REVIEW ARTICLE

Functional architecture in the cell nucleus

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The major functions of the cell nucleus, including transcription, pre-mRNA splicing and ribosome assembly, have been studied extensively by biochemical, genetic and molecular methods. An overwhelming amount of information about their molecular mechanisms is available. In stark contrast, very little is known about how these processes are integrated into the structural framework of the cell nucleus and how they are spatially and temporally co-ordinated within the three-dimensional confines of the nucleus. It is also largely unknown how nuclear architecture affects gene expression. In order to understand how genomes are organized, and how they function, the basic principles that govern nuclear architecture and function must be uncovered. Recent work combining molecular, biochemical and cell biological methods is beginning to shed light on how the nucleus functions and how genes are expressed *in vivo*. It has become clear that the nucleus contains distinct compartments and that many nuclear components are highly dynamic. Here we describe the major structural compartments of the cell nucleus and discuss their established and proposed functions. We summarize recent observations regarding the dynamic properties of chromatin, mRNA and nuclear proteins, and we consider the implications these findings have for the organization of nuclear processes and gene expression. Finally, we speculate that self-organization might play a substantial role in establishing and maintaining nuclear organization.

Key words: chromatin, gene expression, nuclear compartments, protein dynamics.

INTRODUCTION

The nucleus was the first intracellular structure discovered and was originally described by Franz Bauer in 1802 and later popularized by Robert Brown [1]. The organelle attracted much attention because of its fascinating, complex and aesthetically pleasing behaviour during cell division and its evident roles in essential processes, such as fertilization and inheritance [1]. Further analysis of nuclear substructure was difficult because of the limitations of microscope technology. Once the process of gene expression became experimentally tractable, the emphasis in nuclear studies shifted to biochemical and molecular approaches, and the analysis of nuclear structure became a secondary concern. In the 1980s the use of fluorescence microscopy to study particular proteins in the nucleus of chemically fixed cells renewed the interest in uncovering details of nuclear architecture [2]. These studies clearly established the existence of several morphologically distinguishable intranuclear structures. Recently [3], major efforts have been made to relate these structural landmarks within the nucleus to nuclear functions, especially gene expression. These studies have been catalysed by the emergence of genetically encoded fluorescent tags, which now allow the routine visualization and quantitative analysis of chromatin, mRNA and proteins in living cells [4–6]. These studies have revealed two fundamental aspects of nuclear architecture, which are critical for the understanding of nuclear function: first, the nucleus contains distinct subcompartments; and secondly, the nucleus is a highly dynamic organelle.

NUCLEAR COMPARTMENTS AND THEIR FUNCTIONS

The cell nucleus contains distinct substructures [2,3,6,7]. These structures are characterized by the absence of delineating membranes, but they must be considered 'compartments' for several reasons. First, they contain defining subsets of resident proteins. Secondly, they can be morphologically identified by light and electron microscopy, and most of them have recently been visualized in living cells using green fluorescent protein (GFP) technology. Thirdly, at least some compartments can be biochemically isolated in an enriched form [8,9]. The absence of defining membranes dramatically distinguishes nuclear compartments from their cytoplasmic counterparts. The presence of distinct compartments within the nucleus, and the problem of how the multitude of nuclear processes might be organized in nuclear space, has led to the proposal that a nuclear matrix organizes nuclear architecture. The potential structure of a nuclear matrix, however, is far from clear. In fact, the question of whether a nuclear matrix exists at all is highly controversial [10,11]. While indirect experimental observations in support of such a structure have been reported, neither defined elements of a nuclear matrix nor components of nuclear compartments, which might anchor them to a nuclear matrix, have been identified.

The best-studied nuclear compartments are the nucleolus [12–14], the splicing-factor compartments (SFCs) [15], the Cajal body (CB) [16], the promyelocytic leukaemia oncoprotein (PML) body [17], and a rapidly growing family of small dot-like nuclear

Abbreviations used: GFP, green fluorescent protein; CB, Cajal body; RNA pol II; RNA polymerase II; SFC, splicing-factor compartment; rDNA, ribosomal genes; DFC, dense fibrillar components; snRNP, small nuclear ribonucleoprotein particle; snoRNP, small nucleolar ribonucleoprotein particle; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; PNB, pre-nucleolar body; SRP, signal-recognition particle; MDM2, murine double minute clone 2 oncoprotein; PML, promyelocytic leukaemia oncoprotein; APL, acute promyelocytic leukaemia; PNC, perinucleolar compartment; FRAP, fluorescence recovery after photobleaching; GR, glucocorticoid receptor.

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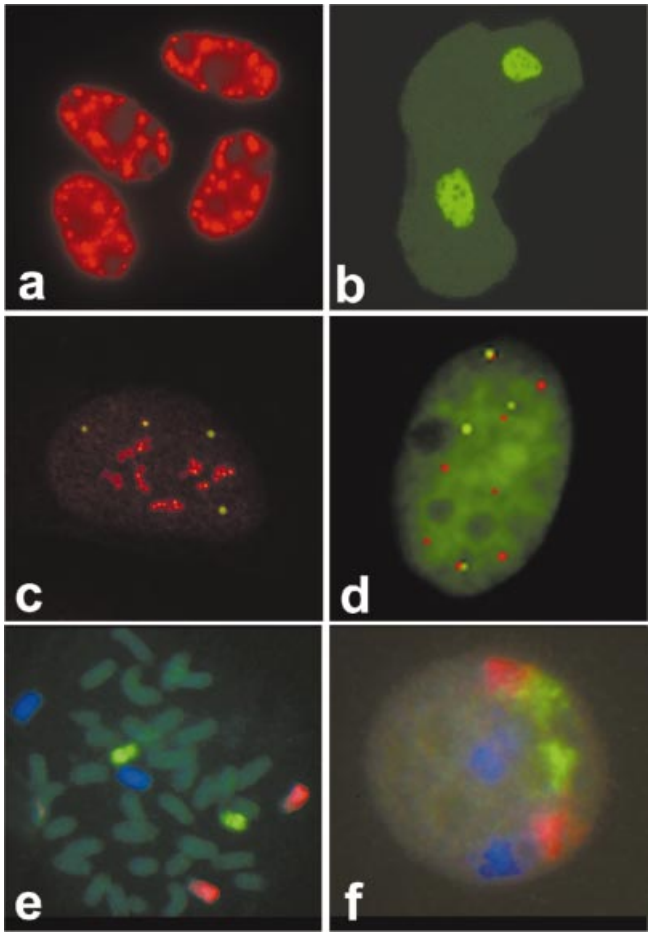

Figure 1 Nuclear compartments visualized by fluorescence microscopy

(**a**) SFCs (red), (**b**) the nucleolus (green), (**c**) the CB (green) and the nucleolus (red), (**d**) PML body (red) and CB (green), (**e**) mouse chromosomes 13 (blue), 14 (red) and 15 (green) in metaphase, (**f**) mouse chromosomes 13 (blue), 14 (red) and 15 (green) in interphase appear as chromosome territories.

bodies [7]. With the exception of the nucleolus and the SFC, the functions of nuclear bodies have not been established.

Transcription sites and SFCs

One of the fundamental cell biological questions in nuclear architecture is: where are genes transcribed? The labelling of nascent RNAs has shown that RNA polymerase II (RNA pol II) transcription takes place in thousands of discrete dots distributed throughout the nucleus [18–20]. As expected, RNA pol II components co-localize with these dots. However, RNA pol II also localizes in numerous domains outside of transcription sites [21]. These sites may be storage pools from which factors are recruited. Using fluorescent *in situ* hybridization, several specific pre-mRNAs have been localized in extended spots or elongated tracks [22,23]. The corresponding active genes are positioned at the end of these tracks [22]. Since splicing often occurs co-transcriptionally, these transcription sites may also represent sites of pre-mRNA splicing [24–26]. However, the majority of pre-mRNA splicing factors are not localized at active transcription sites, but are enriched in distinct domains, termed speckles or SFCs (Figure 1a). Using electron microscopy, SFCs correspond to perichromatin fibrils and interchromatin granule clusters. Perichromatin fibrils are fibres of variable diameter with particles of irregular size associated with them and they are thought to represent nascent transcripts [27]. Interchromatin granule clusters are composed of dense granules of 20–25 nm in diameter connected by fibres [28]. SFCs are not primary sites of pre-mRNA splicing [15]. Most active genes are found at the periphery of SFCs, and only rarely within the compartment [22,26]. The function of SFCs appears to be the storage/assembly of spliceosomal components [15]. Upon expression of intron-containing genes or viral infection, splicing factors are re-distributed from SFCs to the new transcription sites [29–31] (Figure 2d). While these observations suggest that splicing factors generally move towards a gene, it is likely that pre-mRNA also moves toward SFCs [22,30,32].

Recruitment of splicing factors from SFCs to sites of transcription is believed to involve a cycle of phosphorylation and dephosphorylation [15] (Figure 2d). Furthermore, the accumulation of splicing factors at sites of transcription is dependent on the C-terminal domain of the largest subunit of RNA pol II [33–35]. These observations are consistent with the emerging view that all RNA-processing machineries are physically linked to the transcription machinery [36,37].

The nucleolus

The nucleolus is the most prominent nuclear substructure (Figure 1b). It is assembled around clusters of tandemly repeated ribosomal genes (rDNA genes) which are transcribed by RNA pol I. The human rDNA genes are located in approx. 180 copies of a 47 kb rDNA repeat on chromosomes 13, 14, 15, 21 and 22. The regions containing the tandem arrays of rDNA genes constitute the nucleolar-organizing regions, and are the basis of the structural organization of the nucleolus responsible for targeting of all processing and assembly components required for ribosome biosynthesis [12,13].

The nucleolus is morphologically separated into three distinct components, which reflect the vectorial process of ribosome biogenesis. Fibrillar centres are surrounded by dense fibrillar components (DFCs), and granular components radiate out from the DFCs (Figure 2a). In a HeLa cell only approx. 120–150 of a total of approx. 540 rDNA genes are active [38]. Typically, a nucleolus contains approx. 30 fibrillar centres, each accommodating about four genes. Each active gene has associated with it 100–120 engaged RNA pol I molecules (approx. 15000 engaged RNA pol I molecules per cell) synthesizing each primary transcript at a synthetic rate of approx. 2.5 kb/min. Given that each transcript is approx. 13.3 kb, it takes approx. 5 min to transcribe an entire rDNA gene [38,39].

High-resolution *in situ* hybridization and Br-UTP-incorporation studies reveal that active rDNA genes are restricted to the periphery of the fibrillar centres, excluding their interior [40–43]. The absence of rDNA from DFCs [44] indicates that these nucleolar subdomains are sites of transient accumulation of both elongating and full-length primary transcripts released from the template. The series of at least ten separate pre-rRNA cleavage events is initiated in DFCs and is finished in the granular components. In addition, approx. 115 methylations and conversions of 95 uridines into pseudouridines of pre-rRNA probably occur rapidly on nascent transcripts in DFCs [45] (Figures 2a and 2b). While these modification occur, the newly synthesized pre-rRNAs leave their sites of transcription in a radial flow [46].

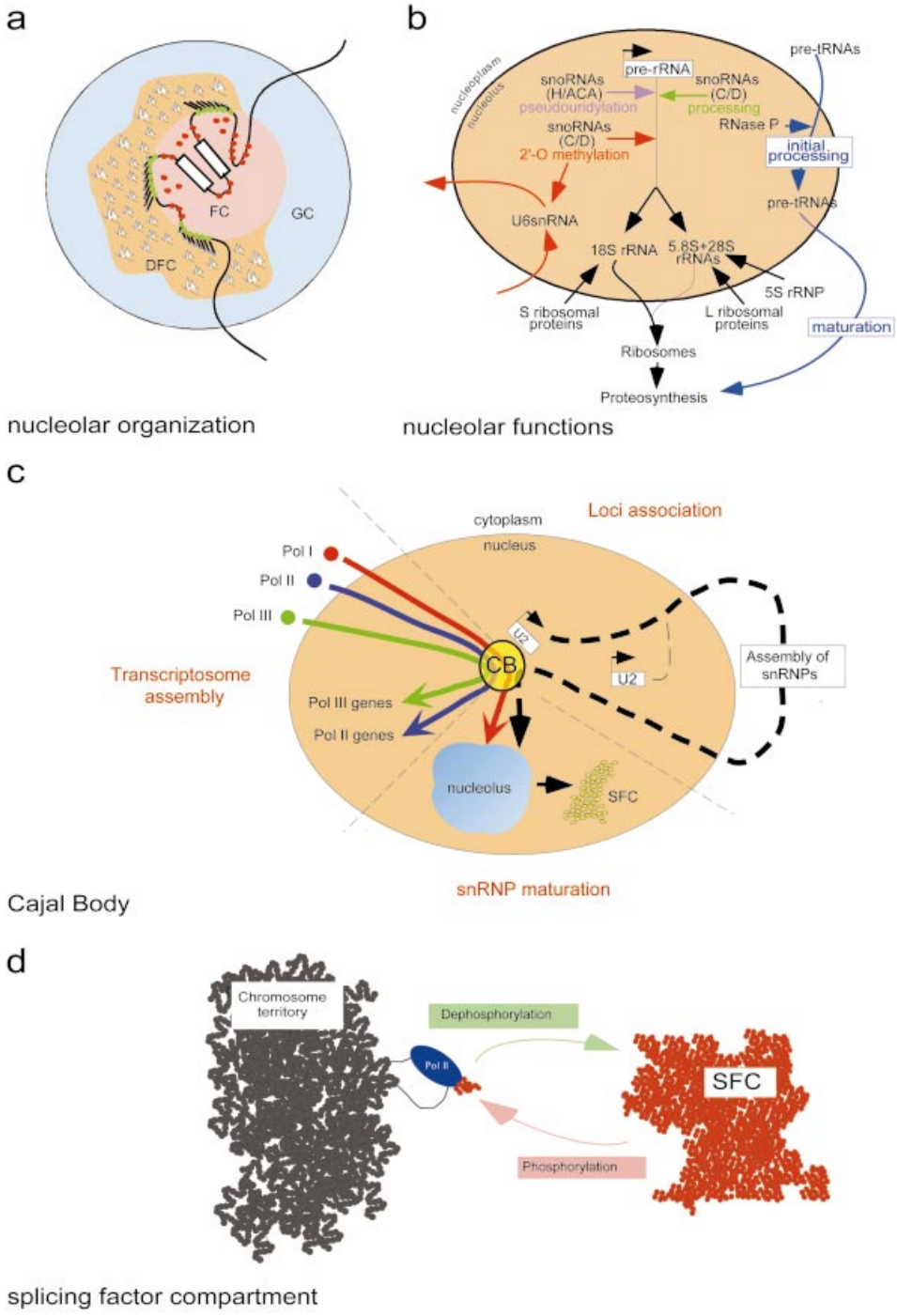

Figure 2 Organization and functions of nuclear compartments

(**a**) The nucleolus is organized from chromatin-containing rDNA genes, which are anchored in the fibrillar centre (FC). Transcriptionally active rDNA genes are located at the periphery of FCs. Nascent pre-rRNA transcripts are entering DFCs, where they undergo a series of processing steps. Later processing and assembly steps occur in the granular component (GC). (**b**) Three different functional pathways in the nucleolus. Pre-rRNAs are co-transcriptionally pseudouridinylated, 2-O-methylated and cleaved by a series of processing steps mediated by specific snoRNAs until they are fully processed, and, with association of ribosomal proteins and 5 S rRNA, assembled into large and small preribosomal subunits. They are then exported to cytoplasm to form a ribosome (black). The nucleolus is also involved in the initial step of the pre-tRNA maturation pathway. The 5′-end of pre-tRNAs are processed by RNase P before they undergo the additional processing steps in the nucleoplasm (blue). Modification of spliceosomal U6 snRNA also occurs in the nucleolus, mediated by specific snoRNAs (red). (**c**) Three possible models for CB functions. snRNA genes are frequently associated with CBs (top right), and the organelle may play a role in their transcriptional activity. The CB plays a role in maturation of snRNPs, which are exported after transcription to the cytoplasm, where they are assembled into snRNP particles and transiently reimported to the CB, from where they pass through the nucleolus and reach the SFCs (top right and bottom). Preassembly of RNA pol I, pol II and pol III transcriptosomes from newly synthesized subunits may occur in CBs before transcriptosomes are involved in transcription of specific genes (left). (**d**) Recruitment of splicing factors from SFCs. A cycle of phosphorylation and de-phosphorylation controls the association of splicing factors with SFCs. Release of factors from the SFC is promoted by phosphorylation. Accumulation of factors at active genes is facilitated by their interaction with the C-terminal domain of RNA pol II.

Processing and modification events of the primary 47 S pre-rRNA transcript are assisted by approx. 200 small nuclear ribonucleoprotein particles (snoRNPs), containing metabolically stable RNAs [47,48]. Two major classes of small nucleolar RNAs (snoRNAs), box C/D and box H/ACA, have been identified based on conserved structural elements [49,50]. In addition, some snoRNAs (U3, U8, U14 and U22) are required for pre-rRNA cleavages [47]. Little is known about the proteins associated with snoRNAs or the enzymes which catalyse these modifications. Yeast protein Cbf5p, and its mammalian homologue, rat NAP57, are candidate pseudouridylases of rRNA [51,52]. Analysis of mutants in Nop1p, the yeast homologue of fibrillarin, reveal that this protein is required for pre-rRNA methylation [53]. The recent crystallization of an archaebacterial fibrillarin homologue identified fibrillarin as the rRNA 2′-*O*-methylase [54] (Figure 2b).

The mechanisms of pre-ribosomal particle export is tightly associated with their biogenesis [55]. In yeast, over 60 trans-acting factors are necessary for efficient assembly of ribosomes [56]. The majority of these factors are localized in the nucleolus, but some are present in the nucleoplasm, and even at the nuclear pore complex. The ordered assembly of ribosomal proteins begins on the primary pre-rRNA transcript leading to the formation of a 90 S pre-ribosomal particle. The 90 S particle subsequently undergoes a series of processing steps that separate it into a pre-60 S large subunit, containing 28 S and 5.8 S rRNAs, and a 43 S small subunit precursor, containing 20 S rRNA [56]. Both of these subunits are then exported separately to the cytoplasm and are further modified to form mature ribosomal subunits (Figure 2b).

The export of ribosomal subunits has only recently become clear. Microinjection experiments in *Xenopus* oocytes show that it is an energy-dependent and receptor-mediated process [57]. In yeast, mutations affecting the function of the small GTPase, Ran, inhibit ribosomal-subunit export, as do mutations in several nucleoporins [58,59]. In addition, the yeast Nmd3p non-ribosomal protein shuttles between the nucleus and the cytoplasm, and deletion of the nuclear export signal from Nmd3p leads to nuclear accumulation of the mutant protein, inhibition of the 60 S subunit biogenesis and export inhibition of 60 S subunits [60]. The export factor Crm1p was identified as the export receptor for the 60 S subunit mediated by the adapter protein Nmd3p [60]. Taken together, these data suggest that export of pre-ribosomal subunits is mediated by specific receptors and some members of the karyopherin family [55].

The complexity of nucleolar structure is visible during mitosis, when the nucleolus disintegrates and reassembles as the daughter cells proceed toward G_1 phase. A pivotal event in this process is the repression of RNA pol I synthesis, which begins in prophase and continues into late anaphase. Recent findings [61,62] suggest that mitotic inactivation of rDNA transcription occurs under the control of the cdc2-cyclin B kinase, which impairs the interaction of selectivity promoter factor 1 with upstream-binding factor, and thus prevents the formation of a pre-initiation complex. The inhibition of cdc2–cyclin B kinase activity induces resumption of rDNA transcription in mitotic cells; however, newly synthesized primary 47 S pre-rRNA transcripts remain unprocessed [63], suggesting an independent mechanism for activation of post-mitotic pre-rRNA processing. The RNA pol I transcription machinery remains associated with nucleolar-organizing regions between nucleolar disassembly in late prophase and reassembly in late anaphase [63,64]. In contrast, partially processed pre-rRNA, in association with processing components, is concentrated in the perichromosomal regions [65] or in numerous large aggregates, termed nucleolus-derived foci, distributed throughout the cell [66]. At late anaphase RNA pol I transcription is reactivated. Simultaneously, pre-nucleolar bodies (PNBs) are formed from the maternally preserved, partially processed pre-rRNA transcripts, in association with processing components released from the peripheral layer of decondensing chromatids. The incorporation of the processing components into newly forming nucleoli is dependent on reactivated transcription within them [67,68], and the order in which nucleolar components enter nucleoli corresponds to their position in the pre-rRNA processing pathway [64], suggesting a role for self-organization in the reassembly process.

There is increasing evidence that the nucleolus not only carries out ribosome biogenesis, but is also the site of assembly of and interaction between multiple ribonucleoprotein machines, many of which are involved in protein synthesis [69]. Among them is the signal-recognition particle (SRP). Three out of the six SRP proteins (SRP19, SRP68 and SRP72) were localized in the nucleolus with SRP RNA as well as in the cytoplasm [70]. These data suggest that SRP pre-assembly occurs during its passage through the nucleolus. Furthermore, it has been shown that post-transcriptional modifications of spliceosomal U6 small nuclear RNA occur in the nucleolus (Figure 2b). The 2′-*O*-ribose methylation and pseudouridylation by three nucleolar box C/D small nucleolar RNA has already been identified and suggests that U6 snRNA is maturated there before it leaves the nucleolus and re-enters the nucleoplasm [50,71]. More recently, it was reported that the U5 snRNA also passes through the nucleolus where it is modified [72].

There is evidence that the nucleolus also plays a role in maturation of some yeast tRNAs [73] (Figure 2b). Removal of the pre-tRNA 5′-leader is performed by RNA-containing endonuclease RNase P, which is primarily found in the nucleolus [74]. Interestingly, the C-terminal domain of the human La protein, which appears to recognize the 5′-ppp ends of nascent RNAs, maintains pre-tRNA in an unprocessed state by blocking the 5′-processing site recognized by RNase P [75]. Phosphorylation of the human La protein on serine-366 reverses this block and promotes tRNA maturation. In addition, tight coupling of rRNA and tRNA synthesis has been demonstrated in yeast [76]. When the synthesis of RNA pol III transcripts is impaired in temperature-sensitive RNA pol III mutants, there is no effect on mRNA synthesis of ribosomal protein genes produced by RNA pol II; however, the formation of the 25 S, 18 S and 5.8 S rRNAs derived from the 35 S primary transcript synthesized by RNA pol I is largely turned off [76]. Thus RNA pol III mutants affect the overall rate of rRNA synthesis. Taken together, these data indicate that yeast cells can regulate pre-rRNA processing in response to RNA pol III activity [76].

The nucleolus also exerts regulatory functions. The HIV-regulatory proteins, Tat and Rev, are localized in nucleoli, where the Rev protein is capable of recruiting proteins to the Rev-export pathway [77]. Because the Rev protein binds HIV-1 mRNA, and facilitates the transport of incompletely spliced and unspliced RNAs to the cytoplasm, it has been suggested that the nucleolus plays a critical role in HIV-1 mRNA export. To test this, a hammerhead ribozyme that specifically cleaves HIV-1 mRNA was inserted into the sequence of U16 snoRNA, resulting in accumulation of the ribozyme within nucleoli of infected cells. HeLa cells expressing this nucleolar ribozyme exhibit significantly suppressed HIV-1 replication. These data suggest trafficking of HIV-1 mRNA through nucleoli of infected cells [78].

The nucleolus has also been implicated in the regulation of the tumour-suppressor protein, p53 [79]. Critical for the regulation of p53 activity is its interaction with the murine double minute clone 2 oncoprotein (MDM2), which regulates p53 degradation

in the cytoplasm through its targeting to the ubiquitination pathway. MDM2 is a shuttling protein and its activity is regulated by the $p19^{ARF}$ protein, which blocks the export of MDM2 to the cytoplasm and sequesters MDM2 in the nucleolus [80]. MDM2 becomes increasingly localized to nucleoli when co-expressed with $p19^{ARF}$. Thus retention of MDM2 in the nucleolus prevents its export to the cytoplasm for p53 degradation. Subsequently, nuclear import of p53 is activated, thereby maximizing the accumulation of p53 in the nucleus [81,82].

The Cajal body

Initially described as "nucleolar accessory bodies" by Santiago Rámon y Cajal at the start of the last century, these cytologically distinct bodies were renamed coiled bodies due to their characteristic appearance as a tangle of coiled fibrillar strands of 40–60 nm in diameter [28]. Recently [83], in honour of its initial discoverer, the coiled body was renamed Cajal body.

CBs are small spherical structures, which are typically present as 1–5 copies per nucleus, ranging in size from 0.1–1.0 μm (Figure 1c). CBs contain a large number of components, including the spliceosomal snRNPs, U3, U7, U8 and U14 snoRNAs, basal transcription factors TFIIF and TFIIH, cleavage-stimulation factor and cleavage and polyadenylation specificity factor, and nucleolar components fibrillarin, Nopp140, and protein B23 [16]. The function of the CB is unknown.

CBs are generally found within the nucleoplasm, but they have been detected within the nucleolus in human breast carcinoma cells, brown adipocytes and hepatocytes of hibernating dormice [84,85]. Several observations indicate that CBs might pass through the nucleolus. Recently [86,87], it has been shown that CBs are highly mobile structures moving throughout the nucleoplasm. In all cases they appear to have the ability to move to and from the nucleolar periphery and within the nucleolus [86,87]. At least two size classes of CBs have been characterized in living cells, the larger (termed CB) and smaller (termed mini-CB). Each appears to arise through joining and separation events [86,87].

The nucleocytoplasmic-shuttling phosphoprotein, p80-coilin, has been identified as a specific marker of CBs [88]. Formation of CBs inside the nucleolus can be induced by a single point substitution (serine to aspartate at position 202) in transiently expressed p80-coilin protein [89]. In addition, Nopp140, which interacts with p80-coilin *in vitro* [90], is present in both CBs and the nucleolus, and affects both structures when non-functional mutants are transiently expressed [90,91]. Furthermore, treatment of HeLa cells with the specific serine/threonine protein phosphatase inhibitor, okadaic acid, led to a redistribution of p80-coilin into the nucleolus [92]. Collectively, these data indicate that CBs and the nucleolus are functionally connected.

Recent evidence suggests that CBs might be involved in transport and maturation of snRNPs and snoRNPs (Figure 2c). Sleeman and Lamond [93] reported that once GFP-tagged core Sm proteins associated with U-snRNA are re-imported into the nucleus, they first localize to CBs before they pass through the nucleolus and reach their final destination in SFCs. In another study [94], the export inhibition of newly transcribed U snRNA from the nucleoplasm to the cytoplasm, and re-import of newly assembled snRNPs into the nucleus by leptomycin B, caused a progressive depletion of U snRNPs in CBs. In addition, when p80-coilin was immunodepleted from *Xenopus* egg extracts before assembly in pronuclei, CBs were still formed within the pronuclei, but they lacked both coilin and Sm proteins [95]. These observations suggest a specific role of CBs in the maturation pathway of snRNPs, which may involve steps in their modification and assembly process into RNP complexes. A similar role for CBs in the assembly and transport of snRNP has been proposed by Narayanan et al. [96]. Fluorescently labelled U3, U8 and U14 snoRNAs were microinjected into *Xenopus* oocytes, and were found to accumulate transiently first in CBs and only later in nucleoli, suggesting that snoRNAs flow from the CB to nucleoli [96].

Substantial evidence suggests that CBs associate with specific chromosomal loci (Figure 2c). They are frequently found preferentially associated with tandemly repeated histone genes on amphibian lampbrush chromosomes, and with tandemly repeated genes encoding U1, U2, U3, U4, U11, and U12 sn(o)RNAs in mammalian interphase nuclei [97–99]. Frey et al. [100] showed that artificial tandemly repeated U2 snRNA genes were associated with CBs, and that their association was dependent on the transcription activity of those genes. Furthermore, when U2 expression levels were increased by increasing the U2 copy number, their association with CBs was also elevated [100]. This indicates that targeting of CBs to this chromosomal site is mediated by an interaction with nascent snRNA transcripts. Gene loci appeared more transcriptionally active when they were associated with CBs [100,101]. Schul et al. [101] found that CBs contain the 45 kDa γ-subunit of the proximal element sequence-binding transcription factor (PTF), which is specific for snRNA genes along with TBP and a subset of RNA pol II subunits. This suggests that CBs may function as distribution centres, which recruit specific transcription and processing factors to the sites of snRNA transcription [100]. In addition, CBs can supply RNA 3′-processing factors to adjacent genes from specific domains associated with CBs, called cleavage bodies [102,103]. In addition to their association with snRNA genes, CBs also appear to preferentially co-localize with histone loci [103–105]. However, Shopland et al. [105] showed that the sustained contact of the HIST2 locus with CBs is not required for gene expression. One reason for this association might be that CBs supply factors, which promote expression of the histone genes. The increased number of CBs in late G_1 and S phase might reflect the increased level of histone gene expression. This is further supported by the finding that the CDK2–cyclin E complex is localized in CBs in a cell-cycle-dependent manner [106].

An alternative, but not mutually exclusive, model for CB function suggests that CBs are the assembly sites for the transcription machinery of the nucleus [16,83,107] (Figure 2c). The observation that factors involved in transcription, capping, splicing, polyadenylation and cleavage of pre-mRNAs are initially targeted to CBs in the oocyte [83], allows for the possibility that the RNA pol II machinery is pre-assembled in CBs with other elements of the processing machinery to form transcriptionally competent large multi-subunit complexes, termed polymerase II transcriptosomes. The existence of coupled transcription/processing machineries is indicated by the recently observed [36,37] coupling of all RNA processing events to transcription. In support, Pellizzoni et al. [108] demonstrated that gemins, which play a role in assembly and regeneration of snRNPs, interact with the C-terminal domain of RNA pol II through RNA helicase A. Expression of a dominant negative mutant of survival motor neuron causes dramatic reorganization of snRNPs into large domains containing markers of CB. This reorganization is accompanied by an inhibition of RNA pol I and pol II transcription and accumulation of the hypophosphorylated RNA pol II in these domains [108]. The observed localization of some pol I and pol III components to CBs indicates that CBs can also function as a platform for assembly of pol I and pol III transcriptosomes [16,83].

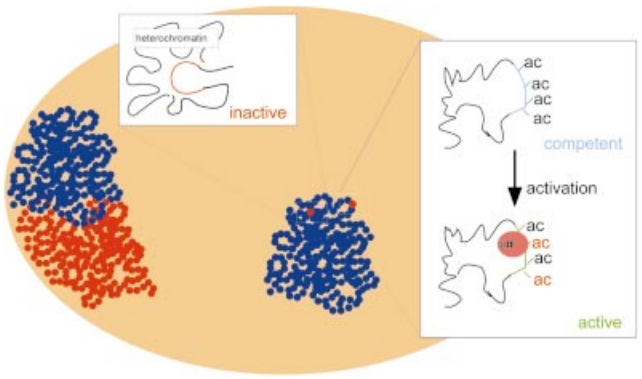

Figure 3 Chromosome territories and gene activation

Genes are found both at the periphery as well as within chromosome territories. Inactive genes are frequently associated with heterochromatin. In contrast, transcriptionally competent genes are displaced from heterochromatin, but their chromatin structure may still not be conducive to transcription. Constitutive acetylation of core histones might contribute to maintaining a particular chromatin state. Upon establishment of a particular acetylation pattern, the gene might become transcriptionally active.

PML bodies

PML bodies are small spherical domains known by a variety of other names, including nuclear domain 10, Kremer bodies, and PML oncogenic domains. PML bodies are found scattered throughout the nucleoplasm, and cells typically contain 10–30 PML bodies per nucleus, ranging in size from 0.2–1.0 μm, fluctuating in number and size through the cell cycle [109,110]. PML bodies are often found associated with Cajal bodies and cleavage bodies [102], sometimes forming triplets. Ultrastructurally, this nuclear domain resembles a dense ring, positively stained by anti-PML antibody as its defining component [111]. In addition to PML, the bodies contain many components, including Sp100, retinoblastoma protein Rb, Daxx and the Bloom syndrome protein, BLM [112]. PML bodies have been implicated in terminal differentiation, transcriptional regulation [112], nuclear storage [113], growth control [112] and apoptosis [114].

PML bodies are of clinical interest in acute promyelocytic leukaemia (APL) since they are disrupted in cell lines derived from APL patients owing to the formation of a fusion protein between the PML protein and the retinoic acid receptor, as a consequence of a reciprocal chromosomal translocation at t(15;17) q(22;21) [114a]. The disruption of PML bodies correlated with APL, and may suggest the involvement of PML bodies in the differentiation of promyelocytes [115,116]. The administration of retinoic acid results in the reappearance of the PML bodies and remission of the cancer [115,116]. Whereas the PML protein appears to be essential for proper formation and structural integrity of PML bodies, the precise function of this protein or the PML body itself remain unknown.

PML bodies have been suggested to play a role in aspects of transcriptional regulation [112]. A stably expressed reporter construct containing the lac operator sequence was found associated with PML bodies only when lac repressor protein is expressed, but no association with PML was detectable without lac repressor [117]. This suggests that the association was related to a local concentration of the expressed lac repressor protein at the gene locus, rather than the locus itself. Thus PML bodies might detect highly localized concentrations of exogeneously introduced specific proteins and potentially regulate their transcription [117].

It has been demonstrated that PML bodies are closely associated with sites of viral transcription and replication in early stages of infection. At later points of infection, some viral proteins accumulate in PML bodies and cause their disruption [113]. This has lead to the proposal that PML bodies serve as nuclear storage depots, and that deployment and decommissioning of various protein components could maintain their steady-state level [113]. In this view, accumulated proteins may not exert their primary function at these sites, but instead accumulate in PML bodies to maintain an intranuclear homoeostatic balance, which could be disturbed by hormone imbalance, viral infection, interferon action or heat-shock [111]. A prediction from this hypothesis is that protein components in PML bodies are in continuous flux in and out this nuclear domain as a response to changes in their nuclear concentration [113]. This prediction has recently been tested. It was found that CBP (CREB-binding protein, where CREB is cAMP-response-element-binding protein) rapidly moves through PML bodies, whereas the PML protein itself and Sp100, a further prominent component of PML bodies, are largely immobile within the PML body. This might either suggest that PML and Sp100 are structural proteins of the PML bodies, which anchor other components such as CBP to the PML body, or, alternatively, that PML and Sp100 are retained within the PML body because they exert a specific function within this structure [118].

Miscellaneous nuclear bodies

In addition to the nuclear structures described above, the nucleus contains a large number of less well characterized, mostly small structures [7]. Two of the most intriguing ones are the OPT (Oct1, PTF transcription) domain and the perinucleolar compartment (PNC).

OPT domains are distinct nuclear domains of unknown function. They are up to 1.5 μm in diameter and their number varies from one to very few [101,119]. OPT domains assemble during G_1 and disappear early in S phase [119]. The domains are transcriptionally active, and they associate preferentially with chromosomes 6 and 7 [119], suggesting that their formation might be related to transcription of particular genes on these chromosomes [119].

The PNC is a unique nuclear domain of irregular shape localized at the periphery of the nucleolus [120]. At the ultrastructural level, the PNC is an electron-dense structure consisting of multiple strands, and is in direct contact with the surface of the nucleolus. The PNC contains several snRNAs transcribed by RNA pol III, including RNase MRP, RNase P and RNase Y RNAs [121,122] and the polypyrimidine-tract-binding protein [120,121]. The function of the PNC is unknown, but the presence of nascent RNAs and RNA processing factors suggests a role in transcription and RNA processing [123]. Using polypyrimidine-tract-binding protein–GFP fusion protein, it has been observed that the PNC is a dynamic structure which undergoes small movements at the nucleolar periphery. During mitosis, the PNC dissociates at prophase and reforms at late telophase when post-mitotic nucleoli are already reassembled. Importantly, the presence of the PNC closely correlates with oncogenic transformation [120].

Chromosomes and nuclear compartmentalization

Chromosomes are distinct entities within the nucleus, but can obviously not be considered nuclear compartments [124]. While the distinct nature of chromosomes becomes readily apparent in metaphase, where chromosomes condense and are separated from each other, their topological organization in the interphase

nucleus was, until recently, unclear. In pioneering experiments [125,126], in which limited areas in the nucleus were irradiated using a microlaser and the damaged chromosomes then identified, the Cremer brothers demonstrated that chromosomes occupy distinct nuclear volumes, the so-called chromosome territories (Figure 1d). Within chromosome territories, active and inactive genes localize to the periphery of the territories, whereas non-coding sequences are more frequently found in their interior [127,128] (Figure 3). The observation that some active gene loci and active sites of transcription have been mapped to internal regions of chromosome territories, indicates that chromosome territories are 'porous' and that transcriptional activators can gain access to internal sites in the territory. This notion is supported by the observation that proteins of up to 500 kDa in size can easily diffuse throughout the entire nucleus [129]. The space between chromosomes can be visualized by overexpression of vimentin, which induces the formation of vimentin arrays between chromosome territories [130]. Furthermore, over-expression of the non-chromatin-binding protein EAST (enhanced adult sensory threshold) in *Drosophila* resulted in formation of an extra-chromosomal protein domain [131]. These observations paint a picture in which chromosomes are folded into a three-dimensional flexible network, thus allowing for the possibility that positioning of genes and chromosome domains can exert regulatory functions in gene expression [124].

Most cell types only express a relatively small fraction of their genome. By positioning genes into regions of chromosomes which are not accessible to chromatin remodelling factors or transcriptional activators, subsets of genes can be silenced, whereas accessible sets of genes can potentially be expressed [132]. A role for nuclear compartmentalization in the regulation of gene activity is suggested by the observation that during most differentiation processes nuclear architecture of chromatin is dramatically altered [133,134]. During differentiation of neuronal PC12 cells, for example, DNAse-sensitive chromatin, as well as pre-mRNA SFCs and Cajal bodies, are translocated from internal regions towards the periphery [135,136]. Furthermore, in cells from epileptic foci, the X chromosome is found more frequently in the interior of the nucleus compared with normal control cells, where it is typically found at the periphery [137]. Consistent with the view that intranuclear positioning of chromosomes can affect the expression of particular genes is the long-standing observation that in female cells the transcriptionally inactive X chromosome is invariably found at the cell periphery [138].

Positioning effects are more likely to act on a local level, rather than affect an entire chromosome (Figure 3). An increasing number of observations [139,140] suggest that positioning of genes near heterochromatic regions promotes gene silencing. A classic example is that of the brown locus in *Drosophila melanogaster*. Insertion of a heterochromatic block into one allele causes the association of both alleles with heterochromatin [141]. Similarly, in differentiating B-cells, transcriptionally repressed genes were typically found associated with heterochromatin [142,143]. In yeast, telomeric sequences and mating-type loci near telomeres are effectively silenced by heterochromatinization in the nuclear periphery [139,144,145]. These observations indicate that nuclear positioning, and specifically positioning of genes into heterochromatin, might be crucial for accurate execution of gene expression programmes. Positioning of genes outside of heterochromatin is not sufficient for their activation. Placement in extra-heterochromatin domains merely confers competence, rather than activity, on a gene (Figure 3). In order for genes to be activated, transcriptionally repressive chromatin must be remodelled. Much effort has recently gone into elucidating the mechanisms that lead to chromatin remodelling linked to gene expression, and numerous chromatin-remodelling activities, most notably acetyltransferases and ATP-dependent remodellers, have been identified [146].

NUCLEAR DYNAMICS

The mammalian cell nucleus is a highly dynamic organelle. The obvious dynamic reorganizations that take place during disassembly and reassembly of the nucleus during M phase have been well documented and studied in detail. However, recent observations have made it clear that many nuclear components are also highly dynamic in the interphase nucleus. The dynamic properties of chromatin, mRNA and proteins in the nucleus are crucial for the co-ordination of gene expression processes and are likely to contribute significantly to ensure accurate and efficient gene expression.

Chromatin dynamics

Time-lapse microscopy observations suggest that chromosomes and chromatin are rather immobile during interphase. *In vivo* imaging, using dyes, fluorescently tagged topoisomerase II or GFP-labelled core histone H2B, failed to reveal large-scale chromatin movements within the interphase nucleus [147–149]. More detailed analysis using labelling of specific loci in yeast [148] only revealed restricted Brownian motion, suggesting that chromatin is positionally stable. However, the independent realization, mostly from biochemical studies, that chromatin structure exerts a major regulatory role in gene expression directly indicates that dynamic chromatin remodelling, at microscopic levels smaller than those detected by in the experiments cited above, does occur and is, in fact, important for controlled gene expression. Recent studies confirm this notion.

The textbook view of chromatin is that the lowest organizational unit of chromatin is the nucleosome, consisting of the core histones H2A, H2B, H3, and H4, linked by the linker histone H1 [4]. Strings of linked nucleosomes are helically twisted into a 10 nm fibre, which, in turn, is folded into a 30 nm fibre. This fibre is suggested to be folded into higher order chromatin loops, although the topological organization of these higher order structures *in vivo* is unclear. Robinett et al. [150] have recently developed the first system to study higher order chromatin structure and its dynamics in living cells using microscopy. Using this GFP-based system in interphase cells, large-scale chromatin fibres with diameters of approx.100 nm could be observed, directly demonstrating in intact cells the existence of higher order chromatin structures, which had previously only been observed in chemically fixed cells by light and electron microscopy [4,150]. Consistent with previous observations, time-lapse microscopy indicated that the higher order chromatin fibres were relatively immobile. However, upon forced transcriptional activation by overexpression of the VP16 transactivator, the labelled chromatin domain unfolded, most likely as a consequence of chromatin remodelling [151,152]. These reorganizations are not the consequence of the artifactual action of the strong VP16 transactivator, since similar observations have been made in more physiological reporter systems using the mouse mammary tumour virus promoter or a reporter system that generates functional mRNA from a tetracycline-regulatable promoter [117,153]. These findings confirm and extend into mammalian cells early observations on polytene chromosomes in the dipteran fly *Chironomus tentans* and in lampbrush chromosomes, where transcriptional activation results in the appearance of chromosomal puffs and the size of a puff is proportional to the amount of transcript made [154,155]. These

new microscopy methods will enable us, in the near future, to determine how the macroscopic unfolding of chromatin fibres observed in living cells is related, if at all, to the microscopic reorganizations of nucleosome structure brought about by chromatin-remodelling activities.

RNA dynamics

The mechanisms of transport of nascent mRNAs away from their sites of transcription and subsequent export from the nucleus have been topics of intense investigation. An attractive model [156] suggested that newly synthesized RNAs moved, possibly by an active and directed process, to the closest nuclear pores. This 'gene-gating' model was consistent with the observation of elongated tracks of mRNA extending from transcription sites [157–159]. Furthermore, in *Drosophila*, several RNA transcripts appeared vectorially transported to the apical, but not the basal, portion of the cytoplasm [160,161]. Closer analysis, however, reveals that the initially observed tracks of RNA radiate in random direction from transcription sites [23,32], suggesting that RNA export is not a directed process. Further investigation of RNA export in *Drosophila* reveals that the unequal distribution of RNAs is most likely due to cytoplasmic 'retention factors', rather than vectorial export [162]. The alternative to an active export process is that RNA moves by diffusion from its sites of transcription to nuclear pores.

The ability of RNAs to freely diffuse throughout the nucleus was demonstrated in *Drosophila* using a highly expressed reporter gene, whose mRNA was found throughout an 'extra-chromosomal channel network' rather than in distinct tracks [163]. The importance of diffusion as the major mechanism of RNA transport *in vivo* has recently been demonstrated by live-cell microscopy experiments. Visualization of mRNA using hybridized oligonucleotides containing an activatable fluorescence group demonstrates non-directed, energy-independent movement of polyadenylated RNAs through the nucleus [5,164,165]. Similarly, results from stereological electron microscopy analysis of labelled RNA particles [166] are consistent with passive diffusion as the driving force in RNA export. Diffusion is a very efficient mode of transport for RNA. The measured diffusion coefficient of fluorescently labelled RNAs within the cell nucleus is similar to that measured for an RNA particle in solution [165]. In fact, using these diffusion coefficients, it can be estimated that an RNA particle can be exported from the nucleus within seconds of release from its transcription site. While these observations demonstrate that RNAs can diffuse through the nucleus, and suggest that diffusion might be the major mode of transport of nuclear RNA molecules, it cannot be excluded that some RNAs are transported by active and, possibly, directed mechanisms.

Protein dynamics

Each mammalian cell nucleus with a typical diameter of approx. 10 μm contains about 2 m of DNA. Obviously, DNA needs to be condensed to fit into the limited nuclear volume. Given the high degree of chromatin packing and the vast number of RNA and protein molecules within the nucleus, it has been generally assumed that the nucleus is a densely packed organelle in which it might be difficult for proteins and RNAs to move about. If this is indeed the case, transport mechanisms (possibly active and directed) are likely to exist to bring proteins to their appropriate sites of action. This model is virtually ruled out by recent live-cell microscopy experiments [6], which demonstrate that proteins can move by a passive, diffusion-based mechanism throughout the entire nucleus.

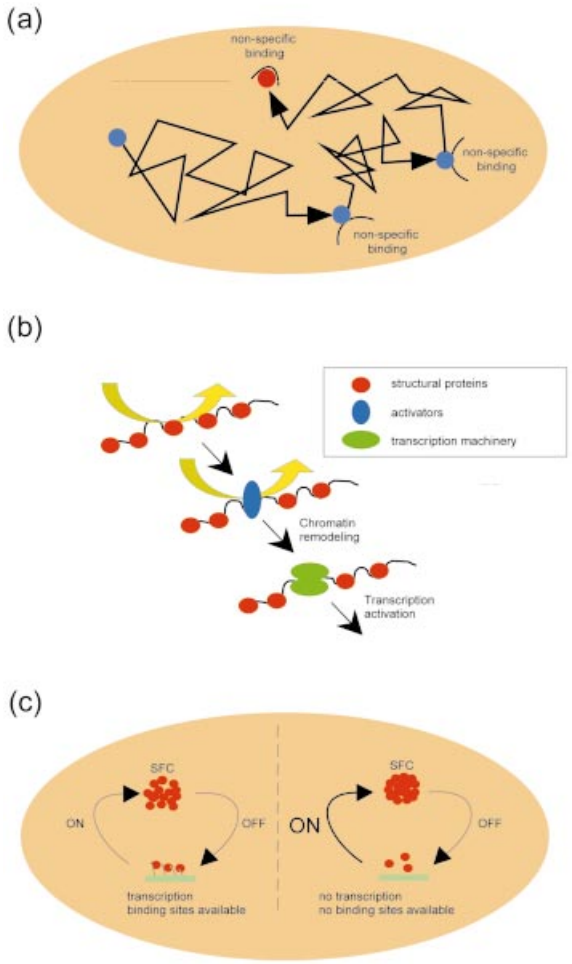

Figure 4 Protein dynamics in the cell nucleus

(**a**) Proteins roam the cell nucleus and scan for potential binding sites. Before finding a specific, high-affinity binding site, each protein is likely to interact with many non-specific, low-affinity binding sites. (**b**) Chromatin-binding proteins, both structural proteins as well as transcriptional activators, only transiently interact with chromatin. The periodic removal of structural proteins allows the access of chromatin-remodelling activities to chromatin. These activities, in turn, have only short residence times on chromatin. (**c**) Nuclear compartments are in constant flux. The morphological appearance of a compartment is dependent on the on/off rates of its components. Splicing factors continuously dissociate and reassociate with SFCs. After dissociation, they search for unspliced RNA to bind to. This establishes a steady-state equilibrium. If no unspliced transcripts are available, the splicing factors spend less time outside of the compartment before they return and the net influx of proteins is increased. Splicing factors accumulate in the compartment, and it appears morphologically more round.

Fluorescence recovery after photobleaching (FRAP) experiments demonstrate that many nuclear proteins are highly mobile within the nucleus (Figure 4a). High mobility is a general feature of nuclear proteins, and has been demonstrated for transcription factors [153,167], pre-mRNA splicing factors [168,169], rRNA processing enzymes [169,170], DNA repair enzymes [171], chromatin-binding proteins [169,172–174], 3′-processing factors and apoptotic caspases (T. Misteli, unpublished work). For most proteins the mobility is independent of energy (in the form of ATP), and is not affected by reduction of temperature, indicative of a passive diffusion process. Diffusion provides an efficient, rapid mode of transport. A protein or

protein complex up to approx. 500 kDa can traverse the nucleus within less than 1 min. Diffusion also provides an energetically economical system to ensure the availability of proteins throughout the nucleus. Importantly, no signals or signal receptors are required to bring proteins to their target sites. Rather, proteins roam through the nucleus in search of a high-affinity binding site where they can exert their functions [6] (Figure 4a). The roaming behaviour of proteins is clearly demonstrated by DNA-repair factors. In the absence of DNA damage, the repair enzyme ERCC1/XPF is freely mobile within a nucleus. Upon induction of DNA damage by UV radiation, the protein is temporarily immobilized because of binding to damaged sites. Once it has acted at the damage site, it dissociates and becomes once again highly mobile [171]. Similar observations have been made for DNA replication factors, which are immobilized at replication sites before they dissociate and diffuse to find the next origin of replication [175].

Of particular interest for the understanding of gene expression is the study of dynamic properties of chromatin-binding proteins. It is typically assumed that chromatin-binding proteins are stably and statically bound to chromatin. While this appears to be true for the core histones [169,172], virtually all other chromatin-binding proteins and components of the transcription machinery associate only transiently with chromatin (Figure 4b). The linker histone H1, which resides outside of nucleosomes but acts as a structural chromatin protein, is dynamically exchanged between binding sites [172,173]. Each molecule of H1 resides for only about a minute on chromatin before it dissociates [173]. Similarly, another group of structural chromatin-binding proteins, the high-mobility group proteins, associate only transiently with chromatin, residing on chromatin for less than 5 s [169]. The short interaction period of these proteins might be crucial to ensure the ability of a cell to respond to external stimuli by rapid changes in its gene expression programme. If chromatin-binding proteins, particularly transcriptional repressors, such as linker histones, were statically associated with chromatin, it might be difficult for chromatin-remodelling factors to gain access to chromatin [6]. However, each time an H1 molecule dissociates, a window of opportunity opens, and transcriptional activators can access chromatin and remodel it (Figure 4b). This interpretation is consistent with the recent observation that the remodelling activity SNF/SWI can only act on chromatin upon removal of histone H1 [176].

Histones and high-mobility group proteins are general chromatin-binding proteins and they do not bind DNA in a sequence-specific manner. Do transcriptional activators, which recognize their specific response elements in promoters, bind more tightly to chromatin? FRAP experiments on steroid receptors indicate that even specific DNA-binding proteins only transiently interact with their targets. The glucocorticoid receptor (GR) binds DNA in a specific GR-response element, and regulates transcription by interaction with components of the transcription machinery. Photobleaching experiments on an array of multiple GR-response elements, which allows visualization of this specific site in living cells, clearly demonstrate that although GR molecules are present at these promoter-binding sites for as long as the target genes are activated, the activating GR molecules are continuously and rapidly exchanged, and reside for only a few seconds at the GR-response element, supporting a 'hit-and-run' mode of binding [153,177]. Identical results were observed with another steroid receptor, the oestrogen receptor [167]. The observation that different ligands of the oestrogen receptor significantly alter the receptor's dynamic binding behaviour strongly suggests that alteration of the dynamic binding properties of a receptor, either by modification of the receptor itself or its interacting proteins, can be used as a regulatory mechanism in gene expression [167].

Compartment dynamics

Nuclear compartments can be observed for several hours by time-lapse microscopy, despite their lack of membrane boundaries, and therefore appear as stable structures. Their stability could suggest that they are inert structures, akin to rocks in the surf. However, when the internal dynamics of compartments were probed by selectively bleaching them in FRAP experiments, a rapid and continuous exchange of proteins between compartments and the nucleoplasm was observed (Figure 4c). Rapid exchange of proteins between compartments and the nucleoplasm has been observed for virtually all nuclear compartments, including the nucleolus [169,170], SFCs [168,169], the CB [170], the PNC [120] and heat-shock granules [178]. The flux of proteins through these compartments is remarkable. For example, more than 10000 molecules of the rRNA processing enzyme fibrillarin are released each second from the 2–3 nucleoli in a cell nucleus [169]. These observations suggest that far from being solid structures, nuclear compartments are in constant flux [6].

Because of the high flux of proteins, compartments must be considered reflections of a steady-state equilibrium. The morphology of each compartment is determined by its residents' on-and-off rates (Figure 4c). These rates, in turn, are determined by the functional status of the resident proteins, both within the compartment and outside of the compartment. Interestingly, virtually all nuclear compartments can be dissolved, and some exaggerated in size, upon experimental manipulation. This rather fragile equilibrium is probably best illustrated in the SFC. In a normally growing cell, these compartments appear as irregularly shaped, morphologically distinct structures of approx. 1 μm in diameter, and pre-mRNA splicing factors are believed to continuously dissociate from them in search of targets, i.e. unspliced RNA [15]. If the number of available transcripts is reduced by inhibition of transcription, splicing factors spend less time outside of the compartment, resulting in an increased association rate [179,180]. Since the dissociation rate is unchanged, a net increase in the on rate results and the compartments increases in size (Figure 4c).

A possible role for self-organization in nuclear architecture

The general theme that emerges from these observations on protein dynamics is that stable configurations can be generated in the nucleus by the interaction of highly dynamic components (Figure 5). The generation of stable configurations from highly dynamic components is consistent with a role of self-organization as a driving force in generation of nuclear architecture [6]. In addition, the observed roaming of proteins through nuclear space, the short residence time of proteins at binding sites, and the resulting ability to repeatedly interact with partners, are prerequisites for self-organization. While no direct evidence for a role of self-organization in establishing and maintaining nuclear architecture exists, numerous observations are consistent with this hypothesis.

Several experimental observations suggest that self-organization plays a role in the structural integrity of many, if not all, nuclear compartments. The above described response of SFCs to the availability of pre-mRNAs, illustrates the self-organizing properties of the SFC. Components of Cajal bodies [181], PML bodies [182] and gems [183] have all been demonstrated to have self-associating properties consistent with their self-organization. The nucleolus is a particularly striking example of a self-organizing structure. The morphological integrity of the

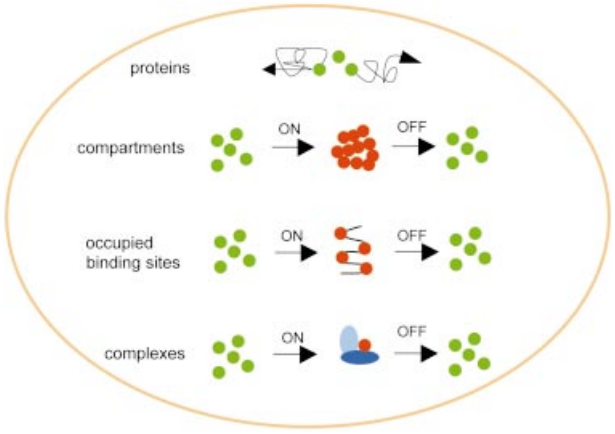

Figure 5 Stable configurations can be generated from dynamic interactions

Proteins are highly mobile within the nucleus. Compartments appear to contain a stable composition of proteins, but each component of the compartment is continuously and rapidly exchanged. Sites on chromatin appear to be stably occupied by proteins, but each binding protein is continuously and rapidly exchanged. Protein complexes appear to be made up of a stable components, but each component may be periodically exchanged.

nucleus is strictly dependent on its transcriptional activity. Upon inhibition of transcription, the nucleolus disassembles and nucleolar components associate with each other, indicating that the normal organization of the nucleolus relies on the preferential interaction of its proteins with rRNA [67,68,184,185]. Conversely, introduction of rDNA minigenes results in the formation of mini-nucleoli in the nucleoplasm, directly demonstrating that rDNA transcription is sufficient for the assembly of the nucleolus [186,187]. The requirement for ongoing transcription in establishing nucleolar morphology becomes evident during reassembly of the organelle at the end of mitosis. At a time when rDNA transcription has not resumed yet, nucleolar components accumulate in the re-forming nucleus in PNBs [14,64]. After resumption of transcription, nucleolar components accumulate at rDNA-transcription sites. The order of accumulation reflects the order of their involvement in rRNA processing, suggesting that their 'targeting' is directly dependent on their function [64]. FRAP experiments have demonstrated that at all times during M phase nucleolar proteins rapidly exchange between PNBs and the nucleoplasm [64]. We envisage that, similar to the situation in SFCs, nucleolar factors continuously dissociate from PNBs in search of appropriate binding sites.

The role of self-organization in determining nuclear architecture and function is not limited to nuclear compartments, but likely also applies to the formation of transcription and replication sites [188]. Replication factors are homogeneously distributed throughout the nucleus, but form distinct foci during S-phase becuase of their recruitment to origins of replication [175,189,190]. The factors dissociate from foci when replication of a particular region is completed. The factors then diffuse through the nucleus in search of a new origin of replication where they reassemble to form a new focus [175]. This behaviour demonstrates that just like in bona fide compartments, replication factors are continuously exchanged from the replication sites, generating a stable, but highly dynamic nuclear structure. Analogously, transcription factors dissociated from transcription sites, may diffuse through the nucleus and associate with a new transcription site. The conclusion from these observations must be that self-organization plays a role in establishing and maintaining nuclear architecture.

CONCLUSIONS

It is now well established that the nucleus contains numerous distinct compartments. An ongoing challenge is to establish the functions of these compartments. A critical step in this endeavour will be the biochemical purification and characterization of nuclear compartments and, perhaps more importantly, the *in vitro* reconstitution of compartments and their functions. Recent observations suggest that the nucleus is a stable, yet highly dynamic, structure and that the dynamic properties are crucial for its accurate functioning. Whereas most studies to date have analysed the dynamic properties of large mixed populations of proteins, important insights will be gained in the future from the study of proteins at specific loci within the nucleus, the visualization of single-molecule dynamics using high-speed microscopy, and from the analysis of protein dynamics within larger complexes. Although we have recently learnt much about protein dynamics, a complete picture of how genomes function *in vivo* can only be obtained by uncovering the organizational principles and the microsocopic dynamics of chromosomes and chromatin. Regardless of the pace of future progress, the ability to visualize dynamic properties of chromatin, RNA and proteins in living cells has already given a more complete picture of how nuclear processes are organized, and has added an additional dimension in the study of nuclear processes and gene expression.

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