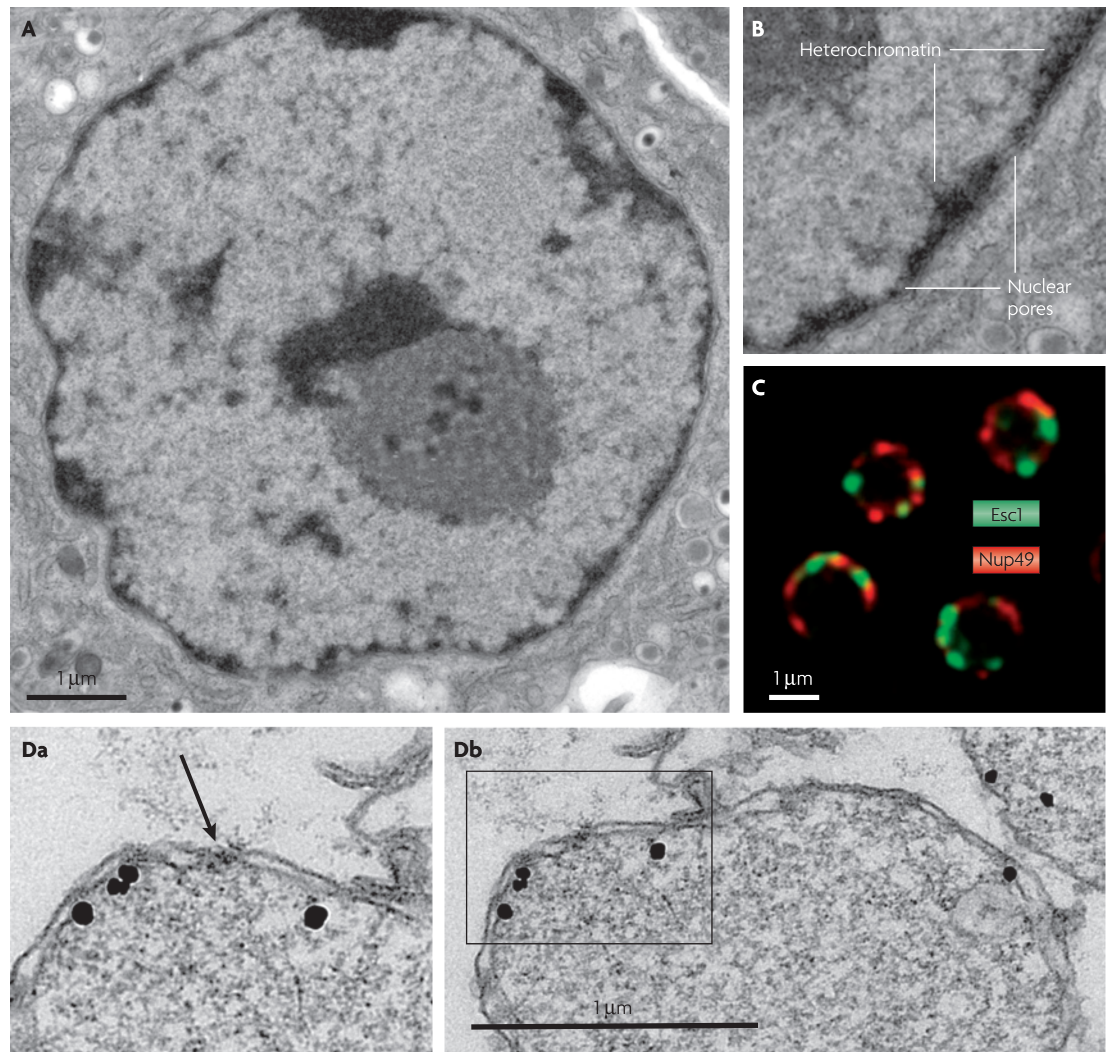

Figure 1 | **Heterochromatin in mammalian and yeast cells is distinct from nuclear pores. A** | An electron micrograph of the mammalian liver nucleus (with an enlarged section shown in part **B**), showing dense-staining heterochromatin located around the nucleolus and against the nuclear envelope. Nuclear pores open onto lighter-staining open chromatin. **C** | In budding yeast, heterochromatin binds the nuclear envelope through Esc1 (enhancer of silent chromatin 1; labelled green), which forms distinct foci alternating with nuclear pores (visualized in red through labelling of Nup49 (nucleoporin 49)). **D** | An electron micrograph showing Esc1 at non-pore sites along the yeast inner nuclear envelope. An arrow indicates the nuclear pore, and black dots represent the labelling of Myc-epitope-tagged Esc1 using fluoronanogold Alexa^{488} anti-mouse antibody[18]. The image in part **c** is reproduced with permission from *EMBO Journal* REF. 18 © (2004) Macmillan Publishers Ltd.

both the establishment and maintenance of chromatin states seems to be their spatial distribution within the interphase nucleus (reviewed in REFS 1,4–7).

Insulator function and nuclear pores

Non-coding DNA elements called boundary elements contribute to chromatin domains in two ways[8]. First, they protect open chromatin regions from the encroaching repression that is caused by heterochromatin. Second, they restrict the interaction of enhancers with promoters. Both events are thought to involve elements of nuclear organization, either through the formation of a chromatin loop or by tethering topologically defined domains to nuclear structures[8–11].

An important aspect of insulator function is the ability to mediate long-range DNA interactions. For example, the *D. melanogaster* insulator element *Fab-7* (also known as *Abd-B*) was shown to physically associate with a second, randomly inserted *Fab-7* copy independently of its position on the chromosome arm. This association in *trans* was disrupted by loss of the Polycomb protein[12], which also compromised insulator function. Another element, the *D. melanogaster gypsy* insulator, which binds a factor called Suppressor of hairy wing (Su(Hw)), might have a similar function. The *gypsy* element itself is often associated with the nuclear periphery and, when it was inserted into the genome, caused surrounding DNA to shift to the insertion site in a Su(Hw)-dependent

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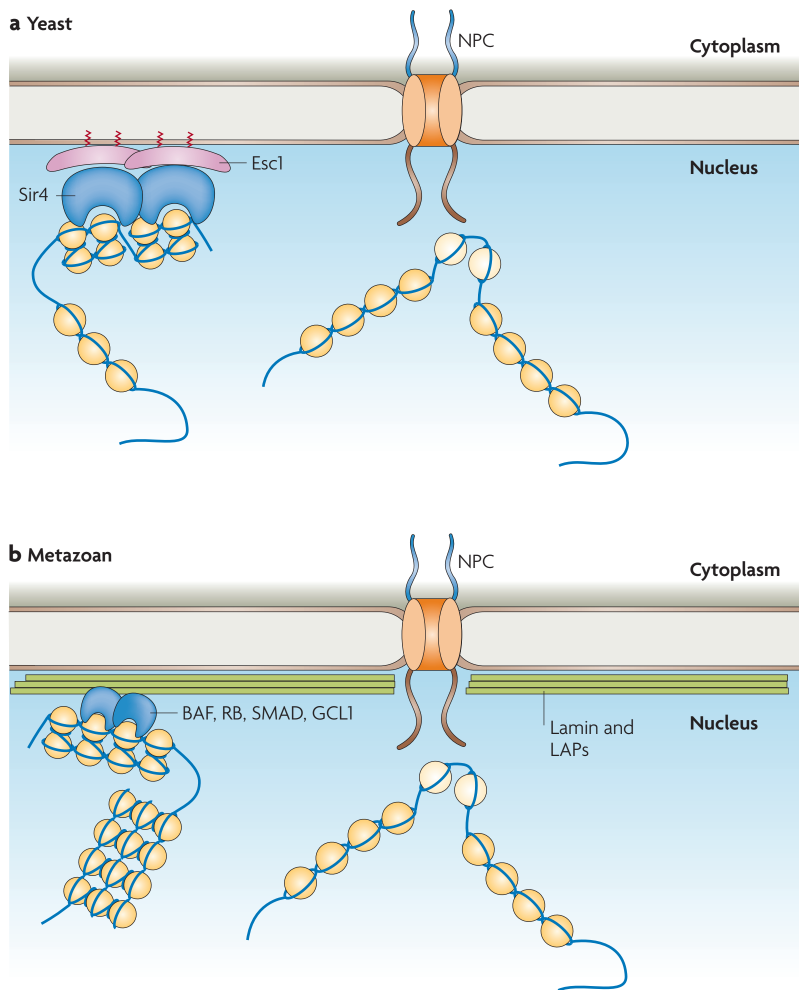

Figure 2 | **The nuclear periphery in metazoans and yeast.** In eukaryotic cells, the nuclear compartment is separated from the cytoplasm by the inner and outer nuclear membranes. This membrane bilayer is perforated by nuclear pores, which are constituted by a large multiprotein complex (the nuclear pore complex (NPC)) that is composed of about 30 proteins (reviewed in REF. 94). This nuclear membrane, together with the pores, is commonly referred as the 'nuclear envelope' (NE). **a** | In yeast nuclei, envelope-associated proteins such as Esc1 (enhancer of silent chromatin 1) are present in foci at the periphery; however, they do not coincide with the pores. Esc1 binds Sir4 (silent information regulator 4), which is an integral component of repressed heterochromatin in yeast[18,19,22]. **b** | In metazoan nuclei, the nuclear envelope is underlaid by a continuous meshwork of lamins and lamin-associated proteins (LAPs), which preferentially associate with inactive chromatin regions[37,38]. Increasing evidence implicates interactions of chromatin with various nuclear-envelope components in gene repression as well as gene activation. BAF, barrier to autointegration factor; GCL1, germ-cell-less homologue; RB, retinoblastoma 1.

manner[11]. Moreover, much like Polycomb-binding sites, two *gypsy* elements that were inserted at distant sites on the same or on different chromosomes were found to self-associate. Although the perinuclear anchoring of *gypsy* was correlated with its insulator function, Xu and colleagues later identified a 340-bp core element that maintained insulator activity without anchoring DNA at the nuclear periphery[13]. This minimal core element could bind Su(Hw) and retained the ability to interact in *trans*[13]. Thus, peripheral position might be a side effect of selective long-range interactions that generate the chromatin loops that are needed for insulator function[8].

Do other insulators depend on attachment to nuclear substructures? In human cells, a conserved sequence-specific factor, CTCF, is implicated in the function of a broad range of insulators, and has been demonstrated to tether an insulator-associated transgene to the nucleolus[14]. It was proposed that CTCF insulator activity might require this localization. Similarly, a screen in budding yeast that scored for insulator activity in a library of targeted fusion proteins[9] yielded many candidates that acted as barriers to heterochromatin spreading. Several seemed to function by creating a DNA loop that encompassed the 'protected' gene and tethered it to Nup2 (nucleoporin 2), a dynamic component of the nuclear pore[9]. Similar tethering models have been proposed for the fission yeast transcription factor TFIIIC, which blocks the spread of heterochromatin apparently by anchorage near the nuclear envelope[10]. The binding of the budding yeast TFIIIC factor at a tRNA gene near the silent mating type locus *HMR* was also necessary for boundary function[15]. In a further study, yeast tRNA genes were shown to be accessible to cleavage by a Nup2–micrococcal-nuclease fusion[16]. Assuming that functional Nup2 is pore-bound, these results collectively argue that pore-mediated anchorage might block the spread of heterochromatin in yeast. It remains to be tested whether TFIIIC or nuclear-pore anchorage are universal mechanisms for insulator and boundary function.

The nuclear envelope harbours repressive zones

Telomeric silencing in yeast. The observation that tethering at the nuclear periphery could shield a promoter from repression seemed surprising initially because, in all organisms from yeast to humans, the nuclear envelope was known to harbour repressed domains (FIG. 2). However, it was shown in budding yeast that the sites that anchor silent chromatin are mechanistically distinct from those for active genes[17,18]. Yeast heterochromatin forms in subtelomeric domains, and basically consists of underacetylated nucleosomes that are bound to a complex of silent information regulators, Sir2, Sir3 and Sir4 (reviewed in REF. 19). Sir-bound chromatin is localized almost exclusively into foci at the nuclear envelope[20], positioned in-between but not coincident with nuclear pores[18,21,22] (FIG. 1c). Although heterochromatin in mammalian cells is also found at the nuclear envelope between pores, anchorage mechanisms differ between yeast and multicellular eukaryotes[1,6].

In budding yeast, silent telomeres are anchored to non-pore sites by two partially redundant pathways[18,23,24]. One of these correlates strictly with repression, being mediated by the binding of Sir4 to an acidic membrane-associated protein called Esc1 (enhancer of silent chromatin 1) [18,22]. The Sir4–Esc1 interaction is both necessary and sufficient to anchor silent chromatin at the nuclear envelope[18], even for silent chromatin on an episome[23]. Immuno-electron microscopy confirmed that Esc1 was peripheral, yet largely excluded from nuclear pores (FIG. 2). In a compelling demonstration of this

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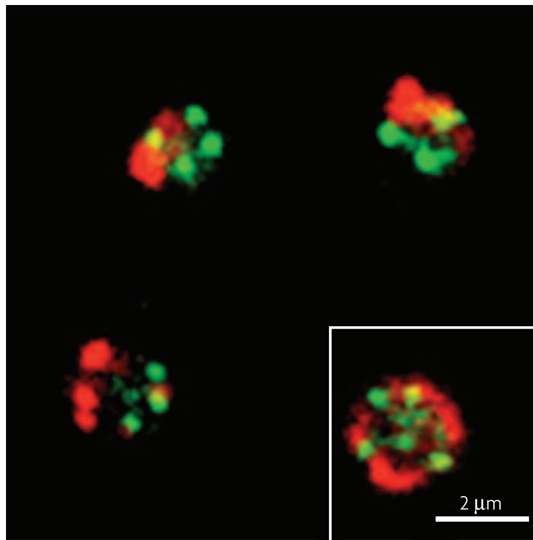

Figure 3 | **Telomere position through Sir4–Esc1 is independent of nuclear-pore positioning.** In budding yeast, mutation of *nup133* shifts nuclear pores (stained red with the monoclonal antibody Mab414, which recognizes the pore proteins Nup116 and Nup96) to one side of the nucleus in a large cluster. This does not affect the position of silent telomeres (stained green with polyclonal antibodies specific for Rap1 (repressor–activator site-binding protein 1)), showing that telomere anchorage is likely to be independent of nuclear pores[18,21,25]. Staining of a wild-type strain is shown in the inset. Identical results were obtained when Sir4 (silent information regulator 4) was localized in the *nup133Δ* strain. Image modified with permission from REF. 25 © (2002) Elsevier Sciences.

separation, Esc1 and silent telomeric foci were shown to maintain a wild-type distribution in the presence of a *nup133Δ* mutation, which allows pores to cluster at one side of the nucleus[18] (FIG. 3). The second telomere-anchorage pathway requires the conserved heterodimeric protein yKu[24,25]. This DNA-end-binding factor can anchor chromosomes independently of Sir-mediated silencing, possibly helping telomeres to cluster together before the silent state is established.

Other factors, including nuclear-pore components, have been suggested to influence telomere anchoring and repression. These effects are likely to be indirect, reflecting loss of the proper sequestering or targeting of the pore-associated desumoylase Ulp1 (ubiquitin-like-specific protease 1) or the SUMO conjugase, Mms21 (methylmethanesulfonate-sensitivity protein 21), which modifies yKu[26,27]. Indirect effects on telomere anchoring may also be attributed to mutations in the structural maintenance of chromatin (SMC) factors or to components of their putative loading complex, such as Ctf18 (chromosome transmission fidelity protein 18)[28]. We expect additional membrane spanning proteins to be involved in tethering yKu- and Esc1-bound telomeres, perhaps including conserved inner-nuclear-envelope proteins, such as those that contain SUN or KASH domains[1].

Nuclear lamina
A meshwork of a nuclear intermediate filament protein that is found at the interface between the inner nuclear membrane and chromatin.

How does the nuclear organization of silent yeast chromatin contribute to heritable repression? Crucially, the silencing factors (Sir2, Sir3 and Sir4) are limiting in concentration everywhere in the nucleus except at telomeres, where the multiple binding sites of repressor–activator site-binding protein 1 (Rap1) sites sequester the silencing factors from histone tails elsewhere in the genome[29]. This sequestration of silencing factors favours the propagation of Sir binding along chromatin close to telomeres, and prevents the promiscuous repression of non-subtelomeric genes[29]. In mutants that release telomeres and allow Sir-protein dispersion, there is both a loss of subtelomeric repression and a gain of repression at internal loci that are positioned near sites that nucleate Sir binding. Consistent with this, Andrulis *et al.* showed that the artificial tethering of chromatin at the nuclear envelope by a membrane-spanning anchor could favour repression of a reporter as long as it contained Sir-nucleating sites called silencers[30]. Although this result is commonly misinterpreted as showing that nuclear-envelope tethering is sufficient to mediate repression, what the paper actually proposed is that the positioning of a *cis*-acting control element (silencer) near Sir pools would facilitate stable repression. Other conditions that enhance weak silencer function to promote silencing include targeting the Sir4-binding domain of Esc1 to the reporter[22] or the coordinate overexpression of all Sir genes[29]. Collectively, these studies argue that the crucial element that is provided by the nuclear periphery is access to limiting pools of general repressors[1].

Nuclear lamina and gene repression. In higher-eukaryotic cells, the nuclear lamina is a layer of intermediate filament proteins at the interface between chromatin and the inner nuclear membrane (FIG. 2b). The α-helical heptad repeats of lamins form coiled-coil dimers, which associate head-to-tail in filaments that span from pore to pore. Loss of lamin A, which leads to death at post-natal week 8 in mice, correlates with dramatic changes in the size and shape of cardiomyocyte nuclei and, importantly, a displacement of heterochromatin from the nuclear periphery[31,32]. Strikingly, the abundance of the most typical repressive histone mark, trimethylation of histone H3 at lysine 9 (H3K9me3), was also reduced. Defects in heterochromatin and H3K9me3 also occurred in cells bearing far-more-minor lamin A mutations, such as the small C-terminal deletion of the premature ageing syndrome Hutchinson–Gilford progeria[33–35]. Finally, in worms, the defects in chromatin organization that are caused by lamin depletion were mimicked by the elimination of two or more lamin-associated proteins, Man1 and emerin, which participate with nuclear lamin-associated proteins to tether chromatin at the nuclear periphery[36]. Thus, the nuclear lamina is implicated either directly or indirectly in the proper distribution and maintenance of heterochromatin.

The abundance and stability of lamin proteins have provided a means to identify the DNA that is associated with the nuclear envelope on a genome-wide scale. To localize lamin-associated sequences, Pickersgill *et al.*

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Box 1 | **Chromatin mobility**

Time-lapse imaging of LacO-tagged chromosomal loci suggests that the random movement of various internal, active loci in the nuclei of yeast, *Drosophila melanogaster* or mammalian cells is similar[23,39,40,49,50,88]. Despite differences in imaging methods, a comparison of the radii of constraint (ranging from 0.5 μm to 1 μm) and diffusion coefficients (ranging from 1×10^{-4} μm^2 s^{-1} to 1×10^{-3} μm^2 s^{-1}) suggests that restricted background movement of chromatin, although influenced by ATP levels, is inherent to the chromatin fibre[23,40,49]. Major alterations in its mobility probably reflect interactions with anchoring proteins or changes in chromatin from repressed to actively transcribed states. The measured degree of random movement would compromise the organization of chromosomes into strict territories in the ~2-μm-diameter nucleus of budding yeast, but would not impair territorial organization in mammalian cells[89], in which nuclear diameters range from 5 μm to 10 μm, as discussed in a recent review[90].

Nonetheless, locus mobility changes with transcriptional status. Most striking is the reduced mobility that correlates with heterochromatin. As shown for yeast telomeres[24,40], the association of heterochromatin with the nuclear periphery (through lamin binding in mammals or through Esc1 binding in budding yeast) restricts its dynamics[23,45,49,50]. The treatment of mammalian cells with DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole), a transcriptional inhibitor that disrupts nucleolar structure, increased the mobility dynamics of a locus that was located close to rDNA repeats, indicating that the nucleolus might also tether certain genes[50]. Finally, double fluorescence *in situ* hybridization (FISH) labelling showed that developmentally induced loci such as the HoxB locus occasionally move far from the condensed chromosome territory to which they belong. It was concluded that locus dynamics can change with developmentally regulated expression patterns[91–93]. From yeast live-tracking studies, one notes that gene dynamics are locus specific: when activated, some become more dynamic, whereas others become peripherally constrained[17,23,46,47,55,56].

adapted an *in vivo* DNA-modification technique called DamID to *D. melanogaster* and coupled it with microarray profiling[37]. This method exploits a fusion of the *Escherichia coli* DNA adenine methyltransferase (Dam) to the lamin gene, which is expressed at a low level in *D. melanogaster* tissue culture cells[37]. Regions of the fly genome that were juxtaposed to the lamins were preferentially methylated by the fusion protein, and could be isolated, labelled and used to probe whole-genome microarrays. Approximately 500 genes were found to be at least transiently lamin-associated; the bulk of these were depleted in active histone modifications, were transcriptionally silent and had late replicating kinetics[37]. They were positioned along all chromosome arms but did not include constitutive centromeric heterochromatin. Although it is not possible to determine from DamID whether the lamin-binding sites are transiently or stably bound, there was significant overlap of these loci with sites that were predicted to be stably nuclear-envelope-bound by high-resolution microscopy[38].

Nuclear space and chromatin mobility

If the subnuclear positioning of any particular chromosomal locus reflects its state of transcriptional activity, then chromatin must be able to move to achieve tissue-specific and developmental-stage-specific levels of organization. How does chromatin move?

Using time-lapse imaging of GFP-tagged chromosomal loci, the Sedat laboratory observed that chromatin in yeast and flies was engaged in a continuous random-walk-like motion[39]. However, these initial studies in yeast monitored a centromere-proximal locus, which is tethered to the nuclear-envelope-embedded spindle pole body (SPB) through short interphase microtubules, limiting chromatin motion[39–43]. Somewhat less constrained random movement was later ascribed to multiple other yeast loci by coupling LacO-tagged genes with a GFP–nuclear-pore marker[40,42], a modification that allows the coincident analysis of mobility and position relative to the nuclear envelope.

In budding yeast, most of the active chromosomal loci moved with a radius of 0.5–0.7 μm in a random walk within the nucleoplasm[23,40,44]. This represents more than one-quarter of the nuclear diameter in yeast, but less than one-tenth of the nuclear diameter in a mammalian cell. Because 50% of the yeast nuclear volume is contained within a peripheral shell that is <0.4 μm thick, most yeast genes probably encounter the nuclear membrane at least occasionally. On the other hand, silent telomeres move in a highly constrained manner along the inner face of the nuclear envelope and only rarely occupy the nuclear core[23,24,40,44]. Yet the movement of a typical telomere is restricted to ~12% of the inner-nuclear-envelope surface[45]. Similar constraint was observed for a subset of active genes, notably the galactose-induced loci that associate with nuclear pores upon induction[46,47]. Given their lateral dynamics and striking radial confinement, it was suggested that these active genes move from pore to pore[47].

By monitoring the dynamics of a fluorescently tagged SPB, embedded in the yeast nuclear envelope[40], it was possible to demonstrate that chromatin movement does not arise from nuclear rotation: SPB movement was clearly more restricted than that of chromatin (r_c = 0.2 μm). Furthermore, the photobleaching of a region of Nup49–GFP-labelled pores showed that lateral movement of unbleached pores was slower than that of chromatin, with recovery requiring minutes, rather than seconds[46,48].

What keeps chromatin at the nuclear envelope? The importance of protein–protein interactions for limiting chromatin dynamics was demonstrated by tracking rings of fluorescently tagged yeast chromatin that were excised from the genome by an inducible recombination event[23]. Whereas non-silenced loci on excised rings traversed the yeast nucleus in rapid jumps, the repressed-chromatin ring remained fixed at the nuclear perimeter[23]. This constraint was due to the affinity of Sir proteins for Esc1 because, if this membrane-associated anchor was compromised, the excised chromatin moved as freely as an active locus, although it remained repressed[23]. Thus, the primary restriction on mobility is not chromatin structure but protein-mediated binding sites at the nuclear envelope, which limits DNA movement to a subnuclear zone[49]. Similar constraint was observed for lamin-bound sites in cultured mammalian cells[50]. Non-coding RNAs might also have a role in heterochromatin anchoring in some species. These studies demonstrate the important principle that nuclear macromolecules, including chromatin, are highly mobile unless anchored (BOX 1). This is confirmed by the equally rapid diffusion kinetics of nuclear proteins and mRNA particles in higher eukaryotes[51,52].

Fluorescence *in situ* hybridization
A technique whereby a fluorescently labelled DNA probe is used to detect a particular chromosomal region by fluorescence microscopy.

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Genome-wide contacts with the nuclear envelope

Given that rapid macromolecular kinetics can easily deliver polymerases to genes and mRNA to pores, it seems unnecessary to propose that RNA synthesis occurs in spatially organized sites. Nonetheless, increasing evidence places transcripts and multiple active polymerases in discrete foci. Perhaps the most surprising results on the localization of active genes came from genome-wide studies that identified sequences that could be either crosslinked to, or modified by, nuclear-pore-associated proteins. Using formaldehyde-mediated crosslinking and chromatin immunoprecipitation (ChIP), Silver and colleagues sought sequences that were bound to nuclear-pore factors and identified three classes of gene-associated pore components[17]. First, they found that several nuclear-pore components (Nic96, Nup116, Nup60, Nup2, Mlp1 (myosin-like protein 1) and Mlp2), and factors involved in mRNA export (Xpo1 (exportin 1), Cse1 (chromosome segregation protein 1) and the karyopherin Kap95) were bound preferentially to genes that were strongly induced by an alternative carbon source, galactose. By contrast, genes that were bound to nucleoporins Nsp1, Nup84, and Nup100 did not change when transcription was altered. Intriguingly, the upstream control regions of genes that were bound by Mlp1, Mlp2 and Nic96 were statistically enriched in binding sites for the multifunctional transcription regulator, Rap1. These results reinforced another set of genetic and biochemical observations, which indicated that Rap1 and its co-activators Gcr1 and Gcr2 could bind Nup84, and that the Nup84–pore subcomplex was needed for full transcriptional induction[53]. These activation events are distinct from those that are induced by galactose, suggesting that more than one pathway makes use of the nuclear-pore complex (NPC) for transcriptional activation in yeast.

A further genome-wide ChIP study used the budding yeast mating pheromone α-factor to induce changes in gene expression, and mapped the association of genes with the pore components Xpo1, Nup116, Mlp1 and Mlp2 (REF. 54). Most α-factor-induced genes had at least a transient association with the nuclear periphery upon activation. Because these genes were distributed on 13 of the 16 yeast chromosomes, global changes in chromatin organization were proposed to occur in response to pheromone[54]. Interestingly, the pore-associated mRNA export factor Mlp1 was recovered with induced genes in an RNase-sensitive manner, and high-resolution mapping placed Mlp1 preferentially at the 3′ end of active genes, suggesting that transcript accumulation might be important for perinuclear tethering. Less clear was why Mlp1 or Mlp2 were also recovered with silent loci, as genetic studies argued against any direct role for Mlp proteins in the anchoring of telomeres[18,22,24,25]. A possible explanation is that, when network-forming proteins are analysed by ChIP, their association with DNA stems in part from indirect interactions that become stabilized by formaldehyde crosslinking. It was therefore essential to couple ChIP with genetic assays and quantitative microscopy on large populations of cells.

An alternative genome-wide approach to the mapping of gene position relied on the regulated cleavage of DNA by micrococcal nuclease (MN). This study indicated that the early steps of initiation, but not the mRNA itself, links genes to nuclear pores. The authors followed the interaction pattern of the dynamic pore protein Nup2 fused to MN[16]. Nup2–MN-dependent cleavage was detected in the promoters of galactose-induced genes, apparently confirming the ChIP results. But, when scored for the entire chromosome 6, Nup2–MN cleavages were found in the promoters of most divergently transcribed RNA polymerase II (RNA pol II) genes, and at tRNA clusters, independently of high levels of transcription. Nonetheless, accessibility increased when genes were induced[16]. At galactose-induced promoters, the transcription factor Gal4 and TATA-binding factor enhanced Nup2–MN cleavage, whereas components of the SAGA histone acetyltransferase complex were not needed, indicating that early steps of initiation increase accessibility for Nup2–MN[16]. It was proposed that transient nuclear-pore contact might even be a prerequisite for RNA pol II activation.

These genome-wide approaches left a somewhat contradictory scenario: although they showed that gene induction and NPC crosslinking were correlated, they failed to establish a functional dependence of transcription on positioning. Were selected promoter factors capable of gene anchoring, or were transcripts and transport factors needed to deliver the induced genes to nuclear pores? One limitation of genome-wide profiling assays is that they tend to trap rapid or one-hit events, scoring both stable and transient interactions with equal efficiency. For this reason, it was unclear from Nup2–MN or ChIP how stable the scored interactions might be. Finally, these results had to be reconciled with the observation that many, if not most, transcribed genes, in both yeast and mammals, did not associate stably with pores. This was confirmed by direct-imaging studies of active loci in both living yeast[23] and mammalian cells[51]. To decipher the functional significance of the NPC–gene link, one needed quantitative analyses of gene position correlated with quantitative transcript-level assessment in the corresponding cells.

Yeast nuclear pores as active zones

The quantitative analysis of subnuclear position for several inducible genes (*INO1* (inositol 1-phosphate synthase gene), *HXK1* (hexokinase isoenzyme 1 gene), *GAL1*, *GAL2*, *HSP104* (heat-shock protein 104 gene)) through the LacO/LacI tagging system confirmed that these become stably positioned at the nuclear periphery when activated. Moreover, they remain there after the gene is shut off[17,46,47,54–58]. By combining the position data with quantitative PCR of mRNA levels, it was shown that maximal expression for at least some loci is coupled with stable NPC association[46,54,56]. However, the elements that are required for the translocation were often gene specific. Despite this, a few principles that guide the positioning of active genes have surfaced.

Chromatin immunoprecipitation
A technique that involves crosslinking methods and is used to identify pieces of DNA or chromatin that contact a protein of interest *in vivo*.

Nuclear-pore complexes
Large, multiprotein complexes (composed of about 30 proteins) that are embedded in the nuclear membrane and serve as gateways for traffic between the nucleus and the cytoplasm.

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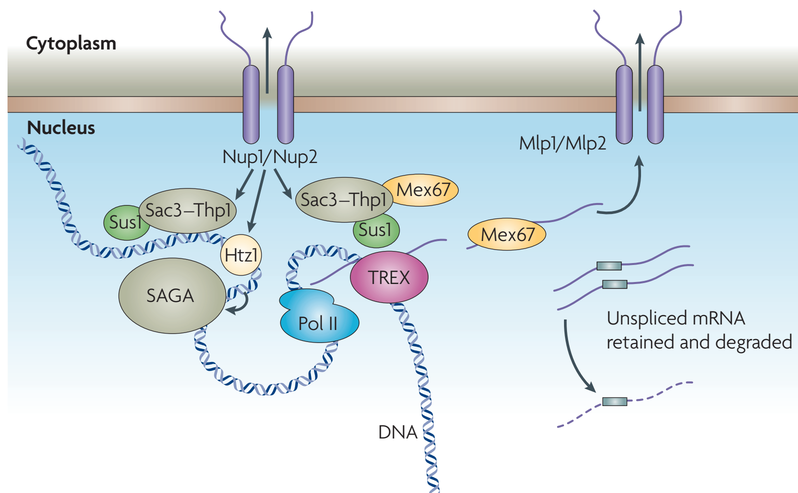

Figure 4 | **A model for the role of the NPC in coupling transcription and mRNA processing by gene looping in yeast.** It is becoming increasingly clear that a number of transcription-coupled processes converge at nuclear pores, and by this virtue nuclear-pore complexes (NPCs) are likely to contribute, directly or indirectly, to transcriptional regulation. Although not all transcriptional activity in the nucleus will be subject to this mode of regulation, the budding yeast NPC seems to work together with transcriptional activation mechanisms to fine-tune gene activity, as proposed in this model. The SAGA chromatin-remodelling complex in yeast has been shown to contain Sus1; this protein is also present in the mRNA-export complex TREX, which interacts with the nucleoporin Nup1 (REFS 59,60). Furthermore, Nup2 has been shown to interact with the promoters of active genes[16], and the NPC-associated protein Mlp1 (myosin-like protein 1) accumulates at the 3′ end of active genes, where it contributes to an RNA surveillance mechanism[54,80,95]. Studies involving individual loci have shown that optimal activation (resulting in a twofold transcriptional effect) can require both localization of the induced gene at the NPC as well as at the 3′ UTR[46,47,56]. Our model suggests that gene looping, which results from the coincident NPC-tethering of an initiation complex and mRNA-processing complexes that are associated with the 3′ UTR, will help to fine-tune the expression of certain genes[62–65]. NPC factors can facilitate efficient transcription elongation and/or efficient mRNA-processing events, or the coordination of the two. If aberrantly processed mRNAs are near the site of transcriptional initiation, their retention or rapid degradation can provide an immediate signal for the production of additional transcripts. Such a mechanism might be important for genes that require immediate high-level induction, such as heat-shock- or galactose-inducible genes. Finally, the pore protein Nup2 was found to tether genes through a histone variant H2A.Z (Htz1) in yeast[55]. This could reflect a heritable localization that contributes to forms of epigenetic control. This might be critical during dosage compensation of X-linked genes in male flies, where a twofold upregulation is crucial. Mex67; mRNA export factor 67; Pol II, RNA polymerase II.

One clear principle is that specific promoter sequences and activators are crucial for determining whether a transcription unit will associate stably with nuclear pores. Importantly, a *GAL1* promoter fused to an artificial reporter at an ectopic site could still mediate NPC relocalization[58]. Conversely, when *HXK1* was activated by two different pathways, only one (induction by low glucose) led to NPC localization[46]. Thus, promoters are selective and essential for NPC binding; a nascent transcript alone seems to be insufficient for pore association. Galactose- and heat-shock-controlled promoters generally require components of the SAGA complex for stable nuclear-pore positioning[47,57,58], involving not only core SAGA components like Ada2 (transcriptional adaptor 2), but the SAGA-associated factor Sus1 (REF. 47). Sus1 is unique in that it co-purifies with both SAGA and the Sac3–Thp1–Cdc31 complex, which is part of the mRNA-export machinery that binds the NPC through Nup1 (REFS 59,60). This might link the promoter with the mRNA-export machinery. Given that SAGA is often required for the induction of stress-responsive genes, which require a highly dynamic range of expression coupled with rapid mRNA export, stress-induced genes might exploit pore association to achieve these ends.

Are promoters sufficient to ensure association with the pore? Here the answer seems to be gene specific. For *GAL2*, the upstream activation sequence (UAS) and promoter were sufficient to support localization at the NPC[57] and, at both *INO1* and *GAL1*, pore relocalization occured independently of elongation[55]. This might reflect promoter strength or context, because an ectopic *GAL1* UAS was not sufficient for stable relocalization; a specific 3′ UTR was also required[58]. Similarly, the subtelomeric *HXK1* gene required its own 3′ UTR for stable association[46]. Because each study exploited a slightly different reporter in a different chromatin environment, we propose that the variable requirement for the 3′ UTR reflects the differential strength of the interactions between promoter-bound factors and the NPC. Strong promoter–pore interactions might obviate the need for anchoring through 3′ UTR-linked factors, but other promoters may require them.

In support of the proposal that mRNA and its processing are important for stable NPC binding, the export-complex component Sac3 and its pore-ligand Nup1 are both needed for relocalization of *GAL1* (REF. 47). Sac3 interacts with the shuttling export factor Mex67, which also affects the stable NPC binding of *GAL10* and *HSP104* (REF. 57). Mex67-dependence might reflect the protein's ability to bind DNA, or its association with a low-level, RNase-inaccessible transcript that would recruit Sac3 and the THO complex, a tetrameric complex composed of Hpr1, Tho2, Mtf1 and Thp2. Together, these mRNA-processing and mRNA-export complexes seem to link the transcribed gene to components of the NPC[47,61]. We conclude that different types of NPC–gene interactions exist; some are mediated by Sus1–Sac3 to Nup1, and others by Mex67 to Mlp1 (FIG. 4). A third pathway seems to involve the histone H2A variant H2A.Z, which is inserted at inducible promoters after activation[55]. In this case, the NPC-retention of *GAL1* and *INO1* is Nup2-dependent and requires the insertion of H2A.Z. Finally, the subtelomeric *HXK1* gene might exploit a fourth pathway involving Med8, a promoter-bound mediator component that interacts with Nup116 (REF. 46).

Generally, the stable association of yeast genes with the NPC seems to occur in two steps. First, promoter-bound factors, including in some cases SAGA and Sus1, promote a transient contact of the gene with pore proteins, which at some promoters is sufficient for relocation. Second, factors that are recruited for transcript processing might stabilize the association and allow the formation of a small single-gene loop[62,63] (FIG. 4). Juxtaposition of promoter and terminator regions resulting in gene looping has been proposed to facilitate transcription reinitiation and thereby increase transcription

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efficiency[63]. The involvement of transcript-processing or quality-control factors in gene anchorage is further supported by the fact that the NPC-association

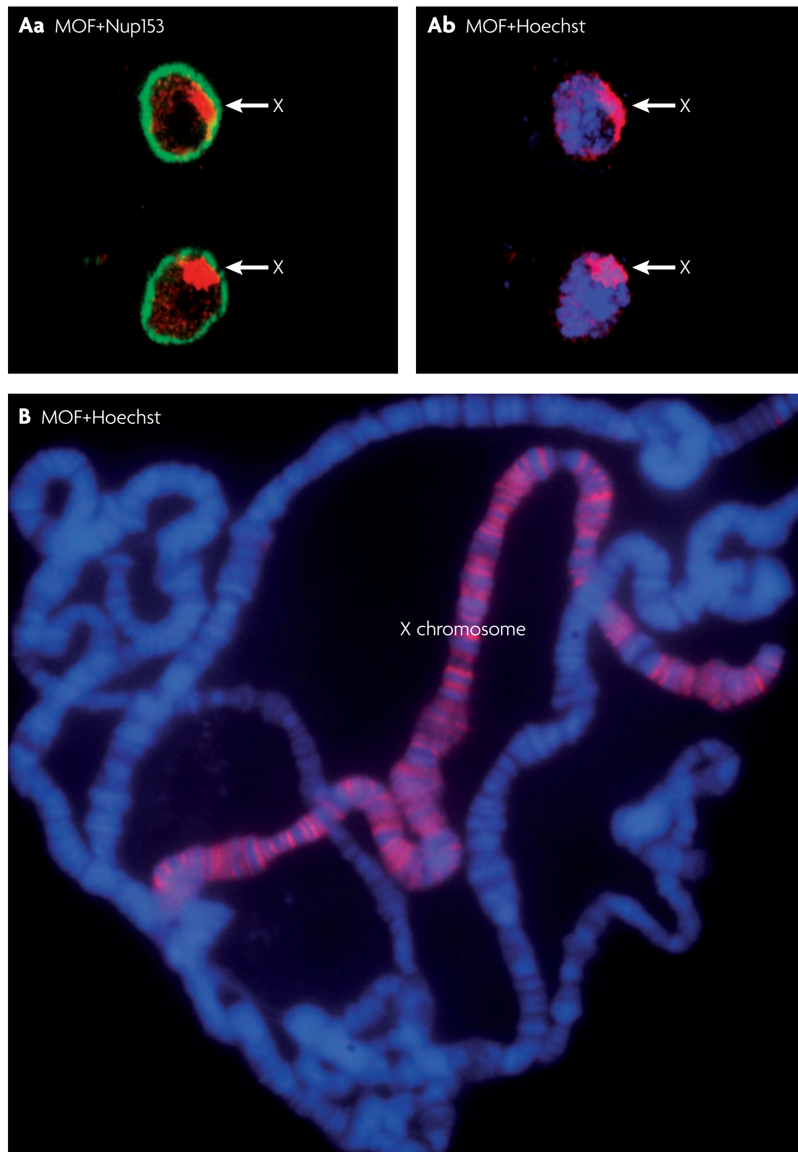

Figure 5 | **The dosage-compensated male X chromosome in *Drosophila melanogaster*.** Dosage compensation of the male X chromosome requires approximately twofold transcriptional activation of X-linked genes in comparison with the female X chromosome, a process that is mediated by the male-specific lethal (MSL) complex (reviewed in REFS 71–73). The X chromosome is immunostained red with an antibody against MOF (Males on absent first), NPC is immunostained green with an antibody against the nucleoporin Nup153, and DNA is stained blue with Hoechst322. **A** | The nuclei of Schneider (SL-2) cells, when immunostained with antibodies against members of the MSL complex (in this case MOF (Males absent on first)), show a distinct X-chromosomal territory within the nucleus. Using confocal microscopy, parts of this X-chromosomal territory appear juxtaposed at or near to the nuclear periphery. Components of the nuclear pore complex (NPC) have been found to be associated with the MSL complex, and are required for dosage compensation of X-linked genes[66]. **B** | Polytene chromosomes isolated from third instar larvae show a distinct binding pattern for the MSL complex on the male X chromosome, as detected by immunostaining with MOF antibody. The MSL complex is enriched on genes along the male X chromosome, with preference for the 3′ end of genes[74–77]. Image courtesy of J. Kind, European Molecular Biology Laboratory, Heidelberg, Germany.

of an activated *GAL1*-reporter persisted for at least 60 minutes after the promoter was switched off[58]. This persistence correlated with the presence of stable mRNA at the pore. Indeed, the persistence of processing and export factors depends on both the transcriptional-elongation rates and the message proof-reading events that precede mRNA export[64,65]. Finally, a recent report argues that the retention of a gene after induction and subsequent repression requires the insertion and recognition of H2A.Z[55]. In this case, NPC binding lasted for seven generations after removal of the inducing conditions.

But do expression levels actually change as a result of NPC association? In two cases, increased transcript levels could be linked to a stable positioning of the gene at the NPC[46,56]; at other genes, however,the impact on transcript accumulation was negligible[47,57,58]. In the first of the positive reports, Brickner and Walter showed that activation of *S. cerevisiae INO1* occurs at the nuclear membrane[56]. In this case, both promoter activation and relocalization required Scs2, an integral membrane protein. By artificially tethering *INO1* at the nuclear periphery, transcriptional activation occurred in the absence of Scs2; in the case of *GAL1*, artificial tethering allowed a more rapid re-induction after an intervening period of repression[55,56]. In a second report, the tagged *HXK1* locus shifted from a telomeric cluster to the nuclear pore upon activation on low glucose[16,46]. When pore binding was averted by targeting a viral transactivator to a region upstream of the gene, the accumulated *HXK1* transcript levels were twofold lower[46]. Finally, much like the results for *INO1* [55,56], *HXK1* induction was rendered twofold more efficient by tethering the gene to the nuclear envelope, this time through a subdomain of Esc1 (REF. 46). Nuclear-envelope-tethering is thought to facilitate transfer to pores; thus, these results indicate that NPC-association can indeed help to fine-tune expression levels.

Conversely, at other inducible loci, mutations that disrupt pore association did not significantly affect levels of accumulated mRNA[47,57,58]. This variation might reflect either the chromatin context in which the reporter gene is found or the strength of the promoter. Strong promoters can overcome a repressive chromatin context but, for weaker promoters, H2A.Z insertion and NPC binding might be needed to insulate the gene from flanking chromatin[9,55].

Known suspects at unexpected places

The association of nuclear pores with chromatin remodelling and gene activation was detected not only in yeast, but by biochemical co-purification in flies. Analogous to the presence of the yeast pore-binding component Sus1 in both the SAGA and mRNA-export complexes, NPC components were recovered in purifications of the male-specific lethal (MSL) complex of both *D. melanogaster* and humans[66]. This complex is involved in the twofold upregulation of X-linked genes in male flies (FIG. 5a,b). In addition to the core MSL complex (which contains MSL1, MSL2, MSL3 and MOF (Males absent on first), the affinity-purified complex contained nuclear-pore

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components MTOR (Megator) and NUP153, and exosome components DIS3 and RRP6. Although these proteins were present in substoichiometric amounts, a depletion of NPC proteins MTOR and NUP153 abrogated the usual MSL staining pattern on the fly X chromosome and compromised dosage compensation of a subset of X-linked genes[66]. Genetic and biochemical studies show that several proteins interact with MSL proteins[66–70], but so far only depletion of MTOR and NUP153 have been shown to cause a significant reduction in MSL staining on the X chromosome[66]. Because MTOR is considered a functional orthologue of the yeast Mlp1, the co-purification of MTOR with MSL parallels the Mex67–Mlp1 link in yeast[17,57].

A speculative link between yeast and flies

What allows the *D. melanogaster* MSL complex to specifically recognize the male X chromosome and produce a twofold transcriptional activation of dosage-compensated genes[71–73] (FIG. 5a,b)? Although recent global chromatin-profiling assays[74–76] have provided clues, the issue remains unresolved. High-resolution ChIP–chip (chromatin immunoprecipitation with tiling arrays) analysis revealed preferential binding of the MSL complex at active genes, with enrichment towards the 3′ ends[74,75]. This 3′ bias resembles the ChIP-determined distribution of yeast Mlp1, which associates with both inducible genes and the NPC[54]. MSL binding is correlated with active genes in both fly embryos and cell lines of embryonic origin[74–76]. Given the co-purification of MSL with MTOR[66], it seems likely that active MSL-bound genes[78,79] are recruited to nuclear pores, even if only transiently. Analogously to the NPC-relocalization of yeast *HXK1*, which required its own 3′ UTR and resulted in a twofold increase in mRNA levels[46], it is possible that NPC association regulates dosage compensation in flies.

What mechanism regulates message level? Although juxtaposition to pores could facilitate mRNA export, the rapid rates of mRNA diffusion within the nucleus argue against subnuclear transport being a rate-limiting event[51]. Rather, it seems likely that transcriptional elongation is crucial in the control of mRNA accumulation[77–79]. By coupling mRNA production with turnover, message accumulation can be precisely regulated. Again using yeast as an example, it has been shown that exosome components Rrp6 and Dis3 (also known as Rrp44) participate at different steps of mRNA maturation and collaborate with the Mlp proteins at other essential steps of gene expression[80,81]. Recent work has linked Rrp6 to the mRNA-export TREX complex[61,82,83], and the exosome components Rrp6 and Dis3 were co-purified with the elongating form of RNA pol II[84]. Because the Rrp6 subunit has been implicated in quality control of mRNP particles at an early step of transcription[82,85,86], it might also contribute to elongation control. By analogy, *D. melanogaster* MSL proteins might bridge the exosome to MTOR, possibly to regulate elongation rates. We propose that, once induced, modulation of transcriptional efficiency occurs at the level of elongation, which is then coordinated with mRNA packaging, scanning and processing[64]. We note that the factors involved in these processes in yeast are precisely those that are implicated in gene–NPC interactions.

Why should the fine-tuning of elongation occur at the nuclear pore? Nuclear pores harbour a range of ubiquitination and SUMOylation enzymes[26,27], which might have an integral role in controlling elongation[87]. Thus, positioning the transcription machinery itself near these regulatory enzymes could control mRNA levels. In addition, the NPC provides a useful scaffolding for gene-loop-forming insulators, possibly ensuring that active domains are separated from adjacent repression zones[9,62,63,65]. Creating an active and stably transcribed gene loop might require that a domain remains in a readily inducible state, which could be achieved by a combination of pore-bound insulator proteins, histone modifications or histone variants such as H2A.Z[55]. This might ensure that MSL-regulated genes need only a transient contact with the NPC in *D. melanogaster*. In yeast, there are multiple pathways for mediating NPC-association but, once positioned, the incorporation of H2A.Z or other histone modifications could ensure that transcription rates are maintained at reproducible levels through mitotic division[55]. If nuclear-pore association can fine-tune gene expression, juxtaposing genes near pores could indeed represent a heritable element of epigenetic control.

Future Directions

It remains to be seen how inner-membrane or pore-complex factors segregate heterochromatin from highly expressed genes, creating distinct zones at the nuclear envelope. Cross-talk between these zones and their mutual relationships to SUMOylating and ubiquitinylating enzymes, or even the proteasome, are still unclear. Not all transcription occurs at nuclear pores, and it is peculiar that stress-inducing conditions alone should favour localization of genes to the NPC. Above all, it remains to be seen whether nuclear pores or other internal structures fulfil a similar function in mammalian cells.

Intriguingly, the mRNA and promoter sequences are not the only determinants for positioning, as different activation pathways can result in different subnuclear localizations for the same locus. This raises the potential for antagonism between activating mechanisms, and it will be exciting to discover how cells or organisms exploit this. Indeed, some genes, such as those in subtelomeric regions or on the X chromosome, could have evolved to use pore association as a mode of regulation. It is unknown whether there are specific pores for specific classes of genes, nor is it clear whether coordinately regulated genes are grouped at selected pores. Finally, given that NPC anchorage can be re-established after mitosis, we assume that positioning might be heritable through cell division. It is unknown which pore-bound components recognize H2A.Z or other histone modifications, and which marks maintain the association to ensure heritable patterns of expression. Just like for silent chromatin maintenance, we expect that there is a logic of heritable gene–NPC association that awaits deciphering.

MSL complex
An RNA–protein complex containing at least five male-specific Lethal (MSL) proteins, MSL1, MSL2, MSL3, MOF and MLE, and two non-coding RNAs, *roX1* and *roX2*. This complex is stably expressed in male flies and regulates dosage compensation of X-linked genes.

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1. Taddei, A., Hediger, F., Neumann, F. R. & Gasser, S. M. The function of nuclear architecture: a genetic approach. *Annu. Rev. Genet*, **38**, 305–345 (2004).
2. Felsenfeld, G. & Groudine, M. Controlling the double helix. *Nature* **421**, 448–453 (2003).
3. Grewal, S. I. & Elgin, S. C. Heterochromatin: new possibilities for the inheritance of structure. *Curr. Opin. Genet. Dev.* **12**, 178–187 (2002).
4. Gilbert, N., Gilchrist, S. & Bickmore, W. A. Chromatin organization in the mammalian nucleus. *Int. Rev. Cytol.* **242**, 283–336 (2005).
5. Fisher, A. G. & Merkenschlager, M. Gene silencing, cell fate and nuclear organisation. *Curr. Opin. Genet. Dev.* **12**, 193–197 (2002).
6. Spector, D. L. The dynamics of chromosome organization and gene regulation. *Annu. Rev. Biochem.* **72**, 573–608 (2003).
7. Kosak, S. T. & Groudine, M. Form follows function: the genomic organization of cellular differentiation. *Genes Dev.* **18**, 1371–1384 (2004).
8. Burgess-Beusse, B. *et al.* The insulation of genes from external enhancers and silencing chromatin. *Proc. Natl Acad. Sci. USA* **99**, S16433–S16437 (2002).
9. Ishii, K., Arib, G., Lin, C., Van Houwe, G. & Laemmli, U. K. Chromatin boundaries in budding yeast: the nuclear pore connection. *Cell* **109**, 551–562 (2002).
10. Noma, K., Cam, H. P., Maraia, R. J. & Grewal, S. I. A role for TFIIIC transcription factor complex in genome organization. *Cell* **125**, 859–872 (2006).
11. Gerasimova, T. I., Byrd, K. & Corces, V. G. A chromatin insulator determines the nuclear localization of DNA. *Mol. Cell* **6**, 1025–1035 (2000).
12. Bantignies, F., Grimaud, C., Lavrov, S., Gabut, M. & Cavalli, G. Inheritance of Polycomb-dependent chromosomal interactions in *Drosophila*. *Genes Dev.* **17**, 2406–2420 (2003).
13. Xu, Q., Li, M., Adams, J. & Cai, H. N. Nuclear location of a chromatin insulator in *Drosophila melanogaster*. *J. Cell Sci.* **117**, 1025–1032 (2004).
14. Yusufzai, T. M., Tagami, H., Nakatani, Y. & Felsenfeld, G. CTCF tethers an insulator to subnuclear sites, suggesting shared insulator mechanisms across species. *Mol. Cell* **13**, 291–298 (2004).
15. Donze, D. & Kamakaka, R. T. RNA polymerase III and RNA pol II promoter complexes are heterochromatin barriers in *Saccharomyces cerevisiae*. *EMBO J.* **20**, 520–531 (2001).
16. Schmid, M. *et al.* Nup–PI: the nucleopore–promoter interaction of genes in yeast. *Mol. Cell* **21**, 379–391 (2006).
This paper uses a technique involving the fusion of a pore protein to MN to show that promoters associate with nuclear pores in yeast.
17. Casolari, J. M. *et al.* Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. *Cell* **117**, 427–439 (2004).
The initial cross-linking study, showing that a range of pore proteins can be crosslinked by formaldehyde to many genes, among which are those induced by galactose.
18. Taddei, A., Hediger, F., Neumann, F. R., Bauer, C. & Gasser, S. M. Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins. *EMBO J.* **23**, 1301–1312 (2004).
Sir4, yKu and Esc1 are able to relocate DNA to the nuclear envelope independently of their silencing function. The authors descibe interdependencies between proteins. Esc1 is found to be co-localized with silent chromatin but distinct from nuclear pores.
19. Gartenberg, M. R. The Sir proteins of *Saccharomyces cerevisiae:* mediators of transcriptional silencing and much more. *Curr. Opin. Microbiol.* **3**, 132–137 (2000).
20. Palladino, F. *et al.* SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres. *Cell* **75**, 543–555 (1993).
21. Gotta, M. *et al.* The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae. J. Cell Biol.* **134**, 1349–1363 (1996).
22. Andrulis, E. D. *et al.* Esc1, a nuclear periphery protein required for Sir4-based plasmid anchoring and partitioning. *Mol. Cell. Biol.* **22**, 8292–8301 (2002).
23. Gartenberg, M. R., Neumann, F. R., Laroche, T., Blaszczyk, M. & Gasser, S. M. Sir-mediated repression can occur independently of chromosomal and subnuclear contexts. *Cell* **119**, 955–967 (2004).
Active and inactive chromatin moves freely through the nucleoplasm once it is released from the chromosome. Sir-mediated silencing is sufficient to anchor DNA at the nuclear envelope, and is not necessary if Sir proteins are dispersed.
24. Hediger, F., Neumann, F. R., Van Houwe, G., Dubrana, K. & Gasser, S. M. Live imaging of telomeres: yKu and Sir proteins define redundant telomere-anchoring pathways in yeast. *Curr. Biol.* **12**, 2076–2089 (2002).
25. Hediger, F., Dubrana, K. & Gasser, S. M. Myosin-like proteins 1 and 2 are not required for silencing or telomere anchoring, but act in the Tel1 pathway of telomere length control. *J. Struct. Biol.* **140**, 79–91 (2002).
26. Zhao, X. & Blobel, G. A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. *Proc. Natl Acad. Sci. USA* **102**, 4777–4782 (2005).
27. Zhao, X., Wu, C. Y. & Blobel, G. Mlp-dependent anchorage and stabilization of a desumoylating enzyme is required to prevent clonal lethality. *J. Cell Biol.* **167**, 605–611 (2004).
28. Hiraga, S., Robertson, E. D. & Donaldson, A. D. The Ctf18 RFC-like complex positions yeast telomeres but does not specify their replication time. *EMBO J.* **25**, 1505–1514 (2006).
29. Maillet, L. *et al.* Evidence for silencing compartments within the yeast nucleus: a role for telomere proximity and Sir protein concentration in silencer-mediated repression. *Genes Dev.* **10**, 1796–1811 (1996).
Telomere foci favour the repression of silencer-bound constructs by sequestering Sir proteins, which are shown to be limiting for repression.
30. Andrulis, E. D., Neiman, A. M., Zappulla, D. C. & Sternglanz, R. Perinuclear localization of chromatin facilitates transcriptional silencing. *Nature* **394**, 592–595 (1998).
Anchoring of a gene to the nuclear envelope can enhance silencer function in yeast.
31. Capell, B. C. & Collins, F. S. Human laminopathies: nuclei gone genetically awry. *Nature Rev. Genet.* **7**, 940–952 (2006).
32. Sullivan, T. *et al.* Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J. Cell Biol.* **147**, 913–920 (1999).
33. Shumaker, D. K. *et al.* Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. *Proc. Natl Acad. Sci. USA* **103**, 8703–8708 (2006).
34. Scaffidi, P. & Misteli, T. Reversal of the cellular phenotype in the premature aging disease Hutchinson–Gilford progeria syndrome. *Nature Med.* **11**, 440–445 (2005).
35. Goldman, R. D. *et al.* Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson–Gilford progeria syndrome. *Proc. Natl Acad. Sci. USA* **101**, 8963–8968 (2004).
36. Liu, J. *et al.* MAN1 and emerin have overlapping function(s) essential for chromosome segregation and cell division in *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **100**, 4598–4603 (2003).
37. Pickersgill, H. *et al.* Characterization of the *Drosophila melanogaster* genome at the nuclear lamina. *Nature Genet.* **38**, 1005–1014 (2006).
This work describes the binding profile of *D. melanogaster* lamins, showing that lamins preferentially bind to transcriptionally inactive genes.
38. Paddy, M. R., Belmont, A. S., Saumweber, H., Agard, D. A. & Sedat, J. W. Interphase nuclear envelope lamins form a discontinuous network that interacts with only a fraction of the chromatin in the nuclear periphery. *Cell* **62**, 89–106 (1990).
39. Marshall, W. F. *et al.* Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr. Biol.* **7**, 930–939 (1997).
The first description of the constrained random walk of LacO-tagged yeast and *D. melanogaster* loci. This work shows that a centromere-proximal locus is constrained in yeast.
40. Heun, P., Laroche, T., Shimada, K., Furrer, P. & Gasser, S. M. Chromosome dynamics in the yeast interphase nucleus. *Science* **294**, 2181–2186 (2001).
Silent telomeres and active non-telomeric loci are shown to have different amounts of spatial constraint in their random-walk movement in the yeast nucleus. This work coupled Nup49–GFP with LacO-tagged loci to measure the absolute movement of genes.
41. Bystricky, K., Heun, P., Gehlen, L., Langowski, J. & Gasser, S. M. Long-range compaction and flexibility of interphase chromatin in budding yeast analyzed by high-resolution imaging techniques. *Proc. Natl Acad. Sci. USA* **101**, 16495–16500 (2004).
42. Heun, P., Laroche, T., Raghuraman, M. K. & Gasser, S. M. The positioning and dynamics of origins of replication in the budding yeast nucleus. *J. Cell Biol.* **152**, 385–400 (2001).
43. Jin, Q., Trelles-Sticken, E., Scherthan, H. & Loidl, J. Yeast nuclei display prominent centromere clustering that is reduced in nondividing cells and in meiotic prophase. *J. Cell Biol.* **141**, 21–29 (1998).
44. Sage, D., Neumann, F. R., Hediger, F., Gasser, S. M. & Unser, M. Automatic tracking of individual fluorescence particles: application to the study of chromosome dynamics. *IEEE Trans. Image Process.* **14**, 1372–1383 (2005).
45. Rosa, A., Maddocks, J. H., Neumann, F. R., Gasser, S. M. & Stasiak, A. Measuring limits of telomere movement on nuclear envelope. *Biophys. J.* **90**, L24–L26 (2006).
46. Taddei, A. *et al.* Nuclear pore association confers optimal expression levels for an inducible yeast gene. *Nature* **441**, 774–778 (2006).
Telomeric foci and pores are distinct functional compartments. A subtelomeric gene shifts from a telomeric focus to a pore upon induction on low glucose. When this relocation is impaired, maximal induction is prevented.
47. Cabal, G. G. *et al.* SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. *Nature* **441**, 770–773 (2006).
Stimulation of galactose-inducible promoters correlates with relocalization to the nuclear periphery, which is mediated by components of the SAGA complex in yeast.
48. Bystricky, K., Laroche, T., van Houwe, G., Blaszczyk, M. & Gasser, S. M. Chromosome looping in yeast: telomere pairing and coordinated movement reflect anchoring efficiency and territorial organization. *J. Cell Biol.* **168**, 375–387 (2005).
49. Marshall, W. F., Fung, J. C. & Sedat, J. W. Deconstructing the nucleus: global architecture from local interactions. *Curr. Opin. Genet. Dev.* **7**, 259–263 (1997).
50. Chubb, J. R., Boyle, S., Perry, P. & Bickmore, W. A. Chromatin motion is constrained by association with nuclear compartments in human cells. *Curr. Biol.* **12**, 439–445 (2002).
51. Janicki, S. M. *et al.* From silencing to gene expression: real-time analysis in single cells. *Cell* **116**, 683–698 (2004).
The first live tracking of an mRNA from a gene to the nuclear pore. The process is rapid and occurs with a random-walk character.
52. Misteli, T. The concept of self-organization in cellular architecture. *J. Cell Biol.* **155**, 181–185 (2001).
53. Menon, B. B. *et al.* Reverse recruitment: the Nup84 nuclear pore subcomplex mediates Rap1/Gcr1/Gcr2 transcriptional activation. *Proc. Natl Acad. Sci. USA* **102**, 5749–5754 (2005).
54. Casolari, J. M., Brown, C. R., Drubin, D. A., Rando, O. J. & Silver, P. A. Developmentally induced changes in transcriptional program alter spatial organization across chromosomes. *Genes Dev.* **19**, 1188–1198 (2005).
55. Brickner, D. G. *et al.* H2A. Z-mediated Localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. *PLoS Biol.* **5**, e81 (2007).
Active genes are retained at the nuclear periphery for several generations. The retention and reactivation requires histone variant H2A.Z. The authors propose that such a mechanism could provide a system by which cells remember the status of the actively transcribed gene through cell generations.
56. Brickner, J. H. & Walter, P. Gene recruitment of the activated *INO1* locus to the nuclear membrane. *PLoS Biol.* **2**, e342 (2004).
Ino1 expression requires contact with the nuclear envelope for promoter activation.

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57. Dieppois, G., Iglesias, N. & Stutz, F. Cotranscriptional recruitment to the mRNA export receptor Mex67p contributes to nuclear pore anchoring of activated genes. *Mol. Cell. Biol.* **26**, 7858–7870 (2006).
58. Abruzzi, K. C., Belostotsky, D. A., Chekanova, J. A., Dower, K. & Rosbash, M. 3′-end formation signals modulate the association of genes with the nuclear periphery as well as mRNP dot formation. *EMBO J.* **25**, 4253–4262 (2006).
3′ UTR sequences and mRNA influence prolonged gene positioning at nuclear pores.
59. Rodriguez-Navarro, S. *et al.* Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery. *Cell* **116**, 75–86 (2004).
60. Fischer, T. *et al.* The mRNA export machinery requires the novel Sac3p–Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. *EMBO J.* **21**, 5843–5852 (2002).
61. Strasser, K. *et al.* TREX is a conserved complex coupling transcription with messenger RNA export. *Nature* **417**, 304–308 (2002).
62. O'Sullivan, J. M. *et al.* Gene loops juxtapose promoters and terminators in yeast. *Nature Genet.* **36**, 1014–1018 (2004).
63. Ansari, A. & Hampsey, M. A role for the CPF 3′-end processing machinery in RNAPII-dependent gene looping. *Genes Dev.* **19**, 2969–2978 (2005).
64. Furger, A., O'Sullivan, J. M., Binnie, A., Lee, B. A. & Proudfoot, N. J. Promoter proximal splice sites enhance transcription. *Genes Dev.* **16**, 2792–2799 (2002).
65. Proudfoot, N. New perspectives on connecting messenger RNA 3′ end formation to transcription. *Curr. Opin. Cell Biol.* **16**, 272–278 (2004).
66. Mendjan, S. *et al.* Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila. Mol. Cell* **21**, 811–823 (2006).
The first report of the novel biochemical and functional link of MTOR and NUP153 to dosage compensation of the male X chromosome in *D. melanogaster*.
67. Jin, Y., Wang, Y., Johansen, J. & Johansen, K. M. JIL-1, a chromosomal kinase implicated in regulation of chromatin structure, associated with the male specific lethal (MSL) dosage compensation complex. *J. Cell Biol.* **149**, 1005–1010 (2000).
68. Deuring, R. *et al.* The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure *in vivo*. *Mol. Cell* **5**, 355–365 (2000).
69. Corona, D. F., Clapier, C. R., Becker, P. B. & Tamkun, J. W. Modulation of ISWI function by site-specific histone acetylation. *EMBO Rep.* **3**, 242–247 (2002).
70. Furuhashi, H., Nakajima, M. & Hirose, S. DNA supercoiling factor contributes to dosage compensation in *Drosophila. Development* **133**, 4475–4483 (2006).
71. Lucchesi, J. C., Kelly, W. G. & Panning, B. Chromatin remodeling in dosage compensation. *Annu Rev Genet* **39**, 615–651 (2005).
72. Mendjan, S. & Akhtar, A. The right dose for every sex. *Chromosoma* **116**, 95–106 (2006).
73. Straub, T. & Becker, P. B. Dosage compensation: the beginning and end of generalization. *Nature Rev. Genet.* **8**, 47–57 (2007).
74. Alekseyenko, A. A., Larschan, E., Lai, W. R., Park, P. J. & Kuroda, M. I. High-resolution ChIP-chip analysis reveals that the *Drosophila* MSL complex selectively identifies active genes on the male X chromosome. *Genes Dev.* **20**, 848–857 (2006).
75. Gilfillan, G. D. *et al.* Chromosome-wide gene-specific targeting of the *Drosophila* dosage compensation complex. *Genes Dev.* **20**, 858–870 (2006).
76. Legube, G., McWeeney, S. K., Lercher, M. J. & Akhtar, A. X-chromosome-wide profiling of MSL-1 distribution and dosage compensation in *Drosophila*. *Genes Dev.* **20**, 871–883 (2006).
77. Smith, E. R., Allis, C. D. & Lucchesi, J. C. Linking global histone acetylation to the transcription enhancement of X-chromosomal genes in *Drosophila* males. *J. Biol. Chem.* **276**, 31483–31486 (2001).
References 74–77 use a ChIP–chip strategy to identify the binding profile of MSL proteins, and show that the MSL complex is enriched on X-linked genes with preferential binding towards the 3′ end of genes.
78. Hamada, F. N., Park, P. J., Gordadze, P. R. & Kuroda, M. I. Global regulation of X chromosomal genes by the MSL complex in *Drosophila melanogaster. Genes Dev.* **19**, 2289–2294 (2005).
79. Straub, T., Gilfillan, G. D., Maier, V. K. & Becker, P. B. The *Drosophila* MSL complex activates the transcription of target genes. *Genes Dev.* **19**, 2284–2288 (2005).
References 78 and 79 collectively show that transcription of MSL-bound X-linked genes is affected by depletion of MSL2 in *D. melanogaster* cells.
80. Galy, V. *et al.* Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1. *Cell* **116**, 63–73 (2004).
81. Sommer, P. & Nehrbass, U. Quality control of messenger ribonucleoprotein particles in the nucleus and at the pore. *Curr. Opin. Cell Biol.* **17**, 294–301 (2005).
82. Libri, D. *et al.* Interactions between mRNA export commitment, 3′-end quality control, and nuclear degradation. *Mol. Cell Biol.* **22**, 8254–8266 (2002).
83. Zenklusen, D., Vinciguerra, P., Wyss, J. C. & Stutz, F. Stable mRNP formation and export require cotranscriptional recruitment of the mRNA export factors Yra1p and Sub2p by Hpr1p. *Mol. Cell. Biol*. **22**, 8241–8253 (2002).
84. Andrulis, E. D. *et al.* The RNA processing exosome is linked to elongating RNA polymerase II in *Drosophila*. *Nature* **420**, 837–841 (2002).
This work describes the co-purification of the RNA pol II complex with components of the nuclear exosome, thus linking the RNA-processing machinery with the transcription machinery.
85. Hieronymus, H., Yu, M. C. & Silver, P. A. Genome-wide mRNA surveillance is coupled to mRNA export. *Genes Dev.* **18**, 2652–2662 (2004).
86. Hilleren, P., McCarthy, T., Rosbash, M., Parker, R. & Jensen, T. H. Quality control of mRNA 3′-end processing is linked to the nuclear exosome. *Nature* **413**, 538–542 (2001).
87. Herrera, F. J. & Triezenberg, S. J. Molecular biology: what ubiquitin can do for transcription. *Curr. Biol.* **14**, R622–R624 (2004).
88. Vazquez, J., Belmont, A. S. & Sedat, J. W. The dynamics of homologous chromosome pairing during male *Drosophila* meiosis. *Curr. Biol.* **12**, 1473–1483 (2002).
89. Cremer, T. & Cremer, C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nature Rev. Genet.* **2**, 292–301 (2001).
90. Lanctot, C., Cheutin, T., Cremer, M., Cavalli, G. & Cremer, T. Dynamic genome architecture in the nuclear space: regulation of gene expression in three dimensions. *Nature Rev. Genet.* **8**, 104–115 (2007).
91. Chambeyron, S. & Bickmore, W. A. Chromatin decondensation and nuclear reorganization of the *HoxB* locus upon induction of transcription. *Genes Dev.* **18**, 1119–1130 (2004).
92. Chambeyron, S., Da Silva, N. R., Lawson, K. A. & Bickmore, W. A. Nuclear re-organisation of the HOXB complex during mouse embryonic development. *Development* **132**, 2215–2223 (2005).
93. Morey, C., Da Silva, N. R., Perry, P. & Bickmore, W. A. Nuclear reorganisation and chromatin decondensation are conserved, but distinct, mechanisms linked to Hox gene activation. *Development* **134**, 909–919 (2007).
94. Tran, E. J. & Wente, S. R. Dynamic nuclear pore complexes: life on the edge. *Cell* **125**, 1041–1053 (2006).
95. Vinciguerra, P., Iglesias, N., Camblong, J., Zenklusen, D. & Stutz, F. Perinuclear Mlp proteins downregulate gene expression in response to a defect in mRNA export. *EMBO J.* **24**, 813–823 (2005).

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Competing interest statement

The authors declare no competing financial interests.

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