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Review

Macromolecular crowding and its potential impact on nuclear function

Karsten Richter *, Michelle Nessling, Peter Lichter

Division of Molecular Genetics, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

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ABSTRACT

It is well established, that biochemical reactions are dependent on pH, ionic strength, temperature and the concentration of reactants. However, the steric repulsion among bulky components of biological systems also affect biochemical behavior: The 'excluded volume effect of macromolecular crowding' drives bulky components into structurally compact organizations, increases their thermodynamic activities and slows down diffusion. The very special composition of the cell nucleus, which is packed with high-molecular chromatin, ribonucleo-particles and associated proteins, suggests that crowding-effects are part of nuclear functionality. Realizing that many nuclear processes, notably gene transcription, hnRNA splicing and DNA replication, use macromolecular machines, and taking into account that macromolecular crowding provides a cooperative momentum for the assembly of macromolecular complexes, we here elaborate why macromolecular crowding may be functionally important in supporting the statistical significance of nuclear activities.

1. Introduction

This review concerns the potential impact of polymer partitioning on the proper functioning of the cell nucleus. The cell nucleus is a huge compartment which manages faithful propagation of the genetic information of a cell. In this compartment, many relevant processes like DNA replication, RNA transcription and maturation use macromolecular machines composed of many factors. Assumed that subunits bind independently, the probability that an essential factor is missing increases with the number of subunits involved. Thus, cooperativity becomes essential for the concerted binding of so many factors. Further challenges for efficient nuclear function are the competition of many hundred, biochemically identical reaction sites for a few available factors [1], and the risk of reaction-abort during site-exchanges as those involved with the migration of nucleotide-polymerizing machines along a DNA-template. Altogether, it appears advantageous, if nuclear reactions, once initiated, could proceed within a microcompartment. This shelter would not only improve the productivity, but would also support a constant product-flow, which is necessary to assure stable expression patterns. Microcompartmentalization is often thought to require the biochemical interaction with scaffolding factors of a putative nuclear matrix. However, as it will be outlined in the following, nuclear microcompartments could also establish by the physical process of phase separation as a consequence of macromolecular crowding [2].

Macromolecules in aqueous solution have a tendency to phase-separate if mixed together. This was realized more than a century ago by Beijerinck [3], who was puzzled about the observation that perfect aqueous solutions of starch and of gelatin turned to water-in-water emulsions when mixed together. Today, phase separation of polymers in aqueous systems has gained technical importance in material science as well as in the pharmaceutical and food industry. Biologists take advantage of this phenomenon e.g. with widely-used protocols for the hybridization of polynucleotides [4] and the mild preparation of macromolecules [5,6]. The functional properties of extracellular matrices are also known to depend on polymer partitioning [5]. On the other hand, an expectable partitioning of cytoplasmic macromolecules appears rather suppressed, suggesting that nature takes efforts to prevent locking of functional players by unwanted segregation [7,8].

2. Volume exclusion effects macromolecular partitioning

Two principles are distinguished which describe the partitioning of polymers in ternary aqueous systems (i.e. aqueous solutions which contain two species of polymers): simple and complex coacervation [9]. The latter, complex coacervation, is readily explained: If the two polymer species have opposed surface charges, their mutual charge-interaction reduces solvability due to the neutralization of surface charges. In consequence, the interacting polymers segregate into a single phase, while the solvent becomes polymer-depleted. Quite different is simple coacervation, also called 'incompatible phase separation', which provokes the two polymer species to segregate into two phases. This phenomenon is not as easy to understand [10]. As a general theme observed by experiments, the efficiency of 'incompatible phase separation' depends on the sizes and the shapes of the polymers involved: Species of higher molecular weight and extended shape like rods and coils start to segregate at lower critical

* Corresponding author. Tel.: +49 6221 424620; fax: +49 6221 424639.
E-mail address: k.richter@dkfz.de (K. Richter).

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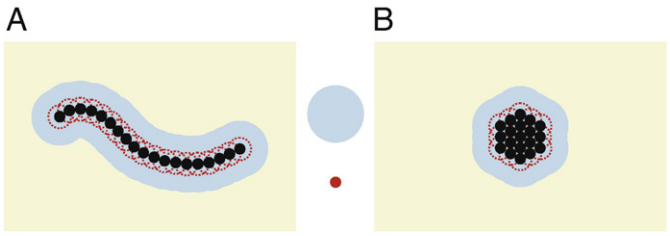

Fig. 1. Scheme to demonstrate the 'depletion-volume', which is inaccessible to the center of a test molecule due to steric repulsion from the macromolecular background of a solution. (A) Shown is the volume segment of a solution (yellow box), which contains one filamentous background molecule (chain of black beads). Considered is the case, that of the two spherical test-particles shown between A and B, either the large one (blue) or the small one (red) is to be placed in this volume segment. Due to steric repulsion, a spherical test-particle can approach the surface of the background particle up to its radial dimension only. The respective depletion layer around the background is large for large test-particles (blue), and small for small one (red). (B) The depleted volume becomes smaller, as the background particle becomes more compact. This gain of accessible volume is more important for the large test-particle compared to the small one.

concentrations than smaller species of compact, globular shape [8]. Hence, 'volume-occupancy' is an important parameter of the effect. 'Phase separation by volume exclusion occurs at about 1–3% for mixtures of rigid, rod-like polysaccharides, about 2–4% for mixtures of linear polysaccharides with proteins of unfolded structure, such as gelatin or casein, about 4% or higher for globular protein-polysaccharide mixtures, and exceeds 10% for mixtures of globular proteins' (cited from [9]).

It is important to note that effects provoked by physical parameters such as size and shape can be contributed by different, biochemically unrelated molecular species. Thus, volume effects are observable in complex solutions composed of many different macromolecular species each of which is below the critical concentration [2,11]. This cumulative relationship is expressed by the notion of 'crowding' and distinguished from concentration dependent effects, which relate to the site-specific interactions of particular molecular species. In this sense, biological media, such as extracellular matrices or intracellular plasmas, are considered to be crowded by macromolecules. The macromolecular content of cell nuclei amounts to roughly 100 mg/ml protein and 50 mg/ml chromatin [12,13]. Two features tend to make the nucleoplasm a crowded environment, its high macromolecular content and the abundance of large, particulate components such as the ribonucleoprotein particles and multi-subunit complexes.

Aqueous phase separation of macromolecules can be quantitatively modeled by the 'volume exclusion effect of macromolecular crowding' [11,14–16]. The concept is based on the steric repulsion of molecules, which are treated as incompressible, hard particles. Interaction potentials such as surface charges and polarities are not taken into account. Using scaled particle theory [17], the thermodynamic cost is estimated, which is required to introduce a 'test-particle' of a certain size and shape into a solution crowded by 'background-particles' of certain sizes, shapes and quantities (Figs. 1 and 2). Though quantitative results are debatable on the approximate nature of assumptions to be made [8,18,19], the 'volume exclusion effect' is readily comprehensible in a qualitative sense: Background-particles dispersed in a solution dissect the body of solvent into a kind of branched lacunae. To introduce a test-particle into this solution, it is required to find accessible space. If the test-particle is small enough to reach everywhere, in a strict sense particles which compare in size with solvent molecules, the accessible volume equals the volume of the solvent. However, if test-particles are larger, they cannot populate the entire body of the solvent. Obviously, they are totally excluded from branches and cavities which are too narrow for their size. More generally, due to steric repulsion, test-particles can approach their center of mass to obstacles like the background-particles up to their radial dimension only (Fig. 1). Thus, a 'depletion-volume' related to the size of a given test-particle covers all free surfaces of the background-particles dispersed in a given solution. Apart from the size and shape of the test-particle, the depletion-volume sensitively depends on sizes, shapes and spatial distribution of the background components (Fig. 2): The volume accessible to a given test-particle increases, as background-surfaces disappear. This happens, e.g., when two background-particles approach each other such that the depletion-volumes associated with the two contact surfaces overlap. In consequence, any impact to reduce the depletion-volumes of a macromolecular solution promotes compaction, assembly and alignment of some macromolecular compounds (Fig. 2B through D). The apparent interaction is called 'depletion interaction'.

The 'volume exclusion effect of macromolecular crowding' explains the depletion interaction as an entropic force. Realizing that the configurational freedom for dissolved molecules increases with the volume available to them, compaction, segregation and alignment of some macromolecular components of a solution is thermodynamically favored if the concomitant loss of mixing entropy is compensated by the increase of configurational freedom for all the other, still dissolved components [14,16,20]. This relationship was also demonstrated in silico by Monte Carlo simulations [21].

Alternatively, the depletion interaction had been modeled on the basis of osmotic pressure [22]: When two large solutes approach each other such that the gap between them becomes so narrow that smaller macro-solutes are excluded, the osmotic pressure of these smaller solutes forces the two large ones into close proximity, up to the maximum overlap of their depletion layers.

Although allowing for comparable quantitative results [23], the two approaches differ substantially: In contrast to the osmotic model, the entropic model, which 'senses' the best compromise between the cost of compaction and gain of configurational freedom, does not converge towards terminal compaction. The entropic model also describes readily the crowding-dependent non-linear behavior of

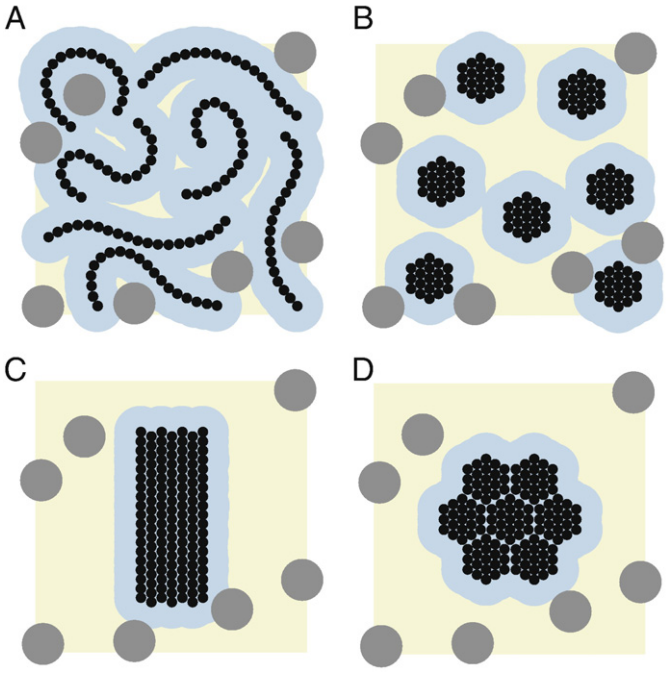

Fig. 2. Depletion interactions force filaments into compact configuration, drive them to self-align, and separate filaments from globules. (A) Within a solution of black polymer-filaments and grey globular macro-solutes in water, the centers of the grey globules can populate a very restricted volume only, due to steric repulsion form the polymers (yellow: accessible volume; blue: volume excluded to the grey globules by the black filaments). (B–D) The accessible volume (yellow) substantially increases, if the black filaments collapse into compact structures (B) align with each other (C) or do both (D).

monotypic solutions, as demonstrated e.g. for solutions of pure hemoglobin [16,24], which cannot be explained by osmotic forces, since solutes cannot exert osmotic pressure on themselves.

With respect to the scaled particle approach, real cellular fluids are much too complex to predict their crowding behavior by crude approximations of bio-molecules as inert hard spheres, rods or dumbbells. A serious problem for modeling are the interaction potentials of biological macromolecules due to surface charges and polarities [14]. Thus, incompatible phase separation can also be viewed from the point of differential interaction strengths among polymer species with water as the solvent: The more hydrophilic species 'drain' the more hydrophobic ones into compact configuration. Experiments show accordingly that salts, as well as small molecules with functional groups that alter the interaction strengths of macro-solutes with water, can substantially interfere with polymer partitioning [6]. Depletion forces furthermore arise from the partitioning of water into a free, bulk phase and a structured, bound phase, taking into account that the bulk water fraction increases, when the bound hydration water becomes liberated due to the compaction or aggregation of macro-solutes [25].

Still another source of complexity in macromolecular phase separation is dynamic asymmetries among segregating components. Without dynamic asymmetry, i.e. when segregating components have similar mobility, initial segregation germs grow with time into macroscopic droplets, which 'coarsen' by fusion and further growth. In contrast, when segregating components are very different in their mobility, the high deformation rate of the fast phase suppresses growth and coarsening of the slow phase. The slow phase then behaves like a viscoelastic body, as it becomes forced to concentrate during ongoing phase separation. Viscoelastic phase separation typically evolves into irregular, reticular segregation patterns [26].

3. Studies on macromolecular crowding in the cell nucleus

Cellular fluids contain large amounts of polymers such as proteins and nucleic acids. Like temperature or gravity, steric repulsion is a physical parameter, which is always there. Thus, bio-molecules need to be adapted to the crowding of macromolecules in their native environments. One may ask whether the crowding-state of cellular fluids is a critical parameter for the proper functioning of cellular life.

Crowding effects in complex fluids involve the entire range of macromolecules contained. Therefore, comprehensive investigation of the nuclear integrity relating to macromolecular crowding should account for its total macromolecular content. A simple experiment which ideally complies with this pre-condition is the osmotic extraction of water from living cells. Our recent studies demonstrate a remarkable sensitivity of nuclear ultrastructure to the hypertonic treatment of cells [27] (Figs. 3–5): Within seconds, chromatin compaction becomes visible as part of a general partitioning of nuclear compounds into several, structurally distinct domains. Not even doubling the isotonic pressure causes severe re-organization of nuclear matter, most evidenced by the establishment of a new nuclear compartment between the peripheral chromatin and the nuclear lamina, which we called the 'peripheral layer' (Fig. 4C). All of these hypertonic-induced re-organizations proceed gradually with time and osmotic load, and reversibly recover to regular phenotype when cells are transferred back to an isotonic environment. The broad spectrum of segregation phases observable corroborates the assumption of a physical rather than a biochemical effect. Both, the adjustability of the degree of structural changes with the osmotic load, and their reversibility indicate that the system reacts within its physiological realms: The phases observed after hypertonic treatment of cells are not caused by the precipitation of denatured matter.

Cells remained alive during hypertonic treatment and, thus, capable to control their internal ionic milieu. Nevertheless, hypertonic extraction of water potentially affects the ionic strengths of the cellular fluids. Accordingly, the compaction of chromatin by hypertonic treatment of cells was attributed to changes in ionic strength rather than macromolecular crowding [28–30]. However, chromatin compaction also occurs in nuclei of permeabilized cells which are equilibrated in isotonic buffers, if polymers, such as PVP (polyvinyl pyrrolidone) or dextran, are added as external crowding agents [27,31] (Fig. 3C). Hancock [32] demonstrated accordingly, that the functional and structural integrity of nucleoli and PML bodies in isolated nuclei can be stabilized adding PEG (polyethylene glycol) or dextran to the incubation media. However, although experiments with permeabilized nuclei allow to demonstrate the influence of macromolecular crowding on the structural organization of certain nuclear components, the native composition of nuclei is substantially disturbed since all dissolved content becomes extracted and the crowding-status needs to be adjusted by the supplementation of ectopic crowders.

4. Possible influences of macromolecular crowding on biochemical reactions

We started this review with arguments that nuclear processes could benefit from sheltering microcompartments, elaborated the concept of depletion interaction as a driving force for microcompartmentalization, and presented experimental work that demonstrated the sensitivity of nuclear organization upon macromolecular crowding.

Apart from polymer segregation, macromolecular crowding reduces diffusion rates [33], and the reduction of available volume to a specific macro-solute increases its effective concentration [16,34]. Macromolecular crowding, therefore, also influences reaction rates

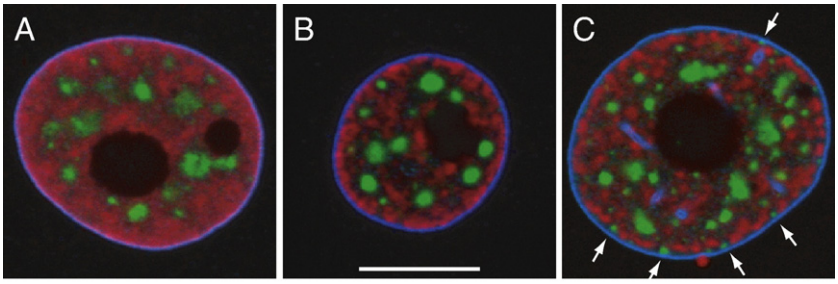

Fig. 3. Structural re-organization of MCF7 cell nuclei upon hypertonic incubation in vivo (confocal fluorescence microscopy; cells were fixed in 4% buffered formaldehyde after 20 minute incubation; red: chromatin labeled with the intercalating dye ToPro3 (Molecular Probes); green: speckles labeled with anti-SC35 (Santa Cruz, California), blue: the lamina labeled with anti-LaminA (H. Zentgraf, DKFZ, Heidelberg)). (A) The control nucleus of a cell grown in isotonic medium (DMEM) shows chromatin at various degrees of packing density throughout the nucleus. The most dense regions preferentially locate to the nuclear periphery and perinucleolar region. (B) After 20 minute incubation with 160 mM sucrose added to the medium, chromatin compacted substantially, leaving large chromatin-empty voids. All speckles reside in such voids, distinctly separated from chromatin by a gap. (C) A load of 260 mM sucrose added to DMEM caused chromatin to detach from the lamina. Although this detachment is not clearly resolved at the light-optical resolution, a distinct sign is the appearance of focal accumulations of the speckle-marker SC35 between peripheral chromatin and the lamina (arrows). Scale bar: 10 μm.

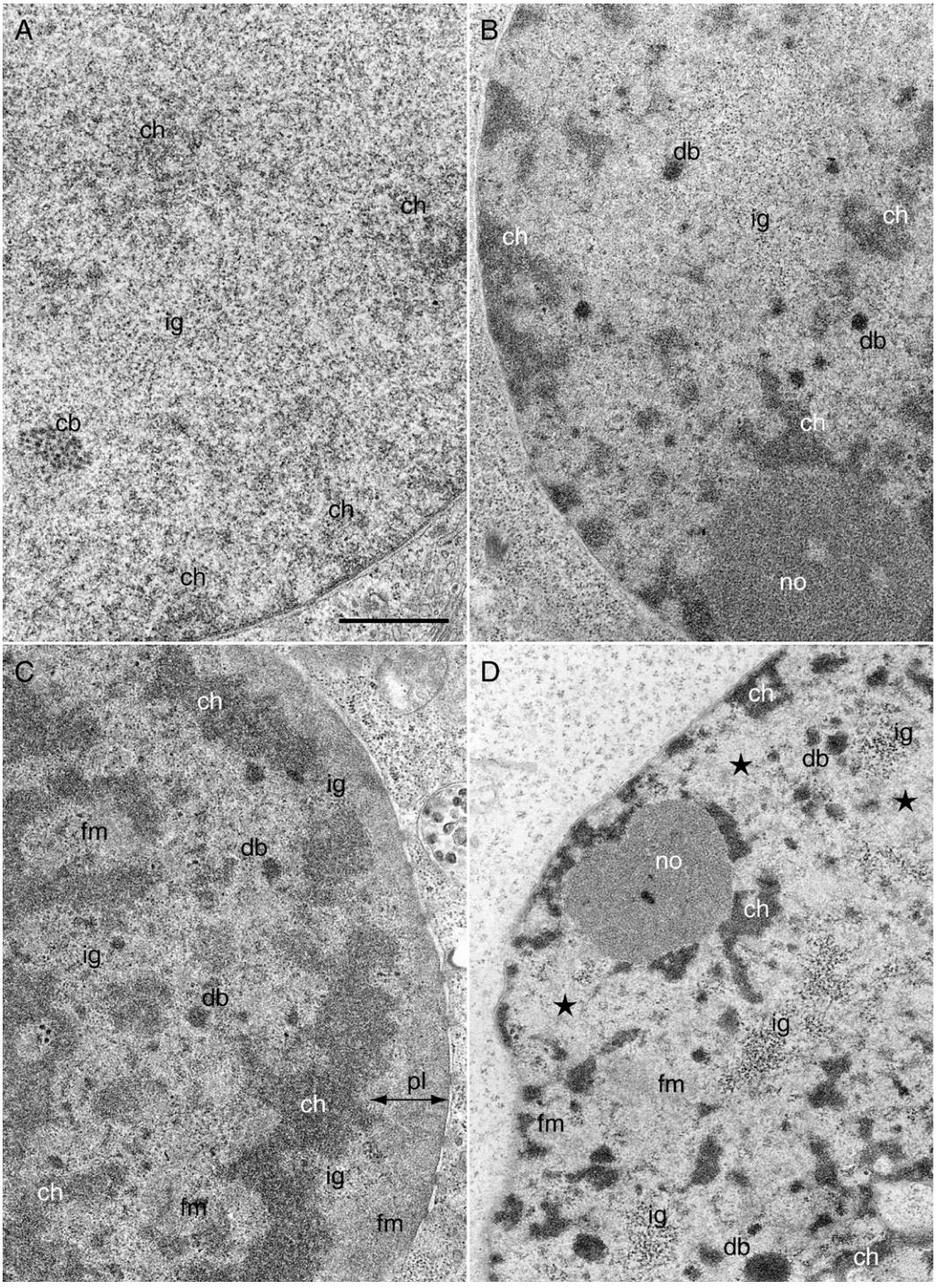

Fig. 4. Electron microscopy of the structural changes induced in MCF7 cell nuclei due to either hypertonic incubation in vivo, or the infiltration of permeabilized cells with dextran (incubation of cells took 20 min; cells were fixed in buffered 4% formaldehyde/1% glutaraldehyde, post-fixed in buffered 1% osmium tetroxide, dehydrated in graded steps of ethanol and embedded in epoxy-resin. Sections were cut at nominal thickness of 70 nm and post-stained in uranyl/lead). (A) Control nucleus of a cell grown in isotonic medium (DMEM). The nuclear content appears finely dispersed. Structural entities like chromatin (ch) and interchromatin granule clusters (ig) are difficult to individualize within the overall fibro-granular background (cb: Cajal body). (B) 20 minute incubation with 160 mM sucrose added to the medium provoked chromatin compaction (ch) and formation of small, dense bodies (db). The nucleoplasmic content became more differentially textured, such that interchromatin granule clusters (ig) are structurally more distinguished (no: nucleolus). (C) Incubation with 320 mM sucrose added to the medium provoked an even more distinguished segregation pattern. Chromatin (ch) compacted into a dense matter of homogeneous granularity, and fine-fibrillar material (fm) segregated into lacunas throughout the interchromatin space. Remarkably, the peripheral chromatin retracted from the nuclear envelope giving rise to a newly established 'peripheral layer' (pl). Fine fibrillar material fills this peripheral space and coarse granular material accumulates at the interface with the peripheral chromatin. Among the latter are small clusters of interchromatin granules (ig). (D) Chromatin compaction also occurs when digitonin-permeabilized cells are pervaded with 10 kDa-dextran. Despite the rich structural diversity which is still preserved, the high contrast and the coarse-textured background (stars) indicate substantial extraction of nuclear content. Also, neither a formation of dense bodies, nor a peripheral layer was observed, even though the amount of dextran added (10% wt/vol) corresponds to the amount of 320 mM sucrose used for part C of the figure panel. Scale bar: 1 μm.

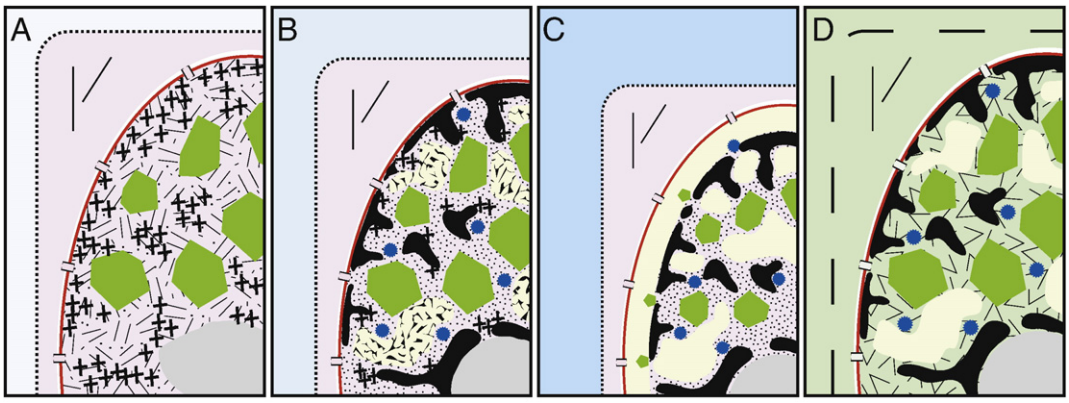

Fig. 5. Scheme to explain the experimental setup used to challenge nuclear architecture by macromolecular crowding (compare with Fig. 4), i.e. (A) control cell in isotonic medium, (B) mild hypertonic treatment (e.g. 160 mM sucrose added to the medium), (C) strong hypertonic treatment (e.g. 320 mM sucrose added to the medium), and (D) permeabilized cell treated with an external crowder (e.g. dextran). (A–C) The intact cell-membrane (dotted line) prevents the free exchange of osmotropes (e.g. sucrose) between the medium (blue) and the cell (rose). The nuclear envelope (white line with pores; red: lamina) does not function as an osmotic barrier. Osmotropes added to the medium extract water from the cell. The cell and its nucleus shrink accordingly (B, C), and the remaining macromolecular content becomes more crowded. The hypertonic treatment drives chromatin compaction (black crosses (A) turn into black areas (C)), and segregation of other nuclear compounds (blue: dense bodies, yellow: fine-fibrillar material). These structural re-organizations proceed gradually with increasing osmotic load. At high osmotic load (C), the peripheral chromatin detaches from the nuclear lamina. Nuclear components invade the peripheral space, such as speckles (green), dense bodies (blue) and the fine-fibrillar material (yellow). (D) Chromatin compaction and nucleoplasmic phase separation is also provoked when permeabilized cells (hatched line) are pervaded by an external crowder. All three spaces of interest (medium, cytoplasm, nucleoplasm) equilibrate with respect to osmotic content and external crowder (light green). In turn, the nucleoplasm becomes extracted from its dissolved content. No peripheral layer is observed.

and equilibria through complex interrelationships of diffusion dependence, thermodynamic activity and steric constraints [35]. Accordingly, it was demonstrated that crowders like dextran or PEG not only raise the melting temperature of DNA, but also increase the renaturation speed [4,36]. Thus, the supplementation of crowders to the reaction-mix of PCR (polymerase chain reaction) improves the specificity as well as the efficiency for the in vitro amplification of DNA [37].

In cellular fluids, biochemical reactions are dominated by the site-specific interactions of distinguished reaction partners. Therefore, experimental observations of the influence of macromolecular crowding on cellular function generally may not be explained by the non-specific depletion interactions alone [38,39]. Macromolecular crowding rather may function to set a pre-load towards the association and compaction of macromolecules, which then are to be put into action by some site-specific interactions and directed molecular modifications, e.g. the activation of proteins by phosphorylation [20].

5. Principles for structural nuclear organization

The genome with its biochemistry for maintenance, gene transcription and DNA replication is separated from the cytoplasm by the nuclear envelope. Nuclear pore complexes control the exchange of macromolecules. Small molecules and ions, in contrast, can pass freely; neither an electrochemical potential, nor an osmotic gradient are being established across the nuclear envelope between nucleoplasm and cytoplasm.

Taking the genomic sequence of organisms as the basis for the organization of life, modern knowledge allows us to appreciate that functional sets of genes are to be expressed according to external triggers and internal schedules, thoroughly controlled by feedback loops. These processes could well happen through random collision and site-specific, stoichiometric interaction of reaction partners. Quite complex structures can self-assemble this way, which then are the consequence of functioning rather than its cause.

Apart from this, we here conceive 'higher level structural organization' as a structural pre-requisite for functional processes, i.e. an investment of the cell for the channeling of future tasks. For example, the nucleolus organizer flanking the 47S rDNA clusters of acrocentric mammalian chromosomes is of 'higher order' in promoting ribosome biogenesis. In contrast, the complex organization of nucleoli into fibrillar centers, fibrillar components and granular components is not pre-determined but arises in consequence of the synthetic activities to produce ribosomal subunits [40]. Also a matrix, for mechanical support or for use as a platform for site-directed activities, is a higher level structure, opposed to mere interaction networks.

The nuclear content is non-randomly distributed on the level of chromatin as well as other nucleoplasmic compounds. Chromatin is packed into chromosome band domains, chromosome arm domains and chromosome territories, which, to a large extent, mutually exclude each other [41–45]. This processive packing is difficult to explain by chemical interaction through crosslinkers, which would require sensing-capabilities to distinguish *cis*- from *trans*-interactions. In contrast, depending on mechanical properties of polymers, depletion interactions favor intramolecular compared to intermolecular compaction, without the need for crosslinking activities. In the case of chromatin compaction, the local binding of chromatin-regulatory proteins would modulate the compaction scheme [27]. Functional importance had been addressed to the territorial organization of chromatin, based on the assumption that chromatin exerts a diffusion barrier to factors necessary for gene activity. To be active, genes would need to become exposed to the more diffusible space between chromosome territories, the interchromosomal compartment domain [46–49]. This model also complies with the above criteria for higher order nuclear organization. In support of this concept, active genes were shown to preferentially localize to the periphery of their chromosome territory [46,50,51]. The observation of intermingling territory borders [52] in front of the otherwise mutually excluded interphase chromosomes further corroborates the active spreading of some chromatin regions into this interchromosomal space. Alternatively, the territorial organization may give credit to conservative constraints for future cell division. Although functional nuclear architecture is expected to relate to interphase-processes like RNA transcription, DNA replication and DNA repair, interphase-activity must not compromise the faithful propagation of the genome during mitosis.

Apart from the territorial organization, chromatin clusters into distinct patterns of replication timing [53,54] and packing status. Dense packing of chromatin correlates with poor accessibility, experimentally demonstrated by its relative resistance to enzymatic degradation and loss of functional activity. Typical locations of close packed heterochromatin are the nuclear periphery and the perinucleolar space. Peripheral heterochromatin associates with the lamina, a proteinaceous layer connected to the nuclear envelope. The lamina

represents a bona fide nucleo-skeleton [55–57]. Its major constituent, the lamins, are members of the intermediate filament protein family. Interaction of chromatin with the lamina functionally acts as a silencer. Interestingly, the layer of peripheral heterochromatin is much thicker than the lamina. A presumptive influence of the lamina towards heterochromatin formation, therefore, would delve deep into the chromatin network. Biochemically, the heterochromatin formation by the protein HP1 had been shown to self-propagate until being stopped by chromatin modifications with barrier function [58–61]. As these insulators compartmentalize the one-dimensional world of DNA-sequence, they also represent a 'higher order' organization. A scenario apart from the propagation of epigenetic markers for heterochromatin can be designed by the biophysical process of viscoelastic phase separation, which would promote compaction of the immobilized peripheral chromatin due to kinetic discrepancy with the internal chromatin [31]. Any mechanism to immobilize chromatin would have this effect and could explain why heterochromatin patches also occur inside the nucleus, notably associated with nucleoli, and in organisms like plants, which lack a lamina.

Many nuclear proteins and ribonucleoprotein particles enrich in prominent, body-like structures of defined morphology and composition but of no obvious synthetic activity; notably speckles, PML bodies and Cajal bodies [62–64], which all reside within voids between chromosome territories [65,66]. Not bound by separating membranes, nuclear bodies represent phases of differential affinity for any given entity which happens to roam the nuclear space. Accordingly, their contents exchange dynamically with the diffusible pool of the nucleoplasm [67–69], which, therefore, becomes buffered to a constant concentration. This buffer-property may be instrumental to prevent substantial depletion of the soluble pools of functional players upon sudden boosts of nuclear activity. In this case, the exact position of a nuclear body within the nucleus would be of minor importance. In contrast, synthetic activities on chromatin, i.e. RNA transcription as well as DNA replication and repair, are site-specific. Except for nucleoli, the factories of RNA-polymerase I activity, sites of synthetic processes in the cell nucleus are structurally poorly defined. Light-optical resolution reveals not more than focal spots, and electron microscopy does not allow a discrimination of their inferred structural complexity from the general fibro-granular background of a nucleus [70] (Fig. 4A). However, it was shown that RNA transcription outside nucleoli occurs in distinct foci throughout cell nuclei [71], that active genes relocate to these transcription foci [72], that several genes cluster in a same transcription focus [73], and that focal accumulations of RNA-polymerase II persist upon transcription inhibition [74]. These observations suggest, that hnRNA transcription occurs at specific nodes in the nucleus. A nuclear matrix, which could account for such nodal attachment sites, can be prepared by high-salt extraction of cell nuclei (see [75] for a historical background of the nuclear matrix, and [76] in response). Chromatin associates with this nuclear matrix via so called scaffold attachment- or matrix associated regions (SAR/MAR), and a marker protein had been identified, SAF-A, capable to link MARs with functional proteins [77]. Content analysis of matrix preparations demonstrates a very heterogeneous composition, which includes factors of the entire spectrum of nuclear activity [78]. Since harsh extraction conditions may well promote the artificial precipitation of an interaction network of many, differently engaged nuclear components, the concept of a nuclear matrix is not commonly accepted [79].

Substantial knowledge about genome function has been gained from in vitro assays without reproducing a nuclear matrix. These studies delineate two structural characteristics, which are common in transcription and replication, the establishment of a stationary initiation complex, and the use of huge macromolecular complexes during mobile polymerase-activity. The packing of DNA into chromatin promotes protection against enzymatic digestion and, in turn, represents a basic level of DNA repression. Further silencing is achieved by epigenetic chromatin modifications at the levels of DNA, nucleosomes and whole chromatin, e.g. DNA-methylation, histone hypoacetylation and HP1 assembly. Activation of chromatin for transcription, replication and repair requires removal of these blocks and the local melting of the DNA double-strand [80,81].

RNA-polymerase II transcription cycles through initiation, elongation and termination in a sequence of steps [82,83]. Regulatory factors bound near the site of transcription initiation recruit the general transcription factors to the promoter, forming the pre-initiation complex, which then melts the DNA locally. Triggered by phosphorylation of its C-terminal repeat domain, the polymerase eventually proceeds into stable transcription. Part of the initiation complex stays behind as a so called scaffold complex to pre-condition the promoter for further transcription rounds, whereas the polymerase associates with elongation factors, which are required to manage downstream chromatin accessibility [84] and co-transcriptional maturation of the nascent RNA-product [85]. Two mechanically opposed features of the transcription machinery are important: 1. Co-operative binding of regulatory factors at the start-site enhances the specificity of the otherwise degenerative promoter. 2. Continuous liberation of the coding region from obstacles like nucleosomes or other DNA-binding proteins assures processivity of elongation after promoter clearance [83].

Similar to RNA transcription, DNA replication also involves sequential processes for initiation, chromatin denaturation and histone re-arrangement, and uses various multienzymatic complexes for chromatin remodeling and DNA-synthesis [86]. Early in G1, origin recognition complexes (ORC) bind to a subset of replication origins to poise them for future firing. Successful firing then requires an open, accessible status of chromatin, and correlates with the nearby presence of promoter regions and chromatin remodeling transcription factors. Maturation of the origin into the initiation complex requires a heterohexamer ring of co-factors (MCM-complex) for unwinding the DNA and starting assembly of the replication machinery [87].

Although these processes may not need the higher order structural organisation provided by a presumptive nuclear matrix, they may well profit from the cooperative momentum of macromolecular crowding on the stability of macromolecular complexes.

6. Potential impact of macromolecular crowding on the functional stability of the cell nucleus

Cellular life signifies the passage through a sequence of distinguished states. Not only the cell cycle, all regulated activities represent a well determined balance among various pathways. Therefore, any establishment of a specific cellular state demands the statistical significance in the time-dependent expression of at least some critical genes. Stochastic noise not only limits the control over expression levels, but also the response-time when expression levels are to be changed upon switch from one state to another. Thus, the statistical stability of nuclear processes appears to be important for proper cellular function.

Noise in gene expression is caused by extrinsic sources, such as the external supply for nutrition or growth stimuli, and intrinsic sources, such as RNA transcription and protein translation [88]. Experiments have shown that the noise rate for transcription is higher than for translation [89]. Therefore, to minimize expression noise at a desired expression level, it is advantageous to combine high transcription rates with respective low translation activities. Accordingly it was shown in *Saccharomyces cerevisiae*, that the expression of essential proteins is biased towards higher transcription-to-translation ratios compared to non-essential proteins. This latter observation also corroborates, that the noise-performance of gene expression is challenged throughout evolution [90].

Nuclear biochemistry is susceptible to statistical fluctuations due to the competition of many, chemically identical, active sites for a limited number of reactive factors [1]. A further source for expression noise is the transition-speed for promoter on and off switching [91]. In this

respect, the temporal stability of the scaffold complex of transcription, which keeps the promoter prepared for ongoing transcription cycles, appears instrumental to delimit transcription noise. But also the stability of other macromolecular complexes, e.g. used for transcription elongation and hnRNA maturation, will influence the statistical noise of gene expression. Therefore, as depletion interactions stabilize macromolecular complexes against their decay, macromolecular crowding in the nucleoplasm could have functional importance to delimit the stochastic fluctuations of nuclear processes.

7. Conclusion

Prokaryotes manage their genome without having a karyon. A classical speculation of why higher forms of life organize their genome within an extra compartment realizes the benefit of improved expression control by splicing, maturation and export due to the separation of transcription from translation. Another aspect is protection of the interphase genome from the influence of cytoplasmic microtubules. Last but not least, the nuclear compartment is well adapted to maintain a controlled degree of macromolecular crowding: Nuclear pores select for the macromolecular content of the nucleoplasm, and the potential of chromatin for adaptive condensation provides buffer-capacity to stabilize a given crowding status. Thus, the consideration of macromolecular crowding as a functional player for the stochastic stability of nuclear processes sheds new light on the functional organization of the cell nucleus.

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References

[1] K. Luby-Phelps, Cytoarchitecture and physical properties of cytoplasm: volume, viscosity, diffusion, intracellular surface area, Int. Rev. Cytol. 192 (2000) 189–221.
[2] H. Walter, D.E. Brooks, Phase separation in cytoplasm, due to macromolecular crowding, is the basis for microcompartmentation, FEBS Lett. 361 (1995) 135–139.
[3] M.W. Beijerinck, Über eine Eigentümlichkeit der löslichen Stärke, Centralblatt für Bakteriologie, Parasitenkunde, und Infektionskrankheiten 22 (1896) 697–699.
[4] R. Wieder, J.G. Wetmur, One hundred-fold acceleration of DNA renaturation rates in solution, Biopolymers 20 (1981) 1537–1547.
[5] T.C. Laurent, An early look at macromolecular crowding, Biophys. Chem. 57 (1995) 7–14.
[6] G. Johansson, H. Walter, Partitioning and concentrating biomaterials in aqueous phase systems, Int. Rev. Cytol. 192 (2000) 33–60.
[7] D.E. Brooks, Can cytoplasm exist without undergoing phase separation? Int. Rev. Cytol. 192 (2000) 321–330.
[8] H.-O. Johansson, D.E. Brooks, C.A. Haynes, Macromolecular crowding and its consequences, Int. Rev. Cytol. 192 (2000) 155–170.
[9] V. Tolstoguzov, Compositions and phase diagrams for aqueous systems based on proteins and polysaccharides, Int. Rev. Cytol. 192 (2000) 3–31.
[10] E. Edmond, A.G. Ogston, An approach to the study of phase separation in ternary aqueous systems, Biochem. J. 109 (1968) 569–576.
[11] R.J. Ellis, A.P. Minton, Protein aggregation in crowded environments, Biol. Chem. 387 (2006) 485–497.
[12] K.E. Handwerger, J.A. Cordero, J.G. Gall, Cajal bodies, nucleoli, and speckles in the *Xenopus* oocyte nucleus have a low-density, sponge-like structure, Mol. Biol. Cell 16 (2005) 202–211.
[13] R. Hancock, Packing of the polynucleosome chain in interphase chromosomes: evidence for a contribution of crowding and entropic forces, Semin. Cell Dev. Biol. 18 (2007) 668–675.
[14] S.B. Zimmerman, A.P. Minton, Macromolecular crowding: biochemical, biophysical and physiological consequences, Annu. Rev. Biophys. Biomol. Struct. 22 (1993) 27–65.
[15] J. Herzfeld, Entropically driven order in crowded solutions: from liquid crystals to cell biology, Acc. Chem. Res. 29 (1996) 31–37.
[16] R.J. Ellis, Macromolecular crowding: an important but neglected aspect of the intracellular environment, Curr. Opin. Struct. Biol. 11 (2001) 114–119.
[17] J.L. Lebowitz, E. Helfand, E. Praestgaard, Scaled particle theory of fluid mixtures, J. Chem. Phys. 43 (1963) 774–779.
[18] O.G. Berg, The influence of macromolecular crowding on thermodynamic activity: solubility and dimerization constants for spherical and dumbbell-shaped molecules in a hard-sphere-mixture, Biopolymers 30 (1990) 1027–1037.
[19] K.E.S. Tang, V.A. Bloomfield, Excluded volume in salvation: sensitivity of scaled-particle theory to solvent size and density, Biophys. J. 79 (2000) 2222–2234.
[20] J. Herzfeld, Crowding-induced organization in cells: spontaneous alignment and sorting of filaments with physiological control points, J. Mol. Recognit. 17 (2004) 376–381.
[21] D. Hall, Protein self-association in the cell: a mechanism for fine tuning the level of macromolecular crowding? Eur. Biophys. J. 35 (2006) 276–280.
[22] S. Asakura, F. Oosawa, Interaction between particles suspended in solutions of macromolecules, J. Polymer. Sci. 33 (1958) 183–192.
[23] D. Marenduzzo, F. Kieran, P.R. Cook, The depletion attraction: an underappreciated force driving cellular organization, J. Cell. Biol. 175 (2006) 681–686.
[24] F.A. Ferrone, M.A. Rotter, Crowding and the polymerization of sickle hemoglobin, J. Mol. Recognit. 17 (2004) 497–504.
[25] K.D. Garlid, The state of water in biological systems, Int. Rev. Cytol. 192 (2000) 281–302.
[26] H. Tanaka, Viscoelastic phase separation, J. Phys: Condens. Matter 12 (2000) R207–R264.
[27] K. Richter, M. Nessling, P. Lichter, Experimental evidence for the influence of molecular crowding on nuclear architecture, J. Cell. Sci. 120 (2007) 1673–1680.
[28] E. Robbins, T. Pederson, P. Klein, Comparison of mitotic phenomena and effects induced by hypertonic solutions in HeLa cells, J. Cell. Biol. 44 (1970) 400–416.
[29] E. Delpire, C. Duchêne, G. Goessens, R. Gilles, Effects of osmotic shocks on the ultrastructure of different tissues and cell types, Exp. Cell Res. 160 (1985) 106–116.
[30] H. Albiez, M. Cremer, C. Tiberi, L. Vecchio, L. Schermelleh, S. Dittrich, K. Küpper, B. Joffe, T. Thormeyer, J. von Hase, S. Yang, K. Rohr, H. Leonhardt, I. Solovei, C. Cremer, S. Fakan, T. Cremer, Chromatin domains and the interchromatin compartment form structurally defined and functionally interacting nuclear networks, Chrom. Res. 14 (2006) 707–733.
[31] F.J. Iborra, Can visco-elastic phase separation, macromolecular crowding and colloidal physics explain nuclear organisation? Theor. Biol. Med. Model 4 (2007) 15.
[32] R. Hancock, A role for macromolecular crowding effects in the assembly and function of compartments in the nucleus, J. Struct. Biol. 146 (2004) 281–290.
[33] E. Dauty, A.S. Verkman, Molecular crowding reduces to a similar extent the diffusion of small solutes and macromolecules: measurement by fluorescence correlation spectroscopy, J. Mol. Recognit. 17 (2004) 441–447.
[34] A.P. Minton, The effect of volume occupancy upon the thermodynamic activity of protein: some biochemical consequences, Mol. Cell Biochem. 55 (1983) 119–140.
[35] A.P. Minton, How can biochemical reactions within cells differ from those in test tubes? J. Cell. Sci. 119 (2006) 2863–2869.
[36] J.-L. Sikorav, G.M. Church, Complementary recognition in condensed DNA: accelerated DNA renaturation, J. Mol. Biol. 222 (1991) 1085–1108.
[37] R.R. Lareu, K.S. Harve, M. Raghunath, Emulating a crowded intracellular environment in vitro dramatically improves RT-PCR performance, Biochem. Biophys. Res. Comm. 363 (2007) 171–177.
[38] M.C. Konopka, I.A. Shkel, S. Cayley, M.T. Record, J.C. Weisshaar, Crowding and confinement effects on protein diffusion in vivo, J. Bacteriol. 188 (2006) 6115–6123.
[39] Z. Hu, J. Jiang, R. Rajagopalan, Effects of macromolecular crowding on biochemical reaction equilibria: a molecular thermodynamic perspective, Biophys. J. 93 (2007) 1464–1473.
[40] D. Hernandez-Verdun, Nucleolus: from structure to dynamics, Histochem. Cell. Biol. 125 (2006) 127–137.
[41] L. Manuelidis, Individual interphase chromosome domains revealed by in situ hybridization, Hum. Genet. 71 (1985) 288–293.
[42] M. Schardin, T. Cremer, H.D. Hager, M. Lang, Specific staining of human chromosomes in Chinese hamster x man hybrid cell lines demonstrates interphase chromosome territories, Hum. Genet. 71 (1985) 281–287.
[43] P. Lichter, T. Cremer, J. Borden, L. Manuelidis, D.C. Ward, Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries, Hum. Genet. 80 (1988) 224–234.
[44] A.R. Leitch, T. Schwarzacher, W. Mosgöller, M.D. Bennett, J.S. Heslop-Harrison, Parental genomes are separated throughout the cell cycle in a plant hybrid, Chromosoma 101 (1991) 206–213.
[45] S. Dietzel, A. Jauch, D. Kienle, G. Qu, H. Holtgreve-Grez, R. Eils, C. Munkel, M. Bittner, P.S. Meltzer, J.M. Trent, T. Cremer, Separate and variably shaped chromosome arm domains are disclosed by chromosome arm painting in human cell nuclei, Chromosome Res. 6 (1998) 25–33.
[46] R.M. Zirbel, U.R. Mathieu, A. Kurz, T. Cremer, P. Lichter, Evidence for a nuclear compartment of transcription and splicing located at chromosome domain boundaries, Chromosome Res. 1 (1993) 93–106.
[47] T. Cremer, A. Kurz, R. Zirbel, S. Dietzel, B. Rinke, E. Schröck, M.R. Speicher, U. Mathieu, A. Jauch, P. Emmerich, H. Scherthan, T. Ried, C. Cremer, P. Lichter, Role of chromosome territories in the functional compartmentalization of the cell nucleus, Cold Spring Harb. Symp. Quant. Biol. Vol LVIII (1993) 777–792.
[48] J.M. Bridger, H. Herrmann, C. Münkel, P. Lichter, Identification of an interchromosomal compartment by polymerization of nuclear-targeted vimentin, J. Cell. Sci. 111 (1998) 1241–1253.
[49] T. Cremer, C. Cremer, Rise, fall and resurrection of chromosome territories: a historical perspective. Part II. Fall and resurrection of chromosome territories during the 1950s to 1980s. Part III. Chromosome territories and the functional nuclear architecture: experiments and models from the 1990s to the present, Europ. J. Histochem. 50 (2006) 223–272.
[50] A. Kurz, S. Lampel, J.E. Nickolenko, J. Brandl, A. Benner, R.M. Zirbel, T. Cremer, P. Lichter, Active and inactive genes localize preferentially in the periphery of chromosome territories, J. Cell. Biol. 135 (1993) 1195–1205.
[51] M.O. Scheuermann, J. Tajbakhsh, A. Kurz, K. Saracoglu, R. Eils, P. Lichter, Topology of genes and nontranscribed sequences in human interphase nuclei, Exp. Cell. Res. 301 (2004) 266–279.

[52] M.R. Branco, A. Pombo, Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations, PLoS Biol. 4 (2006) e138.
[53] D.A. Jackson, A. Pombo, Replicon clusters are stable units of chromosome structure: evidence that nuclear organization contributes to the efficient activation and propagation of S phase in human cells, J. Cell. Biol. 140 (1998) 1285–1295.
[54] D. Zink, The temporal program of DNA replication: new insights into old questions, Chromosoma 115 (2006) 273–287.
[55] S. Vlcek, T. Dechat, R. Foisner, Nuclear envelope and nuclear matrix: interactions and dynamics, Cell. Mol. Life Sci. 58 (2001) 1758–1765.
[56] Y. Gruenbaum, A. Margalit, R.D. Goldman, D.K. Shumaker, K.L. Wilson, The nuclear lamina comes of age, Mol. Cell. Biol. 6 (2005) 21–31.
[57] R.D. Goldman, Y. Gruenbaum, R.D. Moir, D.K. Shumaker, T.P. Spann, Nuclear lamins: building blocks of nuclear architecture, Genes Dev. 16 (2002) 533–547.
[58] Y. Zhang, D. Reinberg, Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails, Genes Dev. 15 (2001) 2343–2360.
[59] B. Burgess-Beusse, C. Farrell, M. Gaszner, M. Litt, V. Mutskov, F. Recillas-Targa, M. Simpson, A. West, G. Felsenfeld, The insulation of genes from external enhancers and silencing chromatin, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16433–16437.
[60] M. Labrador, V.G. Corces, Setting the boundaries of chromatin domains and nuclear organization, Cell 111 (2002) 151–154.
[61] T. Kouzarides, Chromatin modifications and their function, Cell 128 (2007) 693–705.
[62] A.I. Lamond, D.L. Spector, Nuclear speckles: a model for nuclear organelles, Nat. Rev. Mol. Cell. Biol. 4 (2003) 605–612.
[63] M. Cioce, A.I. Lamond, Cajal bodies: a long history of discovery, Annu. Rev. Cell. Dev. Biol. 21 (2005) 105–131.
[64] T.H. Shen, H.-K. Lin, P.P. Scaglioni, T.M. Yung, P.P. Pandolfi, The mechanisms of PML-nuclear body formation, Mol. Cell. 24 (2006) 331–339.
[65] S.M. Goerisch, M. Wachsmuth, C. Ittrich, C.P. Bacher, K. Rippe, P. Lichter, Nuclear body movement is determined by chromatin accessibility and dynamics, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 13221–13226.
[66] K. Richter, M. Reichenzeller, S.M. Görisch, U. Schmidt, M.O. Scheuermann, H. Herrmann, P. Lichter, Characterization of a nuclear compartment shared by nuclear bodies applying ectopic protein expression and correlative light and electron microscopy, Exp. Cell. Res. 303 (2005) 128–137.
[67] R.D. Phair, T. Misteli, High mobility of proteins in the mammalian cell nucleus, Nature 404 (2000) 604–609.
[68] T. Misteli, Protein dynamics: implications for nuclear architecture and gene expression, Science 291 (2001) 843–847.
[69] K. Wiesmeijer, C. Molenaar, I.M.L.A. Bekeer, H.J. Tanke, R.W. Dirks, Mobile foci of Sp100 do not contain PML: PML bodies are immobile but PML and Sp100 proteins are not, J. Struct. Biol. 140 (2002) 180–188.
[70] S. Fakan, The functional architecture of the nucleus as analysed by ultrastructural cytochemistry, Histochem. Cell. Biol. 122 (2004) 83–93.
[71] F.J. Iborra, A. Pombo, D.A. Jackson, P.R. Cook, Active RNA polymerases are localized within discrete transcription 'factories' in human cell nuclei, J. Cell. Sci. 109 (1996) 1427–1436.
[72] C.S. Osborne, L. Chakalova, J.A. Mitchell, A. Horton, A.L. Wood, D.J. Bolland, A.E. Corcoran, P. Fraser, Myc dynamically and preferentially relocates to a transcription factory occupied by Igh, PloS Biol. 5 (2007) 1763–1772.
[73] C.S. Osborne, L. Chakalova, K.E. Brown, D. Carter, A. Horton, E. Debrand, B. Goyenechea, J.A. Mitchell, S. Lopes, W. Reik, P. Fraser, Active genes dynamically colocalize to shared sites of ongoing transcription, Nat. Genet. 36 (2004) 1065–1071.
[74] J.A. Mitchell, P. Fraser, Transcription factories are nuclear subcompartments that remain in the absence of transcription, Genes Dev. 22 (2008) 20–25.
[75] T. Pederson, Half a century of "The nuclear matrix", Mol. Biol. Cell. 11 (2000) 799–805.
[76] J.A. Nickerson, Experimental observations of a nuclear matrix, J. Cell. Sci. 114 (2001) 463–474.
[77] J. Bode, S. Goetze, H. Heng, S.A. Krawetz, C. Benham, From DNA structure to gene expression: mediators of nuclear compartmentalization and dynamics, Chromosome Res. 11 (2003) 435–445.
[78] D. Ottaviani, E. Lever, P. Takousis, D. Sheer, Anchoring the genome, Genome Biol. 9 (2008) 201.1–201.6.
[79] R. Hancock, A new look at the nuclear matrix, Chromosoma 109 (2000) 219–225.
[80] R.G. Roeder, Transcriptional regulation and the role of diverse coactivators in animal cells, FEBS Lett. 579 (2005) 909–915.
[81] H. Kohzaki, Y. Murakami, Transcription factors and DNA replication origin selection, BioEssays 27 (2005) 1107–1116.
[82] S. Hahn, Structure and mechanism of the RNA polymerase II transcription machinery, Nat. Struct. Mol. Biol. 11 (2004) 394–403.
[83] J.Q. Svejstrup, The RNA polymerase II transcription cycle: cycling through chromatin, Biochim. Biophys. Acta. 1677 (2004) 64–73.
[84] H. Boeger, D.A. Bushnell, R. Davis, J. Griesenbeck, Y. Lorch, J.S. Strattan, K.D. Westover, R.D. Kornberg, Structural basis of eukaryotic gene transcription, FEBS Lett. 579 (2005) 899–903.
[85] A. Aguilera, Cotranscriptional mRNP assembly: from the DNA to the nuclear pore, Curr. Opin. Cell. Biol. 17 (2005) 242–250.
[86] A.E. Ehrenhofer-Murray, Chromatin dynamics at DNA replication, transcription and repair, Eur. J. Biochem. 271 (2004) 2335–2349.
[87] A.P. Tabancay Jr, S.L. Forsburg, Eukaryotic DNA replication in a chromatin context, Curr. Opin. Dev. Biol. 76 (2006) 129–184.
[88] N. Maheshri, E.K. O'Shea, Living with noisy genes: how cells function reliably with inherent variability in gene expression, Annu. Rev. Biophys. Biomol. Struct. 36 (2007) 313–334.
[89] J.M. Raser, E.K. O'Shea, Noise in gene expression: origins, consequences, and controls, Science 309 (2005) 2010–2013.
[90] H.B. Fraser, A.E. Hirsh, G. Giaever, J. Krumm, M.B. Eisen, Noise minimization in eukaryotic gene expression, PLoS Biol. 2 (2004) 834–838.
[91] M. Kaern, T.C. Elston, W.J. Blake, J.J. Collins, Stochasticity in gene expression: from theories to phenotypes, Nature 6 (2005) 451–464.