Review

Nuclear dynamics: where genes are and how they got there

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Abstract

DNA is highly organized spatially, both within domains of chromatin along each chromosome and within the nucleus as a whole. Recent studies suggest that chromatin localization can affect transcriptional and replicational activity. The similarity between the movements of chromatin nuclear bodies suggests a common mechanism that regulates nuclear dynamics.

We have grown used to the linear representation of the genome as a cluster of coloured lines representing different chromosomes or sections thereof. Unfortunately, for those of us studying its properties, the structure of the genome *in vivo* is far more complicated. Each chromosome is a complex of protein and nucleic acid, assembled into higher-order chromatin structures and packed into the cell nucleus. It is this chromatin complex that is the substrate for transcription, replication, damage repair and recombination. But chromatin can exist in at least two forms, termed 'open' and 'closed', that differ in their accessibility to nuclear factors including transcriptional activators and the replication machinery. It now appears that processes governing the opening of chromatin may play key regulatory roles in the nucleus. Recent studies have shown that loci do indeed expand upon transcriptional activation [1,2]. Moreover, protein machines and post-translational modifications have been identified that are involved in mediating structural changes in chromatin [3-5].

It is likely that nuclear architecture also influences chromatin structure, just as cytoplasmic organelles are affected by their localization. Efforts to understand the architecture of the nucleus have exposed a remarkable level of organization of chromatin. Individual chromosomes are seen to occupy separate, non-overlapping territories in the interphase nucleus [6-9]. Moreover, a number of nuclear bodies are now known, usually identified by the presence of specific molecular markers and formed in the nucleoplasm without the use of membrane barriers [10,11]. These bodies may be sites where specific nuclear activities take place; for example, the production of ribosomal subunits takes place in the nucleolus.

The nucleus is a dynamic organelle. Its partitioning into different compartments, each formed without membrane boundaries, suggests that the nucleus may use a different organizing principle than that of much of the cytoplasm. It also implies that there is a transport system to deliver factors to their appropriate targets and to move macromolecules between compartments, in addition to the tremendous flux of macromolecules entering and leaving the nucleus through the nuclear pores. So, how do things localize and move in the nucleus? In this review, we discuss the positioning and movement of nuclear components and how these processes may impinge on critical nuclear functions.

Chromatin positioning

There is now a wealth of information about the molecular interactions involved in regulating gene expression and DNA replication, and it is clear that large structural changes occur in chromatin as a consequence of changes in transcriptional or replicational activity [12]. It is now commonly accepted that

chromatin loci that are separated by many kilobases can nevertheless make contact and functionally interact. Changes in higher-order chromatin structure are important for function, although we don't yet understand the mechanisms involved.

A good example of the relationship between function and nuclear location is provided by the inactivated human X chromosome, or Barr body. In human females, the inactive X chromosome is maintained in a condensed, heterochromatic state and predominantly positioned at the periphery of the nucleus, juxtaposed to the nuclear envelope. Transcriptionally inactive heterochromatin is also often observed clustered along the nuclear envelope. Does the location of inactive heterochromatin reflect a regulated localization of chromatin to a specific subnuclear domain? It is possible that parts of chromosomes (or as in the case of the Barr body, whole chromosomes) that are repressed are localized to the periphery of the nucleus because they are simply excluded from the more interior portion. Recent evidence suggests, however, that the localization of chromatin loci is a regulated process with profound consequences for their activity. The key to these observations has been the combination of techniques for fluorescence *in situ* hybridization (FISH) that preserve the structure of the nucleus and high-resolution, multi-colour fluorescence microscopy.

The gene density in human chromosomes is known to vary widely. Bickmore and colleagues [13] have explored the location of gene-rich and gene-poor chromosomes in human cells. Using FISH techniques that 'paint' whole chromosomes, they have visualized the relative position of chromosomes in the nucleus and correlated these with transcriptional activity. A statistical analysis of the positions of the relatively gene-poor human chromosome 18 and the gene-rich chromosome 19 showed that chromosome 18 was preferentially located near the nuclear periphery. After inhibiting transcription, the preferential localization of chromosome 18 to the nuclear periphery was no longer observed. These data suggest that chromosome location can be dependent upon active transcription. Most recently, the same workers have examined the disposition of all human chromosomes in the interphase nucleus [14], and the results reinforce the inverse correlation between proximity to the nuclear periphery and gene density.

It is interesting to compare these results with previous observations showing that late-replicating chromatin is preferentially located at the nuclear periphery (Figure 1) and that high levels of transcription and of acetylated histone H4 are found in the nuclear interior [15,16]. These domains are stably maintained through a number of cell cycles, showing that they are a fundamental aspect of nuclear organization [9,16]. Collectively, these data suggest that in the crowded milieu of the nucleus, chromosome position may be finely tuned and dependent on transcription, replication, and possibly other nuclear activities.

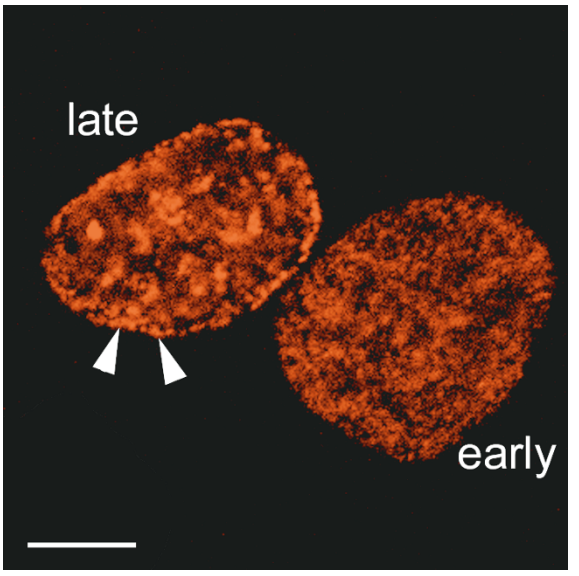

Figure 1
Timing of replication correlates with chromatin location. HeLa cells were briefly pulsed with Cy3-dUTP to mark actively replicating regions of chromatin, then fixed and imaged on a fluorescence microscope. The cell on the left is late in S-phase and preferentially replicating heterochromatic regions, with those in the nuclear periphery marked by arrowheads. The cell on the right is at an earlier stage of S phase, when predominantly internal chromatin is replicated. See [15] and references therein for further details. Scale bar = 5 μm.

If domains of heterochromatin are specifically localized to defined regions within the nucleus, this implies that both *cis*- and *trans*-acting factors must mediate these events. It has been suggested that the nuclear periphery may be repressive by nature and may contain some factors that promote heterochromatin formation. Indeed, in yeast, localization of telomeres to the nuclear periphery requires the presence of the Sir proteins, factors that mediate transcriptional silencing at the telomere [8,17]. The requirement for specific factors that mediate heterochromatin formation and localization suggests that localization is unlikely to be an entirely passive event.

Position-effect variegation

The phenomenon of position-effect variegation (PEV) provides further evidence that the localization of a chromatin locus has strong consequences for its activity, and that proximity to heterochromatin directly represses transcription. PEV was first observed in *Drosophila*, where chromosome rearrangements caused repression of a number of genes by moving them close to a large block of centric heterochromatin [18]. A classic example involves an inversion of the X chromosome that moves the *white* gene to a location near a large block of centric heterochromatin. Expression from the relocated *white* gene was usually repressed, but the level of repression differed between groups of clonally related

cells, generating a variegated phenotype. The overall level of repression also depended on the proximity of *white* to heterochromatin: inversions that placed *white* close to heterochromatin repress more than those that put *white* farther away. This *cis*-inactivation might occur by the spreading of transcription-silencing factors from heterochromatin into the *white* gene [19]. Alternatively, the proximity to heterochromatin of *white* on the inverted X might expose *white* to a repressive environment, perhaps mediated by a cluster of heterochromatin [20].

A clue to the mechanism of PEV came from studies on the *Drosophila brown* gene and a *trans*-inactivation phenomenon. In *Drosophila*, homologous chromosomes pair in somatic cells, allowing the localization of one homolog to affect the other. The *brown-Dominant* allele (bw^D) contains a 1-2 Mb insertion of heterochromatin into the *brown* gene [21]. In a heterozygous animal bearing one wild-type and one bw^D allele (bw^D/+), transcription from the wild-type allele is repressed. In cells bearing two copies of the wild-type allele, FISH analysis showed that both loci were localized away from the nuclear periphery. By contrast, in bw^D/+ flies, both alleles were clustered together on the nuclear periphery in the vicinity of heterochromatin [22,23]. These studies demonstrated that the location of a locus could move. Moreover, they suggest that one part of heterochromatin-mediated repression includes the recruitment of a locus to a heterochromatin zone of the nucleus. In this model, a gene proximal to heterochromatin in linear sequence is guided to a heterochromatic repressive zone, possibly by interactions between separate blocks of heterochromatin [24]. Furthermore, interactions of specific loci with heterochromatin can be transient.

The studies on bw^D suggest that the insertion of DNA sequences that form heterochromatin near to a gene can influence the gene's activity and location. What might these *cis*-acting sequences be? The recent development of synthetic compounds, such as oligomers of pyrrole and imidazole amino acids that bind to specific repetitive tracts of DNA with high affinity, has provided a biochemical tool for probing the effects of heterochromatin structure [25]. These compounds make heterochromatin more accessible to nucleases, presumably by making it more 'open', and they also show interesting effects *in vivo*. For example, *Drosophila* fed these compounds are viable, but show limited homeotic transformations. Moreover, the compounds also reverse the effects of heterochromatin silencing of both the *white* gene and the *brown* locus in bw^D/+ flies [26]. It will be interesting to determine the effects of drugs affecting heterochromatin on the localization of specific gene loci. It is also possible that drugs targeted to other heterochromatic regions (such as telomeres) might be developed in future.

How universal is the regulated localization of chromatin loci in interphase? Since the initial observations in *Drosophila*, targeted localization of heterochromatic sequences has been observed in fission yeast [27], mice, and humans, suggesting that it is does not depend on the somatic pairing of homologs that occurs in insects but is, in fact, a general phenomenon. In humans, the Ikaros DNA-binding protein is required for gene regulation during the development of B and T cells in the immune system [28]. Interestingly, Ikaros localizes to pericentric heterochromatin [29]. It has been proposed that it regulates gene activity by recruiting the loci it binds to the repressive environment of pericentric heterochromatin [30]. Ikaros localization depends on two Zn-finger motifs, suggesting that it associates with pericentric heterochromatin by directly binding DNA [31]. Recent studies have identified a homolog of Ikaros, named Helios, that forms a heterodimer with Ikaros and also localizes to centric heterochromatin [32]. In addition, at least part of the cellular pool of Ikaros is complexed to NURD, a chromatin-remodelling complex, suggesting that Ikaros function may also involve the remodelling of chromatin [33,34].

In summary, there is suggestive evidence that directed localization of chromatin loci is one mechanism for regulating gene expression within the nucleus. Targeted localization can be either permanent or transient. Although it is still unclear how general this process might be, it raises a number of important questions concerning the movement of chromatin domains in the nucleus. For example, do specific chromatin loci differ in their dynamic behaviour? And how rapidly can a chromatin locus move, and what factors mediate the movement of loci?

Chromatin dynamics

Direct methods have recently been used in living cells to analyse chromatin movements. The technique of marking a chromosome with a tandemly repeated array of *lacO* (transcription-factor-binding) sites and expression of a fusion protein made up of green fluorescent protein (GFP) and *lacI* (the cognate transcription factor) provides a way of detecting a specific chromatin locus in a live cell [35]. Once integrated into the *Saccharomyces cerevisiae* genome, the position of the marked locus can be tracked over time [36,37]. A plot of the distance a locus travels as a function of time provides a measure of the type of motion, whether it is passive or active, and its characteristics**.** Results of this type of study showed that yeast chromatin diffuses passively with a diffusion constant $D = 5 \times 10^{-12}$ cm²/sec within a confined nuclear subvolume with a radius $R = 0.3$ μm [36]. Treatment of the cells with sodium azide to inhibit ATP synthesis via respiratory electron transport caused little change in these measurements, suggesting that chromatin diffusion does not require ATP hydrolysis. A similar analysis in *Drosophila* embryonic cells, using a mark in the chromatin generated by the localization of fluorescently labeled DNA topoisomerase II to a heterochromatic region of the X chromosome, gave similar values ($D = 2 \times 10^{-11}$ cm²/sec;

R = 0.9 μm) [36]. An analysis of chromatin movement in living human cells, using Cy3-dUTP-labeled DNA, found similar characteristics [38]. Taken together, these results suggest that bulk chromatin passively diffuses within the nucleus and that, in general, the volume within which it moves is quite limited. We note that these observations do not necessarily rule out the presence of specific paths that nuclear components could move along [39]; but a 'track-like' mode of movement does not appear to be the dominant mechanism used by chromatin or other nuclear bodies so far examined (see below).

It is instructive to consider the scale of these motions relative to free molecules and other nuclear components. Recent studies have used fluorescence recovery after photobleaching (FRAP), a technique in which fluorescence is initially bleached in a spot using a focused laser beam and the subsequent recovery of fluorescence is measured as a reporter of the diffusion of unbleached molecules into the bleached zone. The nuclear diffusion constants of GFP fused with the pre-mRNA splicing factor SF2/ASF, the chromatin-binding protein HMG-17, and the nucleolar protein fibrillarin, were measured in living cells using FRAP [40]. Diffusion constants for all three proteins were found to be approximately 10^{-9} cm²/sec, significantly faster than that measured for chromatin. A diffusion constant of 10^{-9} cm²/sec was also obtained for polyadenylated RNA in living cells [41] and again, no evidence was found for energy-dependent mobility.

We have discussed (above) results indicating that repression of transcriptional activity can be correlated with the location of a chromatin locus. This highlights the importance of analysing the localization of a single locus during transcriptional activation. Recently, Tumbar and Belmont [42] have accomplished this in living cells using a stable cell line bearing 10-20 tandemly repeated copies of the dihydrofolate reductase gene *DHFR* fused to *lacO* and also expressing GFP-*lacI* or GFP-*lacI* fused to the acidic activation domain of VP16 (GFP-VP16-AAD). In cells expressing GFP-*lacI*, the *DHFR-lacO* repeat was seen localized to the nuclear periphery in around 50% of cells examined. In the presence of GFP-VP16-AAD, only about 30% of cells showed peripheral localization. These data suggest that transcriptional activation of a peripheral locus can change its position in the nucleus, at least in a proportion of cells.

Taken together, these results provide a picture of a nucleus in which no components are immobile, but where proteins and other macromolecular complexes rapidly diffuse around, and perhaps within, slower moving, yet still mobile, chromatin. It should be borne in mind, however, that methods such as FRAP assay bulk populations of molecules and so may not identify small subpopulations of molecules with significantly different properties. Thus, even although passive diffusion may be the predominant mode of movement in the nucleus, it cannot be excluded that some factors can move by energy-dependent mechanisms.

If bulk chromatin movement is largely random and well confined, then two events are required to localize a chromatin locus specifically into a regulatory nuclear subcompartment. First, the locus must be liberated from a confined volume - the restriction to its mobility must be removed - and second, a specificity mechanism must be available to target the locus to a specific subcompartment. In view of the large amount of data suggesting passive diffusion mechanisms, specificity probably comes from the binding of chromatin-associated factors, either to one another or to other nuclear components or receptors. This may be similar to the known mechanism of nuclear protein export that involves the selective transport of protein export cargoes to the nuclear pore complex, mediated by the nuclear export receptor Crm1 [43].

When might such movements be detectable? During S phase, all chromatin is disassembled and DNA is replicated and then reassembled into chromatin. This period of disassembly might be associated with increased chromatin dynamics and therefore might afford a chance for chromatin to redistribute within the nucleus. Indeed, in *Drosophila* the association of heterochromatic clusters has been shown to change during S phase [44]. In mammalian cells, analysis of the localization of an inserted late-replicating heterochromatic region showed movement from the nuclear periphery to the nuclear interior that correlated with late S phase [45]. A recent careful study in budding yeast revealed that late-firing replication origins were clustered on the nuclear periphery in G1 [46]. As cells advanced into S phase, late-firing origins lost this constraint and moved into the nuclear interior, apparently before the actual firing of the origin [46]. These data all show that large movements of specific chromatin loci can occur during S phase. It will be interesting therefore to extend the analysis of the dynamic properties of chromatin to see whether large-scale chromatin movements play an important role at other stages of the cell cycle.

Nuclear body dynamics

As well as chromatin, there are different types of nuclear bodies, including nucleoli, speckles, Cajal bodies (formerly called coiled bodies; CBs) and PML bodies, each of which have distinct molecular components and are probably carrying out specific functions (Figure 2) [10,11]. Given the observed mobility of chromatin, it is not surprising to discover that these nuclear organelles are mobile as well. For example, time-lapse analysis following transient expression of a GFP fusion to the splicing factor ASF/SF2 showed that the speckled domains containing these factors were dynamic and could dramatically change shape [47,48]. Changes in the shape of speckled domains were inhibited by

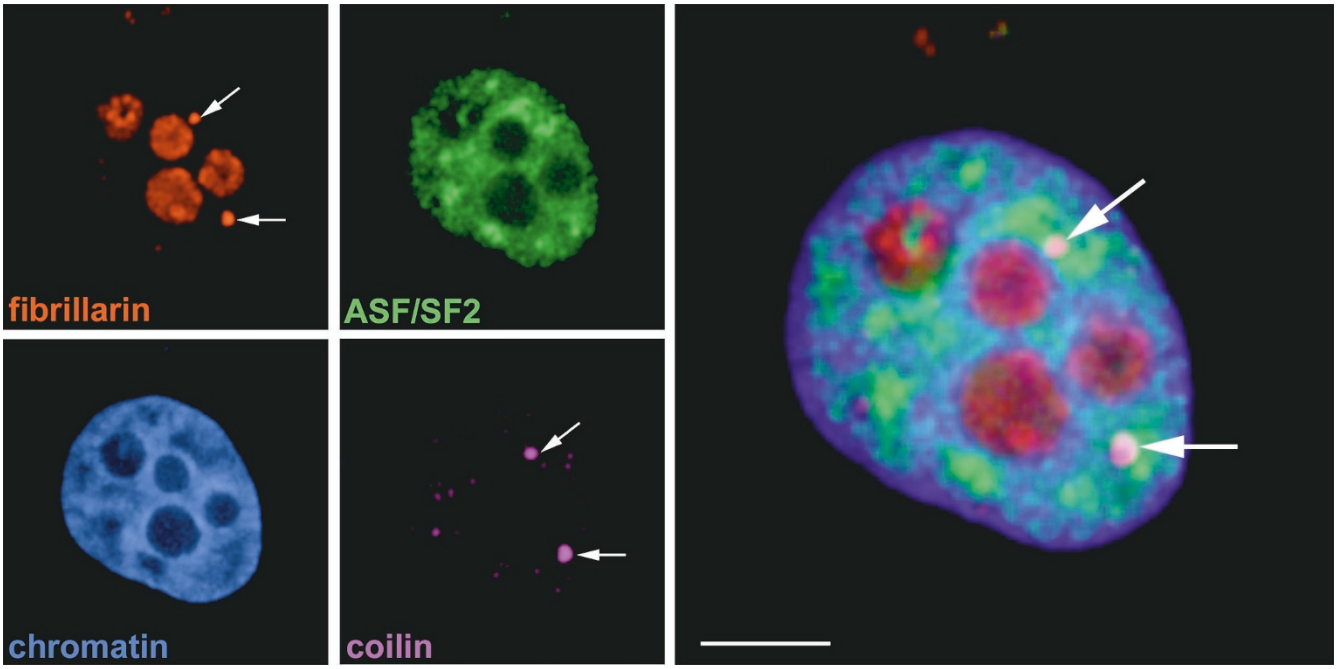

Figure 2
Chromatin and nuclear bodies. The micrograph shows the separate detection in the same HeLa cell nucleus of bulk chromatin (blue), the nucleolar protein fibrillarin (red), the RNA-splicing factor ASF/SF2 (green), and the Cajal body (formerly coiled body) autoantigen p80^{coilin} (magenta). The right-hand panel shows an overlay of the four separate signals. Nuclear Cajal bodies contain both p80^{coilin} and fibrillarin (arrows). ASF/SF2 localizes in nuclear speckles. Scale bar = 5 μm.

treatment of cells with α-amanitin, an inhibitor of transcription by RNA polymerase II. This suggests that dynamic changes in speckled domains were correlated with RNA polymerase II activity, and it was proposed that splicing factors can shuttle between speckles and sites of transcription [47,48].

More recently, time-lapse movies of a stable HeLa cell line expressing GFP fused to the CB marker protein p80^{coilin} have revealed that these can be remarkably dynamic structures. CBs traverse the nucleus and undergo both joining and splitting reactions [49]. CBs could also be detected moving to and from the periphery of nucleoli and rates of movement up to about 1 μm/min were recorded. CB movement has also been detected in HeLa cells expressing GFP fused to the nucleolar protein fibrillarin [49,50] and in the nuclei of plant cells expressing a GFP-fused snRNP protein [51]. The rate of CB movement changes markedly over time. CBs show transitions between making small movements that are apparently confined in a local subvolume and larger movements that involve their transiting more rapidly to more distant sites elsewhere in the nucleus. This behaviour of CBs is consistent with a two-step system for nuclear mobility, where constrained mobility is first relaxed, allowing the bodies to diffuse until they reach a new target or confined location. CBs have been seen to be transiently associated with many different transcriptional loci, suggesting that one mechanism for confining CB mobility may be association with chromatin [52-56]. It is interesting to compare this putative mechanism with the proposed mode of chromatin targeting discussed above, and it will be important to test in future whether a two-step release and target model may provide a general way of organizing large-scale nuclear structures.

In summary, recent studies have underlined the probable importance of regulated localization of specific chromatin loci and nuclear bodies as a way of modulating their function. In this review we have considered the recent data describing the dynamic behaviour of chromatin loci and nuclear bodies. We have discussed a general, two-step model that attempts to account for the movements of the large-scale nuclear structures that have been observed to date (Figure 3). A prediction of this model is that future analysis of the movements of chromatin loci and nuclear bodies will reveal marked changes either in diffusion rates, or in constrained volumes, as they undergo transitions from freely diffusing to tethered or constrained states, and vice versa. A major goal for future studies will be to understand the mechanisms involved in both moving and targeting large nuclear structures. It will be equally important to understand what mechanisms restrict their movement and tether them to specific locations. This will involve the establishment of better assay systems for detecting movement and the identification and characterization of nuclear factors that mediate targeted localization.

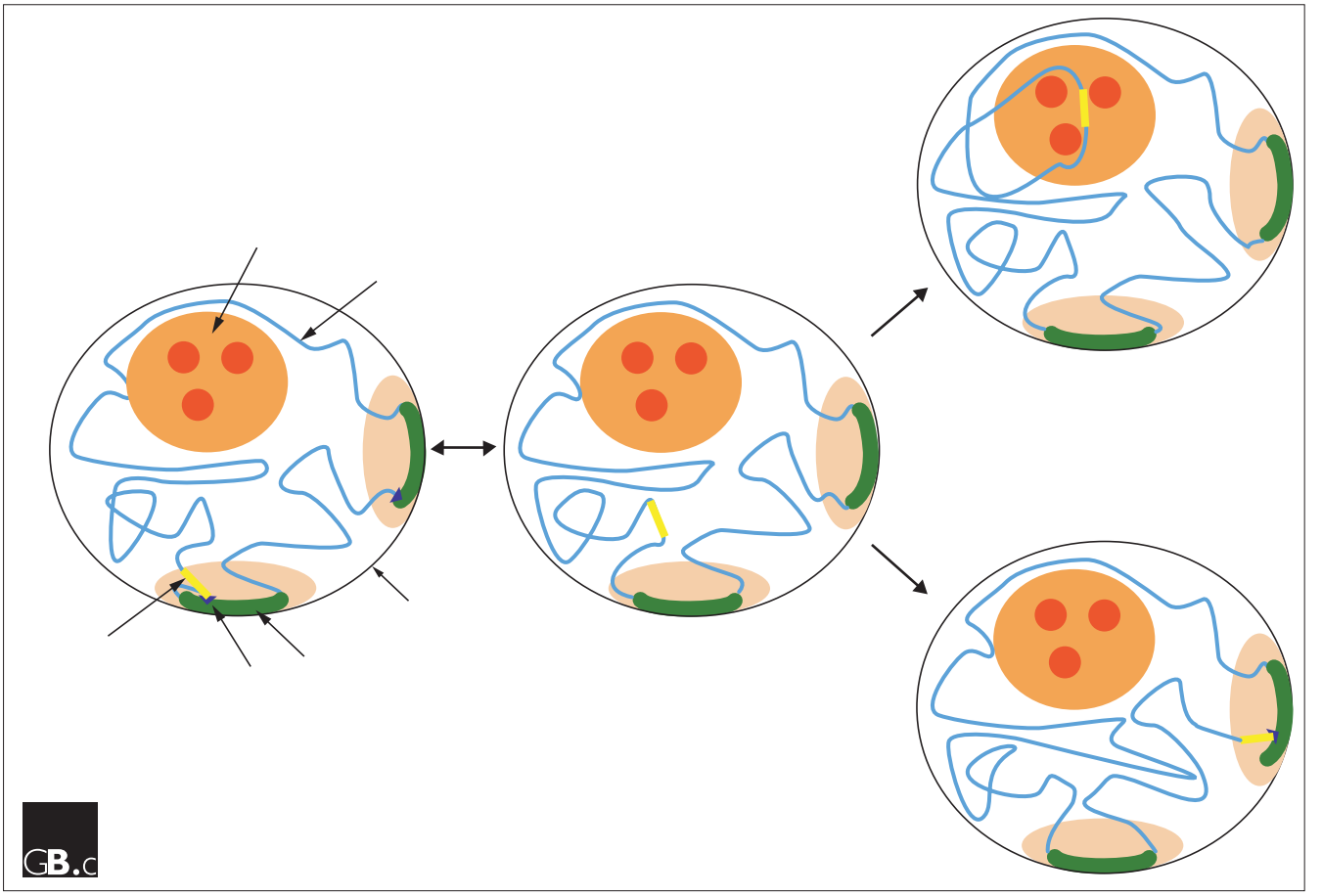

Figure 3
A simplified representation of a model for nuclear dynamics. **(a)** Chromatin loci and nuclear bodies are constrained within a nuclear subvolume, either by tethering to another structure or by physical boundaries to their movements. **(b)** Movement of a locus or nuclear body to a new location entails, first, relaxation of tethering or removal of the physical barrier. This is followed by transit to one or more new sites in the nucleus, and transit ends as a result of either binding via specific factors at a new location or encountering a new physical constraint. The new site can place the locus in either an active **(c)** or a repressive (heterochromatin) **(d)** domain. Current evidence suggests that movement between compartments is likely to be the result of passive diffusion, although at present we cannot exclude the possibility that energy-mediated movements might also occur.

The further development of new and sensitive microscopy techniques to analyse the properties of fluorescently tagged fusion proteins will continue to provide insights into the dynamic properties of the nucleus in living cells. In our opinion, it will be of particular importance to focus such studies on the dynamics of single particles and specific chromosomal loci in living cells. Systems are now available to allow visualization of the events associated with the activation of a specific locus in a living cell [1,42]. While the focus of these studies so far has been on the events associated with transcriptional activation, it will be important to look in future also at specific repression events. We anticipate that quantitative analysis of nuclear dynamics will provide a much clearer picture of how structures are localized and moved around within the nucleus, and should improve our understanding of the effects of such movement on gene expression and replication.

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References
1. Tsukamoto T, Hashiguchi N, Janicki SM, Tumbar T, Belmont AS, Spector DL: **Visualization of gene activity in living cells.** *Nat Cell Biol* 2000, **2**:871-878.
2. Tumbar T, Sudlow G, Belmont AS: **Large-scale chromatin unfolding and remodeling induced by VP16 acidic activation domain.** *J Cell Biol* 1999, **145**:1341-1354.
3. Cheung P, Allis CD, Sassone-Corsi P: **Signaling to chromatin through histone modifications.** *Cell* 2000, **103**:263-271.
4. Sudarsanam P, Winston F: **The Swi/Snf family nucleosome-remodeling complexes and transcriptional control.** *Trends Genet* 2000, **16**:345-351.
5. Pirrotta V: **Polycombing the genome: PcG, trxG, and chromatin silencing.** *Cell* 1998, **93**:333-336.

6. Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC: **Delineation of individual human chromosomes in metaphase and interphase cells by *in situ* suppression hybridization using recombinant DNA libraries.** *Hum Genet* 1988, **80**:224-234.
7. Schardin M, Cremer T, Hager HD, Lang M: **Specific staining of human chromosomes in Chinese hamster x man hybrid cell lines demonstrates interphase chromosome territories.** *Hum Genet* 1985, **71**:281-287.
8. Cockell M, Gasser SM: **Nuclear compartments and gene regulation.** *Curr Opin Genet Dev* 1999, **9**:199-205.
9. Zink D, Cremer T, Saffrich R, Fischer R, Trendelenburg MF, Ansorge W, Stelzer EH: **Structure and dynamics of human interphase chromosome territories *in vivo*.** *Hum Genet* 1998, **102**:241-251.
10. Lamond AI, Earnshaw WC: **Structure and function in the nucleus.** *Science* 1998, **280**:547-553.
11. Matera AG: **Nuclear bodies: multifaceted subdomains of the interchromatin space.** *Trends Cell Biol* 1999, **9**:302-309.
12. Felsenfeld G: **Chromatin unfolds.** *Cell* 1996, **86**:13-19.
13. Croft JA, Bridger JM, Boyle S, Perry P, Teague P, Bickmore WA: **Differences in the localization and morphology of chromosomes in the human nucleus.** *J Cell Biol* 1999, **145**:1119-1131.
14. Boyle S, Gilchrist S, Bridger JM, Mahy NL, Ellis JA, Bickmore WA: **The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells.** *Hum Mol Genet* 2001, **10**:211-219.
15. Ferreira J, Paolella G, Ramos C, Lamond AI: **Spatial organization of large-scale chromatin domains in the nucleus: a magnified view of single chromosome territories.** *J Cell Biol* 1997, **139**:1597-1610.
16. Sadoni N, Langer S, Fauth C, Bernardi G, Cremer T, Turner BM, Zink D: **Nuclear organization of mammalian genomes. Polar chromosome territories build up functionally distinct higher order compartments.** *J Cell Biol* 1999, **146**:1211-1226.
17. Palladino F, Laroche T, Gilson E, Axelrod A, Pillus L, Gasser SM: **SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres.** *Cell* 1993, **75**:543-555.
18. Schultz J: **Variegation in *Drosophila* and the inert chromosome regions.** *Proc Natl Acad Sci USA* 1936, **22**:27-33.
19. Tartof KD, Hobbs C, Jones M: **A structural basis for variegating position effects.** *Cell* 1984, **37**:869-878.
20. Henikoff S: **A reconsideration of the mechanism of position effect.** *Genetics* 1994, **138**:1-5.
21. Henikoff S, Jackson JM , Talbert PB: **Distance and pairing effects on the *brown*^{Dominant} heterochromatic element in *Drosophila*.** *Genetics* 1995, **140**:1007-1017.
22. Csink AK, Henikoff S: **Genetic modification of heterochromatic association and nuclear organization in *Drosophila*.** *Nature* 1996, **381**:529-531.
23. Dernburg AF, Broman KW, Fung JC, Marshall WF, Philips J, Agard DA and Sedat JW: **Perturbation of nuclear architecture by long-distance chromosome interactions.** *Cell* 1996, **85**:745-759.
24. Talbert PB, Henikoff S: **A reexamination of spreading of position-effect variegation in the *white-roughest* region of *Drosophila melanogaster*.** *Genetics* 2000, **154**:259-272.
25. Janssen S, Durussel T, Laemmli UK: **Chromatin opening of DNA satellites by targeted sequence-specific drugs.** *Mol Cell* 2000, **6**:999-1011.
26. Janssen S, Cuvier O, Muller M, Laemmli UK: **Specific gain- and loss-of-function phenotypes induced by satellite-specific DNA-binding drugs fed to *Drosophila melanogaster*.** *Mol Cell* 2000, **6**:1013-1024.
27. Grunstein M: **Yeast heterochromatin: regulation of its assembly and inheritance by histones.** *Cell* 1998, **93**:325-328.
28. O'Riordan M, Grosschedl R: **Transcriptional regulation of early B-lymphocyte differentiation.** *Immunol Rev* 2000, **175**:94-103.
29. Brown KE, Guest SS, Smale ST, Hahm K, Merkenschlager M, Fisher AG: **Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin.** *Cell* 1997, **91**:845-854.
30. Brown KE, Baxter J, Graf D, Merkenschlager M, Fisher AG: **Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division.** *Mol Cell* 1999, **3**:207-217.
31. Cobb BS, Morales-Alcelay S, Kleiger G, Brown KE, Fisher AG, Smale ST: **Targeting of Ikaros to pericentromeric heterochromatin by direct DNA binding.** *Genes Dev* 2000, **14**:2146-2160.
32. Hahm K, Cobb BS, McCarty AS, Brown KE, Klug CA, Lee R, Akashi K, Weissman IL, Fisher AG, Smale ST: **Helios, a T cell-restricted Ikaros family member that quantitatively associates with Ikaros at centromeric heterochromatin.** *Genes Dev* 1998, **12**:782-796.
33. O'Neill DW, Schoetz SS, Lopez RA, Castle M, Rabinowitz L, Shor E, Krawchuk D, Goll MG, Renz M, Seelig HP, *et al*: **An ikaros-containing chromatin-remodeling complex in adult-type erythroid cells.** *Mol Cell Biol* 2000, **20**:7572-7582.
34. Kim J, Sif S, Jones B, Jackson A, Koipally J, Heller E, Winandy S, Viel A, Sawyer A, Ikeda T, Kingston R, Georgopoulos K: **Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes.** *Immunity* 1999, **10**:345-355.
35. Belmont AS, Straight AF: ***In vivo* visualization of chromosomes using lac operator-repressor binding.** *Trends Cell Biol* 1998, **8**:121-124.
36. Marshall WF, Straight A, Marko JF, Swedlow J, Dernburg A, Belmont A, Murray AW, Agard DA, Sedat JW: **Interphase chromosomes undergo constrained diffusional motion in living cells.** *Curr Biol* 1997, **7**:930-939.
37. Zink D, Cremer T: **Cell nucleus: chromosome dynamics in nuclei of living cells.** *Curr Biol* 1998, **8**:R321-324.
38. Bornfleth H, Edelmann P, Zink D, Cremer T, Cremer C: **Quantitative motion analysis of subchromosomal foci in living cells using four-dimensional microscopy.** *Biophys J* 1999, **77**:2871-2886.
39. Meier UT, Blobel G: **Nopp140 shuttles on tracks between nucleolus and cytoplasm.** *Cell* 1992, **70**:127-138.
40. Phair RD, Misteli T: **High mobility of proteins in the mammalian cell nucleus.** *Nature* 2000, **404**:604-609.
41. Politz JC, Tuft RA, Pederson T, Singer RH: **Movement of nuclear poly(A) RNA throughout the interchromatin space in living cells.** *Curr Biol* 1999, **9**:285-291.
42. Tumbar T, Belmont AS: **Interphase movements of a DNA chromosome region modulated by VP16 transcriptional activator.** *Nat Cell Biol* 2001, **3**:134-139.
43. Gorlich D, Kutay U: **Transport between the cell nucleus and the cytoplasm**. *Annu Rev Cell Dev Biol* 1999, **15**:607-660.
44. Csink AK, Henikoff S: **Large-scale chromosomal movements during interphase progression in *Drosophila*.** *J Cell Biol* 1998, **143**:13-22.
45. Li G, Sudlow G, Belmont AS: **Interphase cell cycle dynamics of a late-replicating, heterochromatic homogeneously staining region: precise choreography of condensation/decondensation and nuclear positioning.** *J Cell Biol* 1998, **140**:975-989.
46. Heun P, Laroche T, Raghuraman MK, Gasser SM: **The positioning and dynamics of origins of replication in the budding yeast nucleus.** *J Cell Biol* 2001, **152**:385-400.
47. Misteli T, Caceres JF, Spector DL: **The dynamics of a pre-mRNA splicing factor in living cells.** *Nature* 1997, **387**:523-527.
48. Eils R, Gerlich D, Tvarusko W, Spector DL, Misteli T: **Quantitative imaging of pre-mRNA splicing factors in living cells.** *Mol Biol Cell* 2000, **11**:413-418.
49. Platani M, Goldberg I, Swedlow JR, Lamond AI: ***In vivo* analysis of Cajal body movement, separation and joining in live human cells.** *J Cell Biol* 2000, **151**:1561-1574.
50. Snaar S, Wiesmeijer K, Jochemsen AG, Tanke HJ, Dirks RW: **Mutational analysis of fibrillarin and its mobility in living human cells.** *J Cell Biol* 2000, **151**:653-662.
51. Boudonck K, Dolan L, Shaw PJ: **The movement of coiled bodies visualized in living plant cells by the green fluorescent protein.** *Mol Biol Cell* 1999, **10**:2297-2307.
52. Smith KP, Lawrence JB: **Interactions of U2 gene loci and their nuclear transcripts with Cajal (coiled) bodies: evidence for PreU2 within cajal bodies.** *Mol Biol Cell* 2000, **11**:2987-2998.
53. Smith KP, Carter KC, Johnson CV, Lawrence JB: **U2 and U1 snRNA gene loci associate with coiled bodies.** *J Cell Biochem* 1995, **59**:473-485.
54. Jacobs EY, Frey MR, Wu W, Ingledue TC, Gebuhr TC, Gao L, Marzluff WF, Matera AG: **Coiled bodies preferentially associate with U4, U11, and U12 small nuclear RNA genes in interphase HeLa cells but not with U6 and U7 genes.** *Mol Biol Cell* 1999, **10**:1653-1663.
55. Frey MR, Bailey AD, Weiner AM, Matera AG: **Association of snRNA genes with coiled bodies is mediated by nascent snRNA transcripts.** *Curr Biol* 1999, **9**:126-135.
56. Frey MR, Matera AG: **Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells.** *Proc Natl Acad Sci USA* 1995, **92**:5915-5919.