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Dynamic organization of the cell nucleus

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The dynamic organization of the cell nucleus into subcompartments with distinct biological activities represents an important regulatory layer for cell function. Recent studies provide new insights into the principles, by which nuclear organelles form. This process frequently occurs in a self-organizing manner leading to the assembly of stable but plastic structures from multiple relatively weak interaction forces. These can rearrange into different functional states in response to specific modifications of the constituting components or changes in the nuclear environment.

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Introduction

The cell nucleus is confined by the nuclear envelope and separates the genome from the cytoplasm. It harbors DNA-associated activities like gene expression, replication, recombination and repair, as well as RNA processing and ribosome subunit assembly. This makes the nucleus the central hub for the determination of cell fate (Figure 1). While epigenetic modifications like histone methylation/acetylation or DNA methylation can differentiate between cell states, they often require a readout by other factors to be functional. The complex dynamic organization of the nucleus participates in this process as it directly affects the central molecular processes mentioned above. Thus, the spatial and temporal organization of proteins and nucleic acids in the nucleus can be regarded as an additional control layer for cell function on top of the (epi)genome and the proteome [1–8]. Various nuclear subcompartments or organelles have been described, in which specific biological activities are concentrated. As discussed in a number of recent reviews, the most prominent of these include (i) the nuclear envelope [6,9], (ii) the confinement of individual chromosomes to certain regions

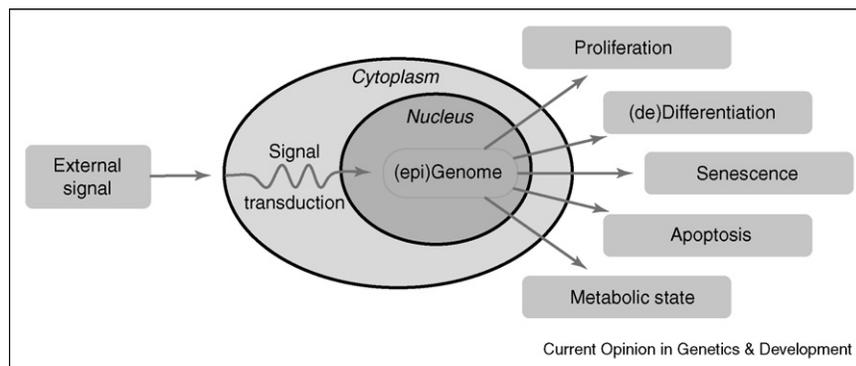
of the nucleus into ‘chromosome territories’ [4,10], (iii) the nucleolus as the site of ribosome subunit biogenesis [7,11], (iv) Cajal bodies (CB) [11], promyelocytic leukaemia (PML) nuclear bodies (PML-NB) [12,13] and SC35 domains (‘speckles’) [11,14], which represent mobile particles involved in RNA processing, transcriptional regulation and apoptosis (v) the assembly of active RNA polymerase II into ‘transcription factories’ [3,5], and (vi) DNA-replication and DNA-repair complexes [1]. Here, I will focus on outlining general principles of nuclear organization during interphase that emerge from recent work. The question addressed is how the dynamic organization of nuclear structures is established and how the rearrangement into different functional states is accomplished.

The nucleus environment

The cell nucleus is densely packed with macromolecules. Protein concentrations of 110 mg/ml in the nucleoplasm, 140 mg/ml in Cajal bodies, 160 mg/ml in SC35 domains and 220 mg/ml in nucleoli have been measured in *Xenopus* oocyte [15[•]]. Typical nuclear ion concentrations are ~ 0.1 M K^+/Na^+ ($K^+ > Na^+$), 0.5–1 mM Mg^{2+} and low μM values of Ca^{2+} [15[•],16]. The apparent viscosity of the nucleoplasm inferred from the mobility of green fluorescent protein (GFP) is 3.1 times higher than water [17]. The above inorganic cations are significantly more abundant than the corresponding mobile anions since nucleic phosphate groups and negative protein charges are in excess of the positive protein charges. This is of relevance for *in vitro* studies of macromolecular interactions with KCl or NaCl concentrations equivalent to the physiological ionic strength. These introduce an artificially high Cl^- concentration that can significantly disturb protein–protein or protein–DNA interactions [18].

The average concentration of nucleosomes has been determined in HeLa cells to be 0.14 ± 0.03 mM on an average and 0.2–0.3 mM for dense chromatin regions [19]. Assuming the packaging of nucleosomes into a 30 nm chromatin fiber with a mass density of 6 nucleosomes per 11 nm fiber this density is in good agreement with the measurements of the nuclear accessibility to microinjected fluorescently labeled dextrans with increasing size from 4 kDa to 2.5 MDa [20[•]]. From these experiments apparent pore sizes of 60–100 nm (bulk chromatin), ~ 40 nm (majority of dense chromatin regions) and ~ 20 nm (small fraction of particularly dense chromatin) have been derived. These correspond to local nucleosome concentrations of 0.06–0.13 mM, ~ 0.2 mM and ~ 0.45 mM [20[•]], with an estimated theoretical upper limit of ~ 1.2 mM nucleosomes for the densest possible

Figure 1



Cell fate decisions in the nucleus. In a simplified global view, the decisions on cell fate are made in the nucleus: An external signal as for example the presence or absence of certain growth factors in the medium is translated via a signal transduction cascade into changes of genome organization and associated activities. This in turn modifies the gene expression pattern and determines whether the cell will differentiate, proliferate, go into senescence or apoptosis or will adopt a specific metabolic state.

chromatin packaging [19]. Thus, chromatin alone provides a concentration of 7 mg ml^{-1} histone proteins and 19 mg ml^{-1} DNA and occupies $\sim 10\%$ of the total nuclear volume. From the above it follows that the total concentration of macromolecules in the nucleus is similar to that in *Escherichia coli* and in the order of 200 mg ml^{-1} occupying 20–30% of the total nuclear volume [21].

Dynamics of the interphase nucleus

As pointed out previously, nuclear substructures may be regarded as self-organizing entities that, because of the intrinsic properties of their components, assemble into distinct but dynamic structures [1,3,22–24]. These maintain their ability to rearrange into different functional states as evident from the fast exchange of protein components with the nucleoplasm on the scale of seconds to minutes. This holds true for the nucleolus [7,11], transcription complexes and replication factories [1], Cajal [25] and PML-NBs [12,26], SC35 domains [11,14] as well as chromosomal proteins, the mobility of which can be described by a reaction–diffusion model [17]. Accordingly, most of the nuclear proteins are present in a freely mobile fraction in the nucleoplasm, which ensures their instant availability for higher order assembly [22]. Even the core histones with typical residence times of $\sim 2 \text{ h}$ in their DNA bound form can adopt a state with highly increased mobility termed hyperdynamic chromatin in mouse embryonic stem cells [27^{••}]. It is also noteworthy that the dynamic assembly/exchange properties of nuclear proteins are both regulated and of functional relevance [1,11–13,27^{••},28[•]].

An additional layer of dynamics results from the translocation of nuclear bodies within a chromatin network that itself is mobile [2,29]. Average apparent diffusion coefficients of $1\text{--}2 \times 10^{-4} \mu\text{m}^2 \text{ s}^{-1}$ have been reported for nuclear particle and chromatin loci movements within

an accessible region ('corral') of 200–300 nm radius that can translocate in the nucleus as part of larger chromatin domains [29]. These relatively slow and confined movements are in agreement with a territorial organization of chromosomes and apparent from the slow mobility of chromatin ends formed after introducing a DNA cut [30]. However, although the mobility of most chromatin loci appears to be quite restricted, long-range movements have been reported under certain conditions [31], and are important in the context of transcription related chromatin reorganizations [1,2,5].

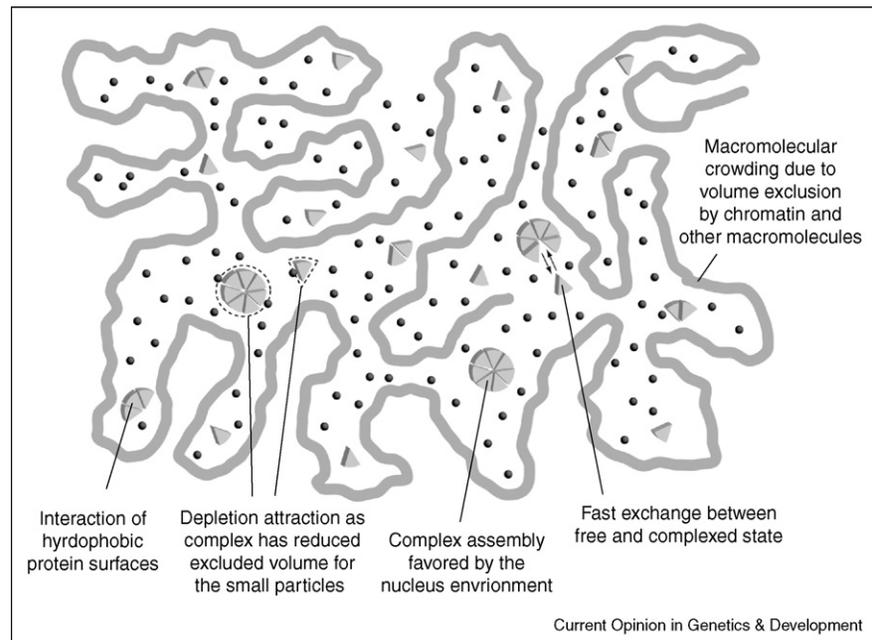
Forces driving macromolecular assembly in the nucleus

To explain the origin of dynamic features of nuclear organization, different forces contributing to macromolecule association have been considered as discussed below and depicted schematically in Figure 2 [3,21,32–36,37[•]]. Their free energy contributions are given in terms of $k_B T$, which is the product of the Boltzmann constant k_B times the absolute temperature T . The thermal energy of $1 k_B T$ or 2.5 kJ mol^{-1} at room temperature (~ 1 hydrogen bond) reflects the energy available to the system for spontaneously occurring reactions and rearrangements. This value compares to $\sim 12 k_B T$ for the hydrolysis of ATP and $\sim 20 k_B T$ (dissociation constant of 10^{-9} M) for the specific binding of a protein to its DNA target sequence.

Macromolecular crowding

Because of the high protein and nucleic acid concentration macromolecules are excluded from a significant fraction of nuclear space in a size dependent manner. This effect is termed macromolecular crowding, and is an important driving force for compartmentalization within the nucleus [21,32,33]: (i) The translational entropy of each species is decreased so that the effective concentration or thermodynamic activity is increased up to a 100-fold under

Figure 2



Subcompartment assembly in the nucleus. The association of a particle into a heptameric complex in the nucleus environment is depicted schematically. The process is reversible and a fast exchange between free subunits in the nucleoplasm and in the complex state exists. The high concentration of nucleic acid and protein components reduces the accessible volume ('macromolecular crowding') so that the effective subunit concentration is increased, which favors association. If a complex is formed the volume excluded to the abundant small particles is also increased. This represents a favorable entropic 'depletion attraction' force. Finally, the displacement of water from hydrophobic surfaces of the protein is also driving the association into a dynamic complex.

typical *in vivo* conditions. This shifts the equilibrium distribution in favor of complex formation [32]. For a bimolecular reaction a 100-fold concentration increase of both reaction partners would correspond to an equivalent favorable free energy of $4\text{--}5 k_B T$. (ii) Phase separation or demixing of different macromolecules can be promoted [33]. (iii) The particle mobility because of diffusion is reduced leading to the confinement of translocations. The latter effect has been recently evaluated quantitatively for the mobility of GFP in an *E. coli* cell in dependence of the biopolymer volume fraction [21]. It is opposing a potential increase of the association kinetics because of the increase of the effective concentration mentioned above. *In vitro* the effect of macromolecular crowding can be reproduced for example by the addition of $50\text{--}100 \text{ mg ml}^{-1}$ of the inert flexible-coil polymer polyethylene glycol (PEG) [18].

Depletion attraction

Recently, a second entropic contribution has been recognized as an important factor in genome organization [3,34,37*]. This 'depletion attraction' force refers to an increase of the entropy because of the interaction of larger particles in a mixture of smaller particles. The association of the larger particles increases the available space for the more abundant smaller particles, which provides a

favorable entropic contribution relevant for the formation of transcription factories and genome organization. The free energy gain estimated from depletion attraction for the assembly of a transcription factory like structure on a 0.4 Mb DNA fragment has been calculated to comprise $\sim 4 k_B T$ [3,34].

Hydrophobic effect

The aqueous environment of the cell with a relatively high ionic strength counteracts any free energy gains from hydrogen bonds and electrostatic interactions forming upon macromolecule association. This is because these interactions can form just as well with the highly abundant water or ion molecules. Accordingly, the hydrophobic effect has been identified as the driving force for protein folding several decades ago [35,36]. It refers to the clustering of hydrophobic surfaces to minimize the unfavorable energy term that is associated with the solvation of these surfaces in an aqueous environment. The hydrophobic effect is well recognized as an essential component for the assembly of lipid bilayers as in the nuclear envelope, the formation of intracellular compartments, and in protein folding with typical energies of $\sim 4 k_B T$ for the burial of an hydrophobic amino acid side chain [35,38]. Importantly, it is also a significant contribution in protein–protein and protein–DNA interactions

and can increase specificity of protein binding to DNA because of induced folding of the protein chain [38]. Recently, a number of advances have been made in the quantitative description of the hydrophobic effect that are relevant for protein–protein interactions involved in self-assembly of large protein complexes [36].

Dynamic organization of PML bodies and interphase chromosomes

The forces described above promote the assembly of subcompartments in the nucleus and are in the order of several $k_B T$. Energies of this magnitude are compatible with self-organizing systems: they are sufficient to promote the association into distinct complexes and subcompartments but are not too high as to impose a kinetic barrier towards reorganization. Both molecular crowding effects as well as the depletion attraction force occur with completely inert particles and represent unspecific purely entropic contributions to the free energy term of association/complex formation. Thus, they can be regarded as forces that facilitate association interactions in general but do not provide any specificity. By contrast, hydrophobic interactions require a spatial match of the interacting surfaces and provide more selectivity while maintaining the flexibility of the associating components in the complex. ‘They lead to structures that are not rigid and are thus uniquely suited for the first critical steps in the organization of living matter’ [35]. To illustrate the above concepts, two examples for nuclear subcompartment organization, namely, PML–NBs as well as interphase chromosome domains are discussed in the following.

PML nuclear bodies

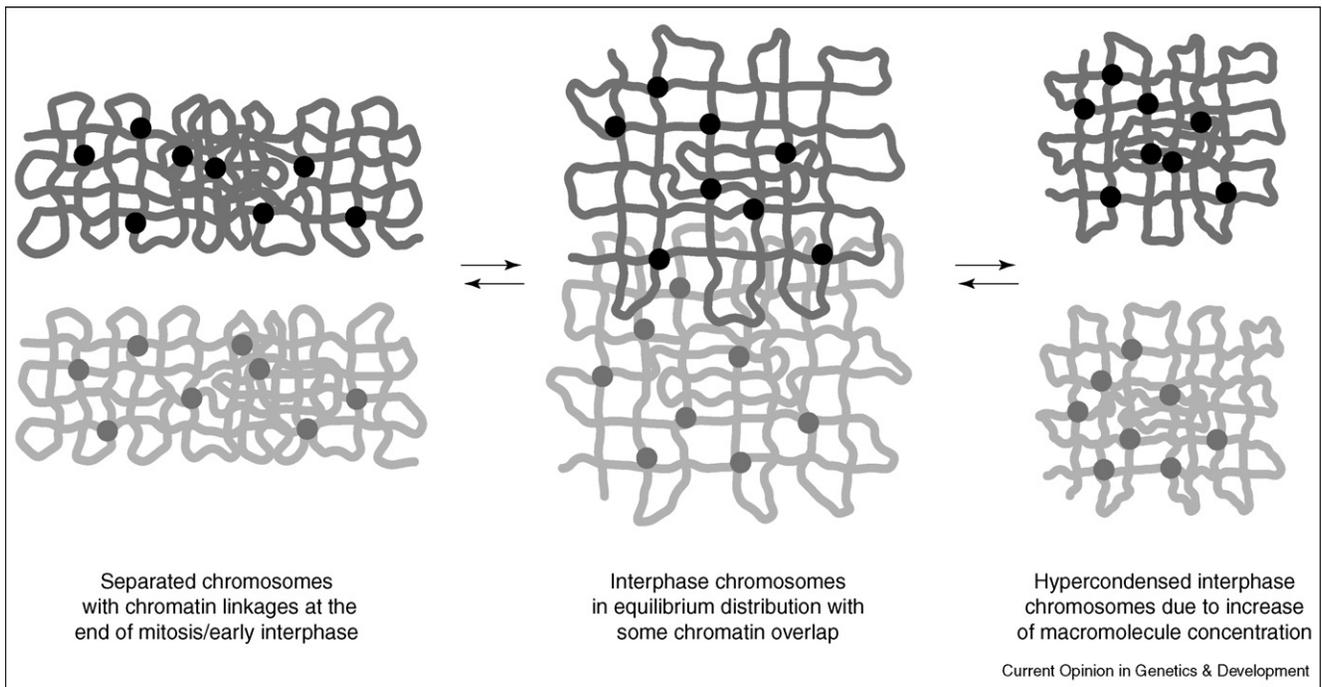
It has been shown that PML–NBs disassemble when cell nuclei are expanded into a medium of low ionic strength [39]. They reassemble when an inert molecular crowding reagent like PEG was added, pointing to a self-organizing mechanism for their formation. The RING protein domain that is found in the PML protein sequence has been identified as a module for supramolecular assembly that also involves hydrophobic interactions [24,40]. In addition, it has recently been demonstrated that sumoylation of PML is a crucial factor to control the (self-) association properties of PML protein [13,28^{*}]. The RING domain appears to be critical for the effects of PML sumoylation. A mechanism has been proposed in which PML sumoylation and non-covalent binding of PML to sumoylated PML regulates PML–NB assembly and the recruitment of other proteins [28^{*}]. The dynamic regulation of PML protein association and interaction properties by post-translational modification and other factors is supported by the occurrence of multiple distinct PML–NB forms. In addition to the canonical PML structures [12], telomere–PML–NB complexes involved in the alternative lengthening mechanism used in telomerase negative cancer cell lines have been observed [41]. Moreover, unusually large PML–NBs composed of several

layers of protein compartments form in lymphocytes of patients with immunodeficiency, centromeric instability and facial dysmorphism (ICF) syndrome [42].

Interphase chromatin organization

A striking demonstration of the dynamic but specific organization of interphase chromosomes in the nucleus has been made in two recent studies [43^{*},44^{*}]. The addition of 100–200 mM concentrations of salt or small inert molecules like sucrose or sorbitol to standard growth medium of physiological ionic strength increases the concentration of macromolecules in the nucleus because of the loss of water. This induces a drastic but reversible chromatin compaction to a hypercondensed state that can be assigned to the effects of macromolecular crowding and attraction depletion forces [44^{*}]. The chromatin conformation changes, albeit more pronounced, appear similar to the effect of ATP depletion [29,43^{*}] or histone hyperacetylation [20^{*},45^{*}]. In all these transitions of the chromatin compaction state, the underlying chromosome structure seemed preserved. The interactions responsible for this behavior are also essential for the territorial organization of chromosomes in order to restrict intermingling of chromatin from different chromosomes to a partial overlap region, the extent of which is somewhat controversial [2,4,43^{*}]. As a structural scaffold for the organization of chromosomes into distinct nuclear regions, the existence of a nuclear matrix has been proposed as critically reviewed previously [46]. Lamins, the intermediate filament proteins of the nuclear lamina are present at low concentrations in the nucleoplasm [9], and actin and myosin have been related to chromatin reorganization [31]. However, neither these nor other proteins have been visualized in a nuclear scaffold structure under conditions where the cell’s native hydration state is preserved. ‘The remaining and entirely plausible possibility is that nothing contributes as much to nuclear structure as does the genome (i.e. chromatin) itself’ [46]. This view is supported by recent single molecule experiments on the structure of mitotic chromosomes [47,48^{*}]. It is demonstrated that their mechanical integrity depends predominantly on the connecting DNA linkage, suggesting a model in which chromosomal proteins serve to link different loci [48^{*}]. A similar net-like organization has been proposed also for chromosomes during interphase [3,4]. The required chromatin linkers could be formed by multiple interactions, including (i) transcription factories of RNA polymerase II or the nucleolus as a polymerase I transcription organelle [3–5,7], (ii) the formation of ‘gene cluster hubs’, in which gene dense regions are separated from gene poor regions [49^{*}], or ‘active chromatin hubs’ between regulatory chromatin elements [50], (iii) the clustering of pericentric heterochromatin, which is affected by DNA methylation [51], (iv) the connection of chromatin fibers or their attachment to other nuclear structures by ATP hydrolyzing chromatin remodeling factors [52] (v) RNA [53] that is likely to involve the increasing number of non-coding RNA sequences that are retained in the

Figure 3



Model for the dynamic organization of chromosome territories. As described in the text inter-chromosomal linkers could form in a self-organizing manner during the M phase of the cell cycle when individual chromosomes are clearly separated. This would result in a stable territorial organization of chromosomes. Upon increasing the concentration of macromolecules in the nucleus because of the loss of water, a reversible chromatin compaction to a hypercondensed state can be induced [43*,44*]. Similar chromatin conformation changes have been observed in response to ATP depletion [29,43*] or histone hyperacetylation [20*,45*].

nucleus [54], and (vi) the differential interactions of active and silent chromatin with the nuclear lamina [9] and nuclear pores [6].

It is noted that the chromatin linkers listed in (i)–(vi) could form in a manner that is characteristic for self-organizing systems as discussed for the RNA polymerase II transcription factories [3]. Inasmuch as they are established already during the M phase of the cell cycle, when individual chromosomes are present as separated domains, they would be established only within a certain chromosome. This would result in a stable territorial organization of chromosomes during interphase where the degree of inter-chromosomal overlap regions is controlled by the temporal relation of chromatin linker formation with the cell cycle (Figure 3).

Conclusions

The nucleus environment is characterized by its high concentration of macromolecules. This favors the formation of nuclear subcompartments in a reversible self-organizing manner that is driven by entropic forces like macromolecular crowding and attraction depletion. Hydrophobic interactions provide an additional favorable free energy contribution to complex formation that is

more specific inasmuch as it requires some spatial complementarity of the interacting surfaces. Given the spontaneous nature of the assembly processes the question arises on how their regulation is achieved. In extension of the examples discussed here, the following principles can be identified:

(i) The global subcompartment structure can be modulated at the expense of chemical energy in the form of ATP hydrolysis. As mentioned above, ATP depletion leads to a more condensed interphase chromatin structure [29,43*]. This is likely to be the effect of the ATP-coupled activity of chromatin remodeling complexes that reposition nucleosomes or evict them from the DNA [52]. Indeed, recent measurements of chromatin assembly from *Xenopus* extracts against an applied force because of DNA tension demonstrate that the absence of ATP leads to significantly more stably bound nucleosome and a less dynamic chromatin organization [55]. It is also noted that chromatin opening and nucleosome disruption during DNA replication is thought to involve ATP-dependent chromatin-remodeling enzymes in addition to the mechanical force generated by the moving replication fork that itself is driven by nucleotide hydrolysis [56]. (ii) Relieving the spatial separation of certain protein and

nucleic acid components into the cytoplasm and the nucleus is an additional regulatory mechanism described for numerous systems. Again an example can be given for changes of the chromatin compaction state: Metaphase chromosome formation requires the condensin I and II complexes that contain the structural maintenance of chromosomes (SMC) proteins SMC2 and SMC 4. In vertebrates the condensin I complex is sequestered in the cytoplasm during interphase. Only after nuclear envelope breakdown in prometaphase can it interact with chromatin to induce the full folding of metaphase chromosomes [57]. During this process the SMC2 protein progressively accumulates in the central chromatid axis serving as a self-organizing inner 'glue' to mediate the hierarchical folding of chromosomes into its fully condensed state [58]. The formation of hypercondensed interphase chromatin discussed above [43*,44*] might have parallels to this condensation process inasmuch as it is also induced by relatively modest changes to the nuclear environment that are likely to be effective by promoting protein–protein and protein–DNA interactions. (iii) Post-translational protein modifications is yet another regulatory mechanism to control the formation and composition of nuclear subcompartments. In the context of the global chromatin condensation state, the acetylation of histones [20*,45*] as well as the Cdk1-dependent phosphorylation of condensin complexes [57] are noteworthy. Further examples refer to the self-assembly of nuclear bodies and their interactions with other proteins and include the sumoylation for PML bodies discussed above [13,28*], the dimethylarginine modification of coilin in Cajal bodies as well as serine phosphorylation of serine/arginine-rich (SR) proteins in nuclear speckles [11].

In summary, a number of principles emerge that govern the dynamic self-organization of nuclear subcompartments [1,3,11,22–24]. The increasing knowledge in this area will serve to decipher the regulatory mechanisms encoded in the spatial and temporal organization of the nucleus. This information can then be related to other types of genome wide information, as demonstrated, for example, for the comparison of the spatial organization of gene rich and gene poor domains with their expression profiles [59]. Thus, it is anticipated that an increasing part of the different functional cell states depicted in Figure 1 can be derived from an interconnected analysis of DNA sequence, epigenetic modifications, gene expression and the genome organization in the nucleus.

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