

Nuclear choreography: interpretations from living cells

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The advent of green fluorescent protein technology, its use in photobleaching experiments and the development of methods to rapidly acquire images and analyze complex datasets have opened the door to unraveling the mechanisms of nuclear functions in living cells. Studies over the past few years have characterized the movement of chromatin, nuclear proteins and nuclear bodies and, in some cases, correlated their dynamics with energy dependence, cell cycle progression, developmental changes, factor targeting and nuclear position. The mechanisms by which nuclear components move or are restrained have important implications for understanding not only the efficacy of nuclear functions but also the regulation of developmental programs and cellular growth.

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Abbreviations

CFP	cyan fluorescent protein
DHFR	dihydrofolate reductase
ER	estrogen receptor
FRAP	fluorescence recovery after photobleaching
GFP	green fluorescent protein
GR	glucocorticoid receptor
lacO	lactose operator
LBR	lamin B receptor
MMTV	mouse mammary tumor virus
NE	nuclear envelope
NPC	nuclear pore complex
PML	promyelocytic leukemia
tetO	tetracycline operator
YFP	yellow fluorescent protein

Introduction

Biochemical and genetic studies have identified a complex array of factors involved in regulating chromatin structure and nuclear processes. An understanding of the organization of these factors, as well as their substrates, within the nucleus will provide significant insight into how nuclear functions are spatially and temporally coordinated. Although immunolabeling and DNA and RNA *in situ* hybridization studies have suggested that

the nucleus is highly organized, they provide only static images of nuclear components. Recent studies in living cells, however, have revealed the highly dynamic nature of proteins in the nucleus and have changed the way that we think about how functional complexes assemble and interact with their substrates. The differential stability of proteins comprising the nuclear envelope/lamina presents an interesting paradigm for the regulation of nuclear import/export processes, while maintaining a structural barrier demarcating the nucleus as an independent organelle. In addition, the association of chromatin with particular nuclear structures and its localized dynamics may significantly influence the regulation of nuclear functions. The fact that nuclear bodies exhibit dynamic properties leaves open the possibility that they may be targeted to particular nuclear domains and/or chromosomal regions and may play previously unimagined roles in nuclear processes.

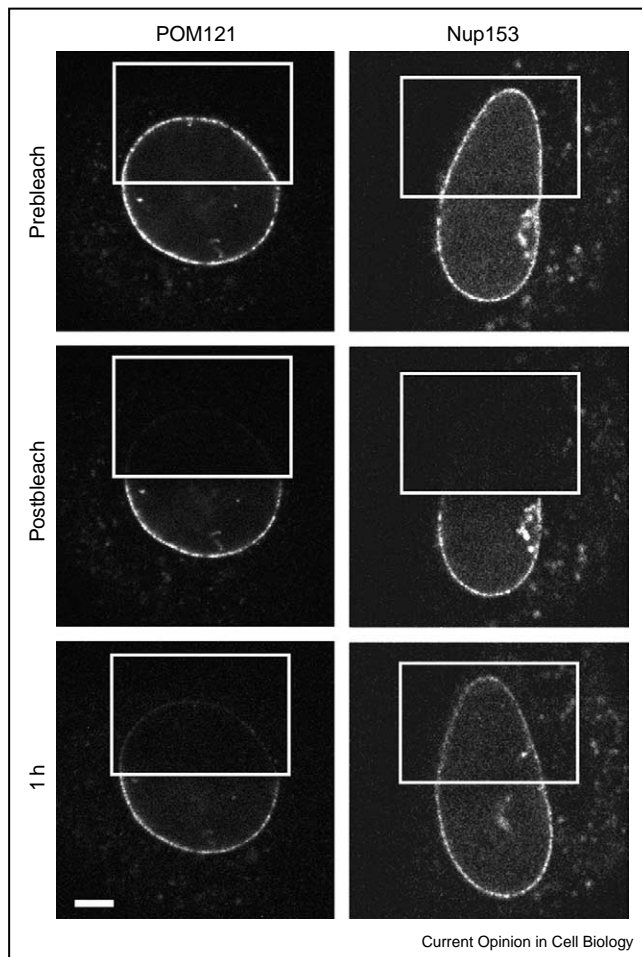
In this review, we discuss recent findings on the dynamics of various nuclear constituents and their impact on nuclear functions.

Turnover at the gate

The nuclear envelope (NE) is composed of two concentric membranes interrupted by a series of nuclear pore complexes (NPC) that mediate factor trafficking between the nucleus and cytoplasm. The outer membrane is continuous with the endoplasmic reticulum. The inner membrane contains a distinct set of integral membrane proteins and is underlined by a meshwork of intermediate-filament proteins called the nuclear lamina. Recently, several groups have expressed NE components and the nuclear lamins as green fluorescent protein (GFP) fusion proteins and used fluorescence recovery after photobleaching (FRAP) to analyze their dynamics in mammalian cells. In FRAP studies, a defined area is bleached irreversibly and protein mobility is determined by measuring the rate that fluorescence returns to the bleached area ($\tau_{1/2}$ is the half-time of recovery) (reviewed in [1,2]). The lamin B receptor (LBR) [3] and emerin [4], the protein mutated in Emery-Dreifuss muscular dystrophy, are integral membrane proteins of the NE, and FRAP analysis suggests that they reach the NE through lateral diffusion from the endoplasmic reticulum. Although highly mobile in the endoplasmic reticulum, at the NE, the LBR is virtually immobilized [3] whereas emerin exhibits limited diffusion (diffusion coefficient $[D] = 0.10 \pm 0.01 \mu\text{m}^2/\text{s}$) [4], suggesting that interactions with nuclear ligands such as chromatin and the lamins mediate their retention.

Characterization of NPCs has shown that whereas individual NPCs do not move within the plane of the NE,

Figure 1



FRAP of NRK cells stably expressing POM121–GFP and GFP–Nup153, respectively. The boxed region was photobleached to background levels and recovery monitored for 1 h by confocal microscopy. Note the lack of recovery for the stably associated POM121 after 1 h and the complete exchange of Nup153 during this time. Bar = 5 μ m. Images were provided by Gwenaél Rabut and Jan Ellenberg, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

they are composed of both stable and dynamic proteins [5**]. POM121 ($t_{1/2} \approx 20$ h; determined in confluent cells) (Figure 1) [5**], hNup107, and hNup133 (little recovery between cell divisions) [6] all exhibit low turnover rates after photobleaching, suggesting that they play structural roles in nuclear pore function. By contrast, Nup153 ($t_{1/2} = 15 \pm 2$ s) (Figure 1) [5**] and Nup98 [7*] are dynamic. Not only does Nup98 localize to the NPC (recovery <3 min), but it also resides in internal nuclear sites (nucleoplasmic pool, $t_{1/2} = 1.16$ s; nuclear bodies: $t_{1/2} = 11.80$ s) [7*]. Additionally, inhibition of RNA polymerase I and polymerase II transcription immobilizes Nup98 in the nucleus, suggesting that it may play a role in RNA export [7*]. The nuclear pool of Nup153 was shown to be diffusionaly isolated from the

cytoplasm [5**] in contrast to Nup98, which shuttles [7*]. Therefore, some NPC components may mediate transport events by directing cargo to pores, shuttling cargo through pores, and/or escorting imported cargo to nuclear sites [7*].

FRAP analysis of A- and B-type nuclear lamins indicates that the nuclear lamina is also a highly stable structure during interphase. Both lamin A [8] and lamin B1 [5**,8] at the nuclear rim and in the nucleoplasmic ‘veil’ [8] recover very little after photobleaching ($t_{1/2} > 180$ min). Interestingly, lamin B1 and POM121 co-expressed as YFP (yellow fluorescent protein) and CFP (cyan fluorescent protein) fusion proteins moved synchronously during global changes in nuclear shape, suggesting that the NE and lamina form interconnected, stable, two-dimensional networks [5**]. The NE/lamina is, therefore, remarkable in that both stable and dynamic proteins associate to form a functional boundary between the nucleus and the cytoplasm.

Visualizing chromatin on the move

DNA wraps around histone octamers to form nucleosomes, and a large number of factors and protein modifications regulate the assembly, maintenance and activity of higher-order chromatin structure. However, it is not well understood how mechanisms that preserve genomic integrity are coordinated with dynamic chromatin-related functions.

Examination of the core histones, H2B [9–11], H3 [11] and H4 [11], in photobleaching studies has shown them to be essentially immobile (little recovery > 4 h), except for a small population of H2B (3%, $t_{1/2} \approx 6$ min) [11], suggesting that DNA associates tightly with histone octamers and that these interactions play a role in stabilizing chromatin structure. By contrast, the linker histone H1 ($t_{1/2} = 18.7 \pm 5.7$ s) [10,12] and the nucleosomal binding protein HMG-17 ($t_{1/2} \approx 3$ s) [9] — both shown to affect chromatin compaction — are more dynamic. In fact, wild-type HMG-17 (HMG1 and 2) competes with histone H1 for binding to nucleosomes, suggesting that chromatin structure is regulated by the dynamic interplay of chromatin associated proteins [13].

Gene disruption experiments have shown that linker histones have highly specific effects of both a positive and negative nature on transcription *in vivo* [14*]. Interestingly, in *Tetrahymena thermophila*, endogenous H1 can be replaced with H1-GFP and the dynamics of H1 mutants with characterized effects on gene expression can be evaluated [14*]. H1 mutated to mimic the fully phosphorylated state has an inhibitory effect on transcription of the *Cyp1* gene, and in this study it was shown to be more mobile than H1 mutated to mimic the fully unphosphorylated state that enhances *Cyp1* transcription. This suggests that with increased residency time, H1 may

create a chromatin conformation that is more amenable to *Cyp1* expression or compete out inhibitory factors, or both [14*]. Kinase and deacetylase inhibitors have also been shown to increase H1 mobility in mammalian cells [10,12]. Therefore, chromatin structure and gene expression may be regulated not only by the association of specific factors but also by the modulation of their residency times through different combinations and levels of histone and chromatin protein modifications.

Analyses of chromosome-associated proteins have provided insight into the global properties of interphase chromatin, but the use of high affinity DNA–protein interaction units to visualize specific chromatin regions in eukaryotic cells has allowed chromatin dynamics and nuclear functions to be correlated in a variety of cell types including mammalian, *Drosophila*, yeast (reviewed in [15]) and plant [16]. Belmont and colleagues pioneered this approach by stably integrating tandem arrays of lactose operator (*lacO*) repeats and expressing *lac* repressor as a GFP fusion protein to visualize the insertion site [15]. Arrays of *lacO* repeats integrated in conjunction with the dihydrofolate reductase (*DHFR*) gene have been shown to form highly condensed heterochromatic structures that move from the nuclear periphery to internal positions during S phase and upon VP16–*lac*-repressor fusion protein targeting [17,18*]. A *lacO*–*DHFR* insertion amplified into a 90 Mb heterochromatic chromosomal region through methotrexate selection was shown to fold reproducibly in mitotic chromosomes [19] and to undergo dramatic unwinding during interphase upon localization of VP16 [20], the estrogen receptor (ER) [21], and BRCA1 [22*] to the repeats. As BRCA1 is involved in several processes including transcription, DNA repair and the maintenance of genome integrity, a chromatin remodeling effect is believed to underlie at least part of its function. This tethering assay identified subdomains of BRCA1 capable of independently unfolding chromatin [22*]. Cancer-predisposing alleles that truncate the protein abolish the chromatin remodeling activity whereas point mutations in the 3' end enhance it, suggesting that the disease phenotype may be sensitive to both increases and decreases in this activity [22*].

In *Drosophila*, plants and vertebrates, interphase chromosome territories occupy distinct, non-overlapping, relatively stable positions within nuclei (reviewed in [23]). However, specific chromosomal regions have been shown to be dynamic and, in certain cases, changes in position have been correlated with the initiation of transcription, DNA replication and development (reviewed in [15]). Imaging of *lacO* arrays targeted to euchromatic sites in *Drosophila* spermatocytes showed that chromatin moves by constrained diffusional motion during interphase [24**], as was previously reported for yeast centromeres [25]. However, chromatin exhibited different *D* values and displacements (Δr_{rms}) over short and long time inter-

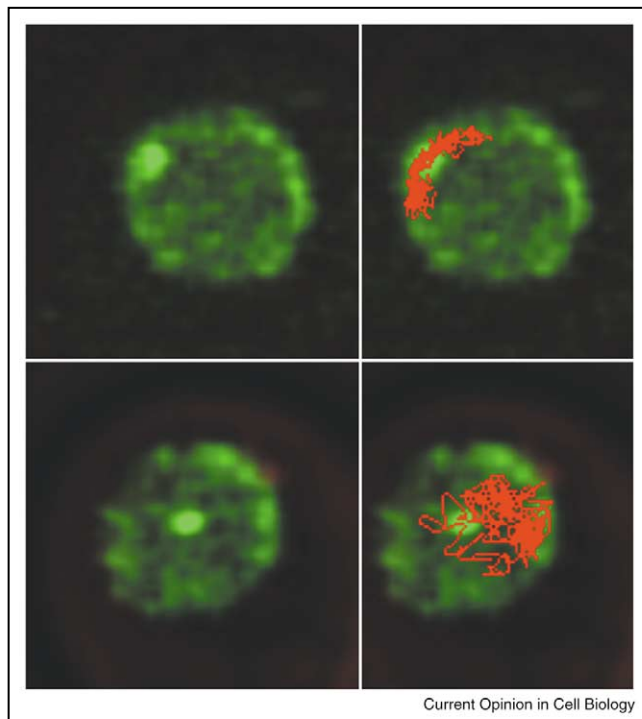
vals and is significantly less restricted in early G2 (short range, $D = 1.3 \times 10^{-2} \mu\text{m}^2/\text{s}$, $\Delta r_{\text{rms}} = 0.30 \mu\text{m}/\text{s}$; long range, $D = 1.0 \times 10^{-3} \mu\text{m}^2/\text{s}$, $\Delta r_{\text{rms}} = 4.6 \mu\text{m}/\text{hr}$) compared with late G2 just before the onset of meiotic prophase (short range, $D = 3.4 \times 10^{-3} \mu\text{m}^2/\text{s}$, $\Delta r_{\text{rms}} = 0.15 \mu\text{m}/\text{s}$; long range, $D = 9.4 \times 10^{-5} \mu\text{m}^2/\text{s}$, $\Delta r_{\text{rms}} = 1.4 \mu\text{m}/\text{hr}$) [24**]. This study showed that chromatin can undergo rapid short-range movements and that over longer time periods it is able to explore chromosome-territory-sized domains.

Observations of individual chromosome territories in mammalian cells, visualized through the incorporation of fluorescently conjugated nucleotides, have also shown that territories persist as stable entities (>4 h) but, in this case, subchromosomal foci occasionally switch from random to directional motion [26–28]. Additionally, nuclear positioning has been shown to influence chromatin dynamics, because *lacO* arrays integrated into mammalian cells in genomic regions that localize to nucleoli or the nuclear periphery (maximum range of movement 0.9 μm) are significantly less mobile than nucleoplasmic loci (maximum range of movement 1.5 μm ; $D = 1.25 \times 10^{-4} \mu\text{m}^2/\text{s}$) [29**]. Together, these studies suggest that in *Drosophila* and mammalian cells, chromosome position and organization are preserved for long periods of time in interphase cells and that chromatin moves by constrained Brownian motion.

By contrast, *lacO*-tagged chromosomal regions near both early and late origins of replication in *Saccharomyces cerevisiae* display a high degree of ATP-dependent mobility during G1 (movement $\geq 0.5 \mu\text{m}/10 \text{ s}$) (Figure 2) [30**]. Although energy dependent, this movement does not appear to be directed but instead may result from the activity of ATP-dependent chromatin-remodeling factors [30**]. DNA replication resulted in a diminution of chromatin mobility, because large-amplitude movements were seen during both G1 and S phase in strains harboring *orc2* mutations, suggesting that the assembly of replication foci restricts movement [30**]. Centromeres and telomeres were shown to move within significantly smaller domains, with telomeres remaining closely associated with the nuclear periphery as marked by a GFP-tagged nuclear pore protein (Figure 2) [30**].

Although the dynamics of yeast chromatin appear to be greater than that observed in mammalian and *Drosophila* cells, this may result from differences in the sizes of the respective nuclei [30**]. For example, a 0.5 μm movement in yeast is equal to nearly one-third of the diameter of a haploid yeast nucleus but only one-thirtieth of the diameter of a typical human nucleus [30**]. In addition, heterochromatin may anchor chromosomes to nuclear sites, and yeast chromosomes may be more mobile because the yeast genome is composed of far less heterochromatin [30**].

Figure 2



Dynamics of yeast chromatin. Shown on the left are equatorial focal scans of a *S. cerevisiae* nucleus, visualized through the fluorescence of a nuclear pore protein (GFP-Nup49), and of a chromosomal domain tagged with an insert of *lac* operators and a GFP-*lac*-repressor fusion. The cells in the upper panels carry a *lacO*-tag of about 15 kb from the right end of telomere VI-R, whereas the lower panel shows a tagged locus in the middle of the left arm of chromosome XIV. In the right-hand panels, the path of movement of the chromosomal loci was captured in 200 sequential frames taken at 1.5 s intervals and is projected as a red trace onto a single focal section, determined after alignment of the focal series through the nuclear pore signal. Images were provided by F Hediger, T Laroche and SM Gasser, University of Geneva, Geneva, Switzerland.

Find your partner: chromatin pairing in somatic and meiotic cells

Recently, using high-affinity DNA-protein interaction units, it has been possible to address chromosome pairing in living cells using non-disruptive techniques. Studies in *S. cerevisiae*, comparing chromosome pairing at both allelic and non-allelic sites in meiotic and somatic cells, through the visualization of *lacO* and/or tetracycline operator (*tetO*)-repeat inserts, revealed that somatic cells display a preference for the pairing of identical sequence tags (*lacO* or *tetO*) whether or not they are in the same allelic position [31]. Therefore, these ‘*trans*-associations’ suggest that a mechanism exists for identical stretches of repetitive DNA to self-associate [31]. As pairing rates among all loci (including non-allelic non-identical tags) were highest during G1, it is possible that the high degree of chromatin mobility reported during G1 [30**] increases the efficiency of chromatin interactions. By

contrast, during meiosis, homologous chromosome pairing between allelic sites is dominant (~90%) suggesting that these associations depend on interactions between flanking sequences [31].

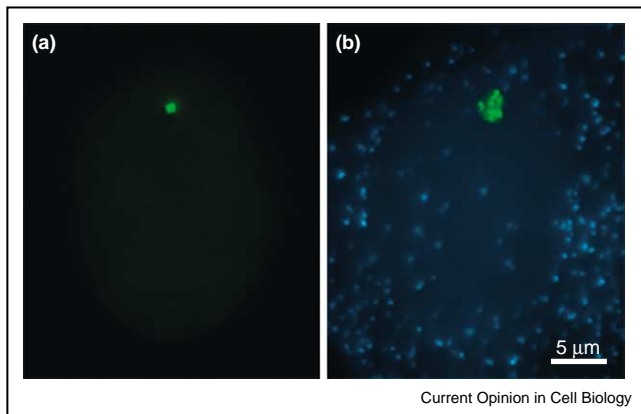
Using the *lacO*/repressor system, the behavior of meiotic chromosomes was studied in live cells during all stages of male *Drosophila* meiosis and a three-step model encompassing initiation, sorting, and maintenance of chromosome pairing was proposed [32**]. In the initiation phase, homologous chromosomes were found to pair throughout euchromatic regions. Sorting occurred via separation of homologs and sister chromatids in mid-G2, near the time when homologous centromeres pair. Changes in large-scale chromosome organization seemed to disrupt non-homologous centromere interactions and thus facilitate pairing between homologs. Finally, the maintenance of homologous chromosome pairing was suggested to involve non-sequence-specific mechanisms such as chromatin entanglement or heterochromatin interactions [32**]. These studies suggest that distinct pairing mechanisms exist in somatic and meiotic cells, and that nuclear organization may play a central role in facilitating these processes.

Caught in the act: transcription

In a cell line containing a stable tandem array of mouse mammary tumor virus (MMTV) reporter elements, ligand activation of a GFP-tagged glucocorticoid receptor (GR) targeted it from the cytoplasm to the repeated regulatory elements in the array and allowed the site of transcription to be visualized in living cells [33,34]. FRAP analysis of the GR associated with these elements substantiated the ‘hit and run’ model by showing that activators exchange rapidly between chromatin-binding sites and the nucleoplasm (recovery occurs in ~12 s) [33]. The discovery that activator-chromatin interactions are highly dynamic suggests that transcription factors do not form stable holo-complexes at promoters and that their activity may be regulated in a similar manner. In fact, photobleaching analysis of the general transcription factors TBP ($t_{1/2} \sim 1$ min; nearly 100% recovery ~20 min) and TFIIB (full recovery in seconds) in the nucleoplasm showed that they are also dynamic [35]. The slower turnover rate of TBP in comparison with TFIIB suggests that TBP remains promoter-bound for more than one round of transcriptional initiation and that TFIIB may dissociate during the transition from initiation to elongation [35].

FRAP analysis of the ER revealed that ligand binding reduces the mobility of the receptor within the nucleus (before ligand addition, $t_{1/2} = 0.8$ s; after ligand addition, $t_{1/2} = 5.0$ – 6.0 s) [36]. This mobility change was concomitant with the well-characterized localization of hormone-responsive nuclear receptors to punctate structures throughout the nucleoplasm after the addition of ligand.

Figure 3



Visualization of a stably integrated genetic locus in the transcriptionally inactive (a) and active (b) states. When transcriptionally inactive (a), the locus appears as a highly condensed dot within the nucleus. After the initiation of transcription, the locus significantly decondenses, and the product of the transcription unit, CFP fused to a peroxisomal targeting signal, accumulates in the peroxisomes in the cytoplasm.

Proteasome inhibitors and ATP depletion were also shown to immobilize the ER [36]. As ER protein levels decrease upon ligand addition and proteins associated with the ubiquitin and proteasome pathways have been identified as ER co-activators, transcriptional activity may be linked to receptor turnover [36]. Interestingly, only the unliganded ER is affected by ATP depletion, suggesting a role for phosphorylation and/or ATP-dependent heat-shock proteins in receptor mobility before ligand binding [36]. FRAP analysis of the ER coactivators, SRC-1 ($t_{1/2} = 8.0 \pm 2.5$ s) and CBP ($t_{1/2} = 4.2 \pm 1.1$ s), co-localized with a ligand bound ER-*lac*-repressor fusion protein at a *lacO* array, also showed that activator-co-activator interactions are highly dynamic [37**].

To examine directly the dynamics of a regulatable transcription site in living cells, *lacO* repeats were placed upstream of a transcription unit composed of tetracycline-inducible response elements, a minimal cytomegalovirus (CMV) promoter, CFP fused to a peroxisomal targeting signal, β -globin intron/exon sequences and a polyadenylation signal [38]. Expression of a YFP-*lac*-repressor fusion protein allowed a stably integrated array of this construct to be visualized in both the transcriptionally active and inactive states (Figure 3). About 20 min after the induction of transcription, chromatin decondensation was detectable and, over time, CFP accumulated in peroxisomes serving as a downstream reporter for gene expression. Using this approach, it will be possible to correlate changes in chromatin structure to factor recruitment and displacement during the transition from transcriptional inactivity to activity (SM Janicki and DL Spector, unpublished data).

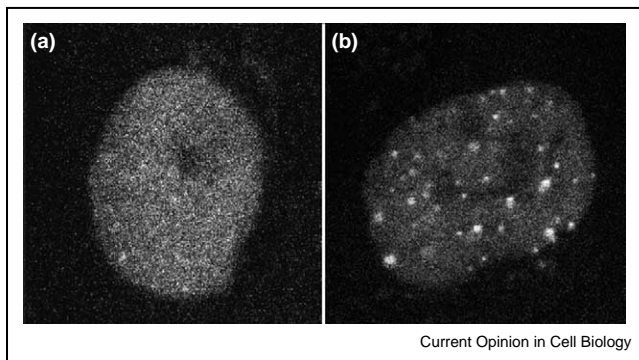
In the three systems described above that have been used to visualize transcriptionally active chromatin in living cells (GR recruitment to repeated MMTV elements [34], VP16-*lac*-repressor tethering to a *lacO*/*DHFR* gene array [20], and induction of the *lacO*/*tet* responsive system, which also utilizes the VP16 activation domain [38]), activator recruitment resulted in dramatic decondensation of the chromatin. Whereas transcriptional inhibition prevented the initiation and maintenance of chromatin decondensation by the GR [34], it did not affect chromatin unwinding induced by the VP16-*lac*-repressor fusion protein [20]. The interaction of the GR with its response elements is likely to be more dynamic than that of *lac*-repressor-VP16 with the operator and, therefore, may require the concerted action of a transcribing polymerase to maintain an open chromatin configuration. During transcriptional inhibition in the MMTV cell line, transcription factors, co-activators and chromatin remodeling factors localize to the array, suggesting that they are not limiting [34].

Given the high affinity of the *lac*-repressor for the operator sequence, VP16 may be more stably associated with the *lacO* array than the GR is with the MMTV array, and thus it could be shown that VP16 alone is sufficient to recruit the factors required to decondense chromatin, and that and chromatin remodeling can be uncoupled from transcription. These studies suggest that transcriptional activators must work in concert with the elongating polymerase to decondense chromatin [34], and activator levels and residence times are likely to be central to the regulation of chromatin remodeling resulting in transcription. Therefore, some cancer- and disease-causing mutations that produce genomic instability may result from the misregulation of chromatin-remodeling processes such as has been suggested for BRCA1 [22*].

Stopping to repair and replicate

Recent studies have suggested that a high degree of protein mobility within the nucleus may serve to provide cells with a rapid response mechanism to contend with events critical to cell viability and growth. Time-lapse image analysis and photobleaching studies of proteins with biochemically and genetically defined roles in DNA repair and DNA replication have provided insight into the functional mechanisms of these processes in living cells. The endonuclease ERCC1/XPF, which is involved in nucleotide excision repair (NER) [39], and the RAD52 group proteins (Rad51, Rad52 and Rad54) [40**], which are required for homologous recombination, are for the most part mobile. However, exposure to ultraviolet light transiently immobilizes a population of ERCC1/XPF in a dose-dependent manner with individual complexes estimated to be immobile for ~ 4 min — the time believed to be required to complete one repair event [39]. In response to ionizing radiation, RAD52 group proteins localized to damage-induced foci (Figure 4) but exhibited different

Figure 4



Visualization of the DNA-damage response of the human Rad52 DNA repair protein in living cells. **(a)** Nucleus of a Chinese hamster ovary (CHO) cell expressing Rad52-GFP. A homogeneous distribution of the protein is observed before the induction of DNA damage. **(b)** Treatment of the cells with ionizing radiation results in the accumulation of Rad52-GFP into nuclear foci. Images were taken 2 h after irradiation with a dose of 12 Gy. Images were provided by Jeroen Essers and Roland Kanaar, Erasmus University, Rotterdam, The Netherlands.

turnover rates within them (Rad51, little recovery after 2 min; Rad52, $t_{1/2} = 26.0$ s; Rad54, $t_{1/2} = 0.5$ s) suggesting that they do not form stable holo-complexes but dynamic multiprotein complexes at sites of damage [40**].

Distinct patterns of DNA replication foci have been linked both to specific time points during S phase and to the replication of euchromatin and heterochromatin; however, it was not known how transitions between these patterns occur. Time-lapse imaging of proliferating cell nuclear antigen during S phase showed that these changes result from the gradual assembly and disassembly of replication foci rather than from the movement of large functional complexes to new sites [41]. Therefore, the execution of many nuclear processes may result from the creation of high-affinity binding sites at which dynamic functional complexes assemble and subsequently disassemble after the completion of the respective functions.

Roaming the nucleus: nuclear bodies and nuclear proteins

A large number of nuclear bodies have been identified by immunofluorescence and ultrastructural localization (reviewed in [42]). However, with the exception of the nucleolus, the functions of these structures have not been well established although many contain biochemically and/or genetically characterized components. Analysis of the movement of nuclear bodies and their proteins has allowed the role that mobility may play in their functions to be considered. In time-lapse images, nucleoli and nuclear speckles remain in their nuclear neighborhoods although speckles exhibit localized peripheral dynamics [43,44]. Photobleaching analysis of the pre-mRNA splicing factor SF2/ASF ($t_{1/2} \approx 3$ s) [9,44],

which is present in both speckles and a diffuse nuclear pool, and the nucleolar rRNA processing factor, fibrillarin ($t_{1/2} \approx 3$ s) [9], revealed that the majority of molecules within these structures are highly mobile and move by an energy-independent diffusion mechanism. It has been suggested that the diffusion rates of nuclear proteins are lower than free GFP (100-fold difference) because of transient interactions with nuclear components [9,44].

Analysis of promyelocytic leukemia (PML) body dynamics showed that in a given nucleus some bodies are stationary, some exhibit localized movements and others move by an energy-dependent directed mechanism (average velocity $4.0\text{--}7.2 \mu\text{m}/\text{min}^{-1}$) [45**]. FRAP analysis of the PML body proteins SP100 and PML suggests that they are structural components of these bodies (little recovery after 10 min) [46**] whereas CBP, which recovered in a time frame comparable to the nucleoplasmic pool ($t_{1/2} = 92$ s), may transiently pass through PML bodies to be modified and/or to form subcomplexes [46**]. In addition, PML bodies may themselves be functional complexes that contain dynamic components.

Cajal body mobility was first reported in plants [47] and subsequent characterization in mammalian cells revealed that these bodies move by anomalous diffusion [48,49**]. Cajal bodies have been found associated with the histone, U1, U2, and U3 snRNA gene clusters and, in fact, the association with the U2 coding region was suggested to be mediated through nascent U2 snRNA transcripts or a polymerase complex [50]. In living cells, Cajal bodies were found to be closely associated with chromatin, as visualized by a YFP-histone-H2B fusion protein, but to switch between chromatin-associated and diffusing states [49**]. They have also been shown to merge with and bud from one another [48,49**]. Additionally, ATP depletion and transcriptional inhibition increase Cajal body mobility, suggesting that Cajal-body-chromatin interactions require ATP and active transcription [49**].

As discussed above, a variety of conditions and associations have been shown to affect the mobility of some nuclear proteins and bodies. The fact that chromatin is highly structured and for the most part confined to chromosome territories suggests that it may play a role in restraining nuclear bodies or restricting their movement to the inter-chromosome-domain compartment (reviewed in [23]). The structure and large volume of chromatin may also require that many nuclear proteins be highly mobile to quickly access the large number of potential functional sites on chromatin. For example, proteins involved in DNA repair and transcription diffuse freely until the creation of high-affinity binding sites induces the assembly of dynamic functional complexes. In addition, the existence of mobile populations of PML and Cajal bodies suggests that they may also need to move to functional targets which, in turn, may influence nuclear organization.

Given the dynamic nature of many of the proteins present in nuclear bodies, it is surprising that these structures exist at all. However, high affinity binding sites may also induce the formation of these structures. For example, at the end of mitosis, the nucleolar organizing regions, containing clusters of rDNA genes, fuse to form nucleoli that concentrate the factors involved in RNA polymerase I transcription and processing (reviewed in [51]). FRAP analysis of the PML protein suggests that it is a structural component of PML bodies [46**] and, in fact, these bodies do not form in its absence, suggesting that it may, itself, create high affinity binding sites that result in the formation of PML bodies (reviewed [52]). Interestingly, energy depletion has been shown to both increase [49**] and decrease [30**,36,45**] the mobility of some nuclear components, suggesting that a variety of mechanisms exist to regulate dynamics within the nucleus.

Conclusions

In recent years, studies in living cells have offered a previously unimagined view of the mechanisms of nuclear functions and dynamics literally through the window of the microscope. The NE/lamina is composed of both stable and dynamic components integrated to form a functional barrier between the nucleus and the cytoplasm. Though dynamic, chromatin seems to impart a high degree of structure upon the nucleus because of its functional organization into, for example, chromosome territories and heterochromatin. However, many nuclear bodies and proteins have been shown to be highly dynamic, which may serve to facilitate molecular interactions. For example, initiation of an event that increases the affinity of a protein or nuclear body for chromatin and/or other factors may result in an equilibrium shift and factor assembly into functional complexes. As these associations are also highly dynamic, it may allow regulatory changes to be effected rapidly and crosstalk between parallel functional pathways, such as DNA repair, DNA replication and transcription, to be facilitated.

GFP technology and the development of techniques to visualize chromatin and proteins in living cells not only have changed the nature of the questions it is possible to address concerning the dynamics of nuclear functions but also have re-established the microscope as an integral tool in the search for answers.

Update

Recent determinations of the mobility of factors involved in transcription, DNA repair, DNA replication and RNA export continue to provide insight into how functional complexes assemble and move in living cells. Characterizations of the movement of transcription factors suggest that active complexes form in a stochastic fashion from freely diffusing pools and do not exist as components of large stable holoenzymes [53**,54**,55]. When photobleached in nucleoli, RNA polymerase I pre-initiation

factors all recovered rapidly, suggesting that they disengage from promoters after the start of transcription; this is in contrast to elongation factors, which exist in two pools — a smaller one engaged in active transcription, and one freely diffusing [53**]. TFIIH was shown to participate for different lengths of time in RNA polymerase I (~25 s) and II (2–10 s) transcription and DNA repair (3–4 min) events, with the cellular pool able to freely switch between these processes [54**]. Likewise, RNA polymerase II is present in two kinetic populations, with 25% transiently immobilized ($\tau_{1/2} \approx 20$ min) and transcriptionally active, while 75% exhibited rapid dynamics [55]. Earlier studies suggested that nuclear RNA dynamics is a diffusion-based process (reviewed in [56]); however, recent examination of proteins involved in the export of messenger ribonucleoprotein particles by photobleaching of poly(A)-binding protein 2 (PABP2) and the export factor TAP showed that they move in the nucleus by a combination of passive diffusion and ATP-dependent processes [57*].

DNA replication factors were also shown to have differential affinities for replication foci. Proliferating cell nuclear antigen (PCNA) is stably associated with the replication machinery, suggesting that it remains bound through multiple rounds of Okazaki fragment synthesis, in contrast to RPA34, which is more dynamic [58**]. The localization of new replication sites in close proximity to earlier replication foci suggests that local changes in chromatin structure may trigger DNA synthesis at neighboring origins [58**].

A recent study of chromatin dynamics in yeast provides insight into the interplay between nuclear organization and transcriptional status [59**]. Telomeres, though dynamic, remain associated with the nuclear envelope throughout interphase. Anchoring was shown to depend on two redundant pathways mediated by yKu and Sir proteins.

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