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Review

The 4D nucleome: Evidence for a dynamic nuclear landscape based on co-aligned active and inactive nuclear compartments

Thomas Cremer [a,*], Marion Cremer [a], Barbara Hübner [a,1], Hilmar Strickfaden [b], Daniel Smeets [a], Jens Popken [a], Michael Sterr [a,2], Yolanda Markaki [a], Karsten Rippe [c,*], Christoph Cremer [d,*]

[a] Biocenter, Department Biology II, Ludwig Maximilians University (LMU), Martinsried, Germany
[b] University of Alberta, Cross Cancer Institute Dept. of Oncology, Edmonton, AB, Canada
[c] German Cancer Research Center (DKFZ) & BioQuant Center, Research Group Genome Organization & Function, Heidelberg, Germany
[d] Institute of Molecular Biology (IMB), Mainz and Institute of Pharmacy and Molecular Biotechnology (IPMB), University of Heidelberg, Germany

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ABSTRACT

Recent methodological advancements in microscopy and DNA sequencing-based methods provide unprecedented new insights into the spatio-temporal relationships between chromatin and nuclear machineries. We discuss a model of the underlying functional nuclear organization derived mostly from electron and super-resolved fluorescence microscopy studies. It is based on two spatially co-aligned, active and inactive nuclear compartments (ANC and INC). The INC comprises the compact, transcriptionally inactive core of chromatin domain clusters (CDCs). The ANC is formed by the transcriptionally active periphery of CDCs, called the perichromatin region (PR), and the interchromatin compartment (IC). The IC is connected to nuclear pores and serves nuclear import and export functions. The ANC is the major site of RNA synthesis. It is highly enriched in epigenetic marks for transcriptionally competent chromatin and RNA Polymerase II. Marks for silent chromatin are enriched in the INC. Multi-scale cross-correlation spectroscopy suggests that nuclear architecture resembles a random obstacle network for diffusing proteins. An increased dwell time of proteins and protein complexes within the ANC may help to limit genome scanning by factors or factor complexes to DNA exposed within the ANC.

1. Introduction

In this review we describe current progress and perspectives of the emerging field of 4D nucleome research. This new term was chosen by the US National Institutes of Health (NIH, USA) as heading for a new Common Fund's program, which was announced in December 2014 with the goal "to understand the principles behind the three-dimensional organization of the nucleus in space and time (the 4th dimension), the role nuclear organization plays in gene expression and cellular function, and how changes in the nuclear organization affect normal development as well as various diseases." (https://commonfund.nih.gov/4Dnucleome/index). Two international 4D Nucleome Workshops, held in Germany (2013) and Japan (2014), have initiated efforts to coordinate this research worldwide [1]. These various initiatives indicate that we are currently on the verge of a concerted effort to dissect the structure–function relationships that operate in the cell nucleus. In this review, we will attempt to integrate published work on nuclear architecture into a model that may serve as a starting point for upcoming studies within the framework of 4D nucleome programs.

To set the stage, a brief historical introduction seems appropriate. The nucleus appeared on the scientific agenda in the early 19th century as a characteristic entity of both plant and animal cells (for review see [2]). Studies performed toward the end of the 19th century and during the beginning of the 20th century culminated in the discovery of the nucleus as the bearer of heredity and in the Boveri-Sutton chromosome theory of heredity, but this theory was severely doubted [3]. According to Rabl (1885) [4] and Boveri (1909) [5] interphase chromosomes occupied distinct regions within a cell nucleus, for which Boveri introduced the term chromosome territories (CTs). Notably, Boveri was keen to

* Corresponding authors.
E-mail addresses: Thomas.Cremer@lrz.uni-muenchen.de (T. Cremer), Karsten.Rippe@dkfz.de (K. Rippe), c.cremer@imb-mainz.de (C. Cremer).
[1] Present address: Nanyang Technological University, Singapore.
[2] Present address: Helmholtz Center Munich, German Research Center for Environmental Health, Garching, Germany.

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reconcile structural and functional aspects of chromosomes within a unified theory of heredity, but lacked the experimental tools to prove the concept of chromosome territories at his time [3,6]. He considered chromosomes as individuals, which retain their identity throughout interphase notwithstanding any possible structural transformations. In contrast, contemporary cytologists preferred the view that chromosomes would dissolve into chromatin particles during interphase and that these particles would only aggregate into chromosomes at the onset of the next mitosis. Confronted with this argument, Boveri pointed out that the chromosome theory of heredity required in such a case that "all particles belonging to a given chromosome possess an affinity toward each other of such a kind that they would come together again in one chromosome" at the onset of mitosis [5].

The rise of molecular biology during the second half of the 20th century with its goal to understand mechanisms by which genome information is processed was paralleled by a great interest into the structure of molecular components, such as nucleic acids and proteins. The nucleus was considered as an organell with a complex biochemistry. While great emphasis was laid on the structural elucidation of proteins relevant for nuclear functions, as well as DNA and nucleosome structure, quantitative studies of higher order chromatin arrangements and nuclear organization at large were considered as less important for nuclear functions. Quite frequently they were labeled as 'only' descriptive, less rewarding and consequently less fundable [7]. It seems that we are currently observing a paradigm change due to the development of advanced biophysical and biochemical tools, including the means for the 3D mapping of entire genomes, as well as advanced microscopic approaches. As pointed out in detail below, the nucleus has emerged as a telling example where changes of higher order chromatin structure may precede or follow changes of nuclear functions, making nuclear structure and function two inseparable sides of the same coin. We now have come a full circle from the early days, where it was without doubt that understanding the function of the nucleus with its chromosomes depended essentially on understanding higher order chromatin structure. A few recent examples may serve to illustrate this intimate correlation between nuclear structure and function:

(1) Features of chromatin contribute to DNA damage signaling and repair. Both chromatin condensation and decondensation play a significant role in the DNA damage response: Upon DNA damage, chromatin regions transiently expand before undergoing compaction [8].
(2) During differentiation, changes in transcription and replication timing are apparently correlated with changes in their nuclear positions. Using synthetic transcription factors, it was found that transcriptional activation of endogenous genes by a viral trans-activator is sufficient to induce gene repositioning toward the nuclear interior in embryonic stem cells [9].
(3) Chromosome conformation capture techniques have provided evidence for topologically associating domains (TADs). These findings are consistent with microscopic evidence for chromatin domains in the order of 100 kbp to 1 Mbp (see below). Using a polymer model, actual physical distances have been predicted [10]. The predictions of such physical models can be tested using high-resolution fluorescence in situ hybridization (FISH) in combination with super-resolved fluorescence microscopy.
(4) Nuclear features such as heterogeneity of local molecule concentrations, crowding effects, phase separation and entropic forces are coming to the forefront of research [11–13]. We anticipate that the understanding of these biophysical properties for the space–time dynamics of nuclear organization will become an important foundation for the 4D nucleome program to fully understand nuclear structure and function.
(5) The nuclear architecture of rod photoreceptor cells differs fundamentally in nocturnal and diurnal mammals. The rods of diurnal retinas possess the conventional architecture found in nearly all eukaryotic cells, with most heterochrom atin situated at the nuclear periphery and euchromatin residing toward the nuclear interior. The rods of nocturnal retinas have a unique inverted pattern, where heterochromatin localizes in the nuclear center, whereas euchromatin, as well as nascent transcripts and splicing machinery, line the nuclear border. The inverted pattern forms by remodeling of the conventional one during terminal differentiation of rods. The inverted rod nuclei act as collecting lenses, and computer simulations indicate that columns of such nuclei channel light efficiently toward the light-sensing rod outer segments [14].

The emerging field of 4D nucleome research appreciates that structure–function relationships need to be explored at all levels of nuclear organization from molecules to the entire system [15]. Notwithstanding the major advancements in nucleome research during the last few years both with respect to experimental progress and modeling (for reviews see [10,16–25]) we face major gaps in our current understanding of how nuclear functions are interconnected with dynamic changes of nuclear organization. A unified theory of the functional nuclear organization with an internationally accepted nomenclature is still lacking and appears as a major goal of an international nucleome program. Since contributors to FEBS letter special issues are encouraged to express personal views and provocative ideas, we take advantage of this freedom. Our main focus is laid on the space–time organization and functional interplay of the major structural components. We propose an integrative model of functional nuclear organization based on two co-aligned, active and inactive nuclear compartments, abbreviated below as ANC and INC. We review the empirical basis for this model view, provided by electron microscopy, fluorescence microscopy and correlation spectroscopy, as well as new biochemical approaches of 3D genome mapping. We discuss evidence that the nuclear interior represents a porous medium or sponge like structure. A protein, such as a transcription factor, that enters the nucleus via a nuclear pore complex, diffuses preferentially within the ANC and explores this structure along the chromatin lining channels. This concept provides a functional interpretation of a higher order chromatin organization based on the principle of a three-dimensional network of chromatin domain clusters pervading the nuclear interior rather than extended chromatin fibers.

Issues of failed or successful attempts to propose a nomenclature have accompanied studies of chromosomes and nuclei from their beginnings and this process continues today [3,26]. In the following description of the ANC–INC network model we describe features of nuclear assemblies with terms, such as chromatin domain cluster (CDC), interchromatin compartment (IC) and perichromatin region (PR), which are not generally accepted or in use. The preliminary nature of such terms emphasizes that we still have a long way to go in order to achieve an integrated view of the relationships between nuclear structures and functions in space and time.

2. Current state: a model of functional nuclear organization based on co-aligned active and inactive nuclear compartments

Fig. 1 presents our view that nuclear organization reflects two co-aligned, three-dimensional networks – the active (ANC) and

the inactive nuclear compartment (INC). Evidence supporting this ANC–INC network model is shown in Figs. 2 and 3. Our model of the functional nuclear organization summarizes progress of knowledge about the space–time organization of CTs since the 1990s. To our knowledge the hypothesis of a functionally important nuclear subcompartment of interchromatin channels, pervading the nuclear space, first emerged during the 1980s and 90s [27–37]. A nuclear landscape with an ANC and INC as major structural components may be compared with a natural landscape, where brushwood growing at the shore zone of an interconnected system of little streams and ponds invades their interior. The ANC contains two essential structural entities, the interchromatin compartment and the perichromatin region. The IC reflects a network of channel-like regions mostly devoid of DNA, which start/end at nuclear pore complexes (NPCs), penetrate the layer of heterochromatin beneath the nuclear lamina and expand through the nuclear interior both between and within CTs. This network pervades the space between chromatin domains (CDs) in the order of 1 Mbp and a network of chromatin domain clusters (CDCs). Splicing speckles (interchromatin granules) and other types of nuclear bodies are located within enlarged regions of the IC, called IC lacunas [38–40].

The IC is delineated by the PR, which represents decondensed chromatin located at the periphery of CDCs [40–43]. The PR is enriched with regulatory and coding sequences of active genes and represents the preferential nuclear subcompartment for transcription, RNA-splicing, DNA replication and repair. Recent super-resolved fluorescence microscopy studies of a variety of mammalian cell types from human, mouse and cattle demonstrate a highly significant enrichment of RNA polymerase II, histone H3 lysine 4 trimethylation (H3K4me3), an epigenetic marker for transcriptionally competent chromatin, nascent RNA, as well as nascent DNA in the PR. In contrast, histone modifications that mark transcriptionally silent chromatin, such as trimethylation of histone H3 at lysine 9 (H3K9me3) and 27 (H3K27me3) are enriched in the interior of CDCs [43–45]. Small chromatin loops may expand from the PR into the interior of IC channels, but we lack quantitative data on their size, compaction and arrangements within the ANC. The dynamic structural and functional interactions between the compact interior of CDCs, the PR and the IC are not well understood. Similarly, like the shore zone interacts with the land outside, dynamic interactions are expected to occur between the ANC and INC, including local repositioning of chromatin from the INC into the ANC to allow transcription, replication and repair. Continuous, constrained movements of chromatin were observed from the level of individual ~1 Mbp CDs to entire CTs. Such movements enforce continuous changes of the actual width of IC-channels and lacunas, and provide possibilities for dynamic changes of chromatin interactions [46–48].

The fraction of the nuclear volume occupied by the IC channel system varies in different somatic cell types [41], changes massively during cell differentiation [45] and can be experimentally manipulated [38]. Incubation of living cells in hyper-osmolar medium induce a rapid change of normally condensed chromatin (NCC) to hyper-condensed chromatin (HCC) with a massive, relative increase of the IC. This effect is fully reversible, when cells are again incubated in normo-osmolar medium. The higher-order chromatin arrangements observed in individual nuclei was stably maintained, when single living cells were subjected to several NCC-HCC-NCC cycles and such cells continued to proliferate. Notably, chromatin domains are also preserved, when living cells are incubated in hypo-osmolar medium [38]. Major differences of nuclear phenotypes with regard to higher order chromatin arrangements and the relative size of the interchromatin compartment were discovered in studies of mammalian cell nuclei from preimplantation embryos, embryonic stem cells and somatic cell types [14,43,45,49,50]. For example, in vitro fertilized bovine preimplantation embryos on their way to major genome activation (MGA) at the 8-cell stage show particularly large nuclei with a major IC lacuna in their interior, enriched with splicing speckles,

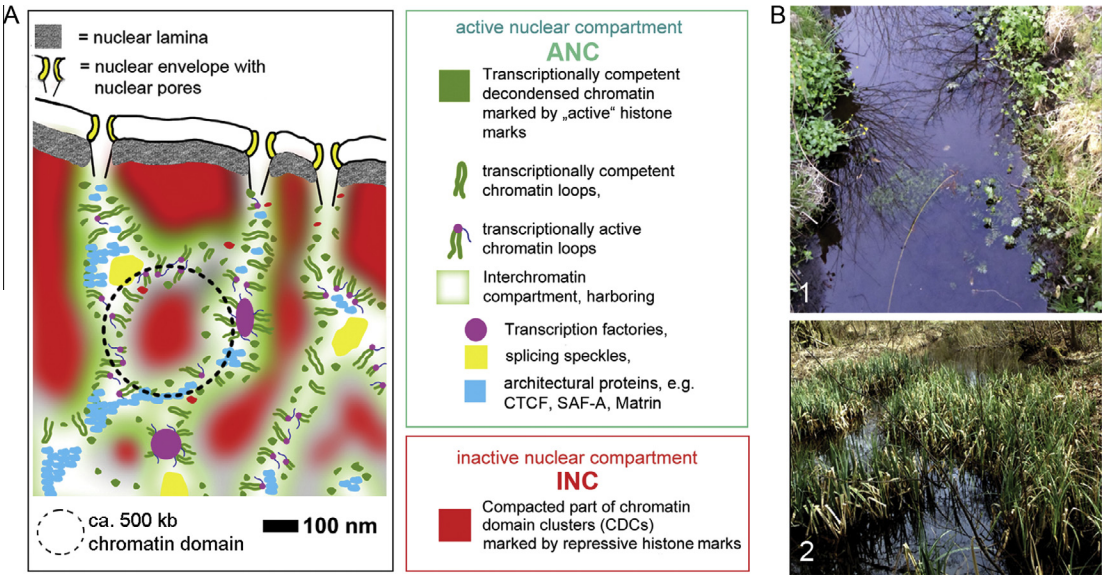

Fig. 1. ANC–INC network model of nuclear organization based on spatially co-aligned active and inactive nuclear compartments. (A) Nuclear organization according to co-aligned 3D networks of an active (ANC) and an inactive nuclear compartment (INC). The ANC is a composite structural and functional entity of a 3D-channel network, the "Interchromatin-Compartment" (IC) together with the decondensed periphery of a higher order chromatin network, which pervades the nuclear space and is built up from chromatin domain clusters (CDCs). The decondensed periphery of CDCs is known as the perichromatin region (PR). According to this model the PR harbors the regulatory and coding sequences of active genes and represents the preferential nuclear subcompartment for transcription, RNA-splicing, DNA replication and repair. Small chromatin loops expand from the perichromatin region into the interior of IC channels which start/end at nuclear pore complexes. Nuclear bodies are located within the IC, which serves as a transport system for macromolecule complexes. The INC is represented by the compacted core of CDCs enriched in markers for silent chromatin (modified from [43,45]). (B) Comparison of the co-aligned 3D networks of the ANC and INC with a natural swampland, where brushwood growing at the shore zone invades to a different extent (1,2) into the interior of an interconnected system of little streams and ponds.

whereas chromosome territories show a markedly peripheral location with individual CTs often separated by wide IC channels [45]. In line with features observed in somatic cell nuclei from various mammalian species, including fetal bovine fibroblast nuclei, the central lacuna disappears in nuclei of post-MGA embryos. Nuclei become significantly smaller and are characterized by chromatin expanded throughout the nuclear space with nuclear borders and nucleoli marked by a rim of heterochromatin. Nuclear phenotypes of bovine preimplantation embryos generated by somatic cell nuclear transfer (SCNT) of bovine fetal fibroblasts reveal a surprisingly similar pattern of architectural changes during preimplantation development. Despite these stage-specific major differences of global architecture, nuclei of IVF- and SCNT- embryos share basic features of the ANC–INC model with bovine fibroblast nuclei [45]. Rod cell nuclei in the retina of nocturnal mammals demonstrate the most radical deviation from a conventional nuclear phenotype of somatic, mammalian cell types [14]. An electron microscopic study [51] indicates a displacement of the IC toward the nuclear periphery. H3K4me3 is also enriched at the nuclear periphery, whereas the large interior mass of facultative and constitutive heterochromatin is enriched in H3K9me3 and other epigenetic marks for silent chromatin [51]. Despite major and sometimes dramatic differences of nuclear phenotypes, present evidence argues in favor of evolutionary conserved features of nuclear architecture. To test the possible extent of evolutionary conservation of such features further studies of eukaryote species are necessary, representing evolutionary distant phylogenetic taxa. Such studies will shed light on structural features, which were possibly maintained in all taxa because of their basic functional relevance. Studies of the 3D genome architecture of archaea (as an outgroup) will shed light on functionally important structural features, which may have already originated in the prokaryote world [52–55].

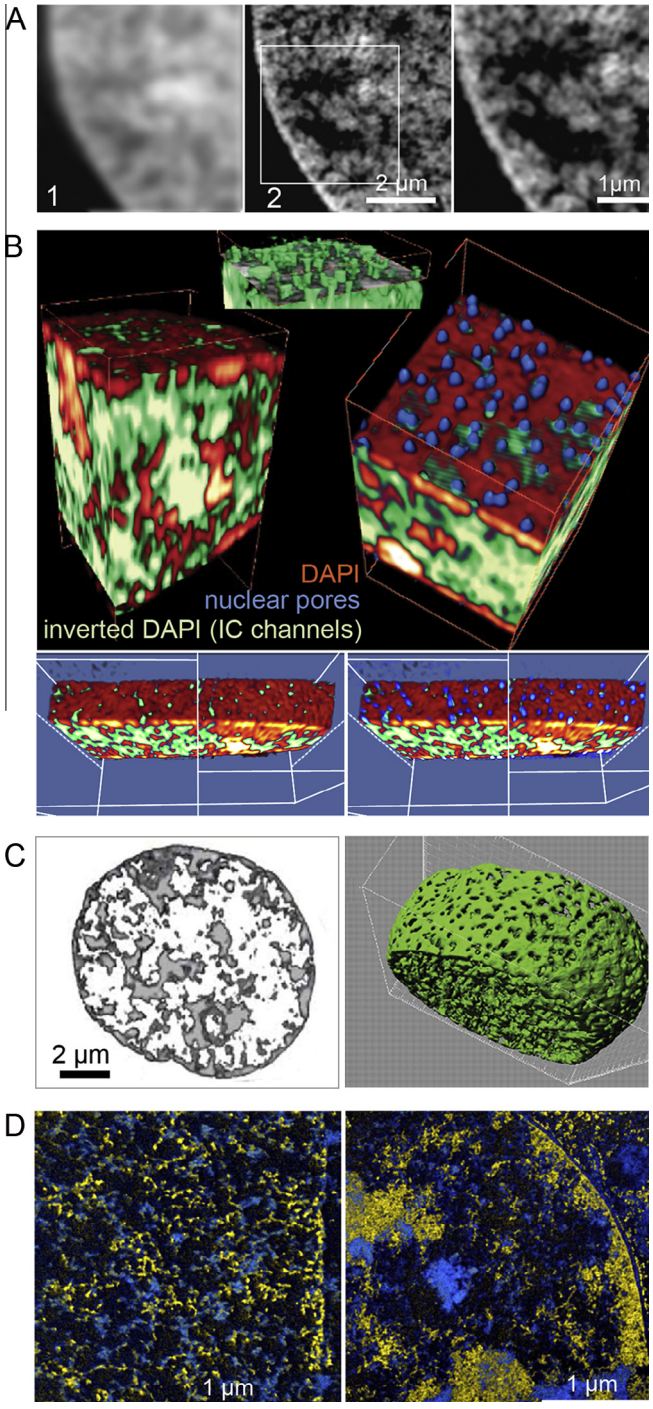

Fig. 2. Chromatin domain organization and an interchromatin compartment revealed by super resolved fluorescence microscopy (A, B) and electron microscopy (C, D). (A) Optical section of part of a DAPI stained mouse cell nucleus obtained by conventional laser scanning confocal microscopy (1) in comparison to structured illumination microscopy (2) exemplifies the gain of information by super-resolved microscopy revealing a network of chromatin domain clusters pervaded by an IC channel system occasionally forming large lacunae (adapted from [43]). (B) 3D-SIM reconstruction of a nucleus delineating segmented DAPI stained chromatin (brown), IC channels (green) and nuclear pores visualized by NUP153 immunostaining (blue) illustrates the connection of IC channels with nuclear pores (adapted from [43]; compare also supplementary movie 1). (C) 3D topography of specifically stained DNA and the interchromatin compartment (IC) in a rat hepatocyte nucleus studied with serial block face scanning electron microscopy (adapted from [41]). *Left:* 3D reconstruction of a 250 nm thick nuclear slice demonstrates chromatin domain clusters (CDCs; gray) pervaded by the interchromatin compartment (IC; white). *Right:* View on the surface of a several µm thick, 3D reconstructed central part of this nucleus shows little holes in the peripheral layer of heterochromatin, representing IC-channels expanding to the nuclear pores (compare Fig. 2B). (D) 50 nm thick sections of nuclei recorded by electron spectroscopic imaging (ESI), an electron microscopic method that allows the distinct visualization of nucleic acids (yellow) and of proteins (blue); (Hilmar Strickfaden, unpublished images; for methodological details see [64]). The two images exemplify profound differences of nuclear architecture in different cell types. *Left*: Detail from a HeLa cell nucleus shows a fairly homogeneous pattern of co-aligned proteinaceous and nucleic acid networks (for immuno-electron microscopic visualization of DNA and histone H2B in a HeLa cell nucleus see Fig. 3 in [38]). *Right:* Part of a SC35 hybridoma cell nucleus shows massive areas of heterochromatin both at the nuclear periphery and the interior. Large proteinaceous clusters (lacking in HeLa cell nuclei) and extended nucleic acid fibers are noted in the interchromatin compartment.

3. Nucleome organization studied by electron microscopy

Transmission electron microscopy (TEM) of nuclei yielded the first insights into the functional compartmentalization of cell nuclei [56–59]. The first evidence for and structural definition of the perichromatin region (PR) as the nuclear subcompartment, where chromatin with functional competence for transcription, co-transcriptional splicing, DNA replication and repair is located, was achieved in EM studies (for review see [60]). Nascent RNA in the PR was first demonstrated in an EM study by Stan Fakan and colleagues in a human cancer cell line, pulse-labeled with BrUTP [61]. This study demonstrated the enrichment of bromine-labeled RNA in the PR [61]. In situ hybridization with sense and anti-sense RNA probes combined with immunoelectron microscopy demonstrates that most transcribed DNA is concentrated in the PR [62]. Based on an EM pulse-chase-pulse labeling protocol with iododeoxyuridine and chlorodeoxyuridine, the Fakan group also demonstrated the formation of replicating DNA within the PR. DNA replication was followed by movements of nascent DNA over distances of about 100 nm into the interior of lining chromatin domains [63].

Despite the astounding advancements of biochemical approaches for 3D genome mapping and of super-resolved fluorescence microscopy beyond the classical Abbe limit (see below), EM will maintain an essential role in future studies, not only because of its superior resolution. Current EM approaches, such as 3D block face scanning EM [41] (Fig. 2C), electron spectroscopic imaging [64] (Fig. 2D) and focused ion beam-scanning electron microscopy [65,66], have a huge potential of their own. Based on cryo-EM [67] and X-ray scattering studies [68,69], Maeshima and colleagues proposed a liquid drop model of chromatin domain organization, arguing that the interior of domains is so densely packaged that nucleosome interactions between 10 nm thick chromatin fibers prevent the formation of 30 nm thick fibers. Since non-random contacts between chromatin fibers may be enforced even in such a situation [70,71], the liquid drop model does not exclude an ordered topography of a chromatin domain.

4. Evidence for a nested doll organization of chromatin domains from super-resolution fluorescence microscopy and chromatin conformation capture

A combination of recent microscopic and biochemical studies has shown that CTs – like Russian Matryoshka dolls, which consist of a series of nested dolls of decreasing size placed one inside the other – are built up from chromosome arm domains, chromosome band domains, and chromatin domains (CDs) (for review see [16]). Major progress became possible by advanced microscopic approaches, including 3D electron microscopy (see above) and super-resolved fluorescence microscopy (SRM) [60,72]. Recently, SRM approaches achieved an optical and structural resolution in the few tens of nanometer regime (for reviews see [73–76]). These developments have the potential to revolutionize structure–function studies in cell biology. SRM has already been employed in a series of studies for 'nano-imaging' of nuclear structures [40,43–45,77–80]. The results of these studies support the ANC–INC network model of nuclear organization described above.

Microscopic approaches to elucidate 3D genome/chromatin arrangements were complemented by the invention of a seminal approach called chromosome conformation capture [81]. As Job Dekker put it in an interview, this method "is akin to a 'molecular microscope' to detect physical interactions between chromosomes." It is based on formaldehyde fixation of cells followed by DNA digestion with restriction enzymes, ligation of cross-linked fragments and sequencing. In Hi-C, relative contact frequencies are studied genome wide using deep sequencing. Contact frequencies are used as a measure for the average nuclear proximity of any given pair of cross-linked fragments. These studies confirmed the microscopic evidence for CTs and chromatin domains in the order of ~1 Mbp. Domains defined by Hi-C are referred to as topologically associating domains (TADs) and are in turn built up from smaller globules [82–84]. The higher the number of sequenced ligation products, the better the resolving power of Hi-C. Although single-cell Hi-C has become possible [85], its coverage is limited. Current Hi-C work is routinely conducted with some millions of cells. Billions of sequence-identified DNA–DNA contacts are required for high resolution 3D maps. A 3D map of the human genome at kilo-base resolution, for example, was based on sequencing of 4.9 billion contacts [86]. The authors identified ~10.000 major loop domains, which frequently link promoters and enhancers. Loop anchors bind the CCCTC-binding factor CTCF and typically occur at domain boundaries [87]. Individual contact domains (with an average size of ~185 kbp) fold in different ways and form larger subcompartments with at least six distinct patterns of histone marks, reflecting the active or silent state of genes. How active and silent TADs are organized with respect to each other and to the nuclear architecture at large is currently not clear. The introduction of the new term 'origami code of gene regulation' (based on the Japanese art of paper folding) emphasizes the potential importance of local chromatin folding in gene regulation with conservation between humans and mouse [82]. The authors of Ref. [86] have suggested "that folding is regulation. When you see genes turn on or off, what lies behind that is a change in folding." (http://news.harvard.edu/gazette/story/2014/12/creating-genomic-origami/).

Notwithstanding the breakthrough accomplishments of Hi-C in providing high-resolution maps of the 3D folding of the entire linear DNA sequence, several limitations of this approach need to be considered. First, conclusions about spatial genome arrangements are based on the questionable assumption that all possible DNA–DNA contacts are captured with the same probability. Although two pairs of DNA segments A/B and C/D may show the same average proximity in a given population of cell nuclei, their relative contact frequencies may differ because of different probabilities of the formaldehyde fixation procedure to capture DNA–DNA contacts, established by cross-linking proteins with different DNA binding efficiencies [88]. Second, Hi-C provides average contact probability data for TADs without a defined scale. Third, the size of a protein complex, which links two DNA segments, may largely vary. Accordingly, a contact captured by Hi-C does not tell unambiguously, how close in terms of nm the two DNA segments were actually arranged in a given nucleus. Notably, a significant increase of average contact frequencies obtained by a Hi-C study of a large cell population between genes, located on different CTs, as a result of a certain experimental stimulus does not necessarily support a long-range motion of the respective CTs or of giant chromatin loops moving over μm distances toward each other. Even in case of two non-homologous CTs, which are widely separated on average in such a population, their actual positioning varies largely in different nuclei. Accordingly, an increased contact frequency measured by population Hi-C after a given stimulus, may reflect induced small scale movements of the respective genes to, e.g., the same transcription factory in a subpopulation of nuclei, where these genes were located by chance already rather close to each other before the stimulus was applied. These considerations emphasize a necessity for single cell studies and approaches that integrate microscopy and sequencing based methods.

5. Dynamics of higher order chromatin organization

Live cell approaches have provided direct insight into the stability and dynamics of higher order chromatin arrangements. Labeling of chromatin was achieved by incorporation of nucleotides tagged with a fluorophore into replicating DNA during S-phase of the cell cycle. Labeled cells were allowed to grow for several additional cell cycles resulting in the segregation of labeled and unlabeled chromatids. Nuclei of cycling interphase cells, which contained only a few labeled CTs, were ideally suited to study movements of CTs [48]. Alternatively, cells were grown expressing histones H4 tagged with photoactivatable green fluorescent protein (H4-paGFP) and H2B tagged with red fluorescence protein (H2B-mRFP) [47]. Patterns of fluorescent paGFP were induced with a uv-microbeam. The results of such studies indicated that once established at early G1 CT proximity patterns were maintained through interphase and prophase. However, during prometaphase chromosome neighborhood arrangements undergo likely random changes. Accordingly, chromosome neighborhood arrangements differ profoundly in different metaphase plates, so that CT proximity patterns in the progeny of a given cell change from one cell cycle to the next. In contrast, radial CT arrangements are maintained in cycling cells. For example, gene dense and

transcriptionally active chromatin is preferentially located in the nuclear interior of most cell types, whereas the nuclear periphery contains mostly gene poor, transcriptionally silent chromatin [89]. In addition, transcriptional activation or silencing of genes can affect their radial nuclear position (for review see [90,91]. Chromatin domains were formed and remodeled by RNA polymerase and topoisomerase activities [92] and changes of chromatin architecture were detected at hundreds of sites during differentiation of mouse embryonic stem cells into lineage committed neural precursor cells and terminally differentiated astrocytes [93]. The visualization of focal DNA replication in living cells [38,48] confirmed previous studies of replication foci in fixed cells [94,95]. These live cell studies confirmed the structural stability of focal domains outside S-phase and during subsequent cell cycles, as well as constrained Brownian movements [46,48,96–98]. Quantitative motion analyses suggested that random movements may occasionally switch to directional movements [46]. Recent studies also demonstrated unidirectional, curvilinear movements of HSP70 loci upon heat shock toward nuclear speckles over ~0.5–6 μm distances at velocities of 1–2 μm per minute [99]. Movements of two chromatin sites, each carrying a double-strand break (DSBs), over distances up to several μm were observed in an experimental system to visualize the formation of translocations in living cells [100]. When two sites met in space they moved occasionally thereafter in synchrony as paired arrays, possibly suggesting a translocation event. While these examples demonstrate long-range movements of chromatin at the single cell level, further studies are necessary to elucidate the mechanisms responsible for them. It will be interesting to see whether apparently directed movements reflect movements along IC-channels.

6. Functional implications of an interchromatin channel system

Proteins involved in transcription, splicing, replication and repair need to access chromatin embedded in the nuclear interior, while ribonucleoprotein particles carrying messenger RNAs have to reach nuclear pore complexes (NPCs) for export. We propose that the connection of NPCs with the ANC serves as a system for nuclear import–export functions, including the supply of target regions involved in DNA replication, repair or epigenetic modifications. The integrated NPC/ANC system may help to speed up both the access of relevant proteins entering the nucleus to their target sites and the export of messenger RNA toward NPCs along the IC channel system. As described above, current evidence demonstrates that essential nuclear functions like transcription, DNA replication and repair occur within the ANC, more specifically within the perichromatin region (PR). We expect, although compelling evidence for this is still lacking, that both regulatory and coding sequences of genes are exposed within the PR and possibly also on chromatin loops, which expand into the interior of the IC (Fig. 1). Several lines of evidence were recently obtained in favor of this view.

Mor and co-workers [101] reported that interchromatin channels in mammalian cell nuclei ensure "a steady and continuous wave of mRNPs traveling toward the NPC." Recent studies of blastomere nuclei in bovine pre-implantation embryos shed new light on the interplay between contact sites of chromatin at the nuclear envelope and the formation of NPCs, as well as functional advantages of the direct connection of NPCs with the ANC [49]. Up to the onset of major embryonic genome activation (MGA) at the 8-cell stage blastomere nuclei show a non-uniform distribution of nuclear pore complexes (NPCs). NPCs are exclusively present at sites where DNA contacts the nuclear lamina, whereas extended regions of the lamina without such contacts lacked NPCs. In post-MGA embryos, both chromatin contact sites and NPCs are distributed with similar density throughout the entire nuclear envelope. The switch from maternal to embryonic production of mRNAs is accompanied by multiple invaginations of the nuclear envelope covered with NPCs. These invaginations likely served the increased demands of mRNA export and protein import at this stage, but disappeared during further pre-implantation development. In the absence of such invaginations, proteins, which enter the nucleus through NPCs, need to overcome distances up to several μm in order to reach specific target sites located deep in the nuclear interior.

We assume that chromatin domain clusters (CDCs, Fig. 1) are organized in a way that their interior is much less accessible for these factors. The functional benefit of such a topography could be that the local search time of factors entering the nucleus may be considerably shorter for DNA sequences positioned within the ANC as compared to sequences embedded in the compact interior of CDCs. Such a functional topography may help to limit genome scanning by factors or factor complexes to a minor fraction of the entire genome exposed within the ANC (see below).

Studies in living mammalian cells, based on green fluorescent protein (GFP) and its multimers as a marker, have indicated larger deviations from normal diffusion of individual proteins in more condensed chromatin regions than in the interchromatin space [102]. How the nuclear interior is 'sensed' by a moving protein was mapped in a comprehensive manner by high-resolution multi-scale fluorescence cross-correlation spectroscopy (msFCCS) analysis of the mobility of inert monomers, trimers and pentamers of GFP, as well as GFP fusions with other proteins in nuclei of living cells [103,104]. The msFCCS experiments conducted in these studies measured how long it takes proteins to move over distances in the range from 0.2 to 3 μm in the nucleus of living cells. The resulting distance versus time maps revealed that the nuclear interior appears as a porous medium or sponge like structure from the point of view of a protein that diffuses within the nucleus (Fig. 4). According to this model, the mobility of the particle – its time-dependent diffusion coefficient $D(t)$ – is determined by a relation that contains only three parameters:

$$D(t) = (D_0 - D_\infty)\exp\left(-\frac{4\sqrt{D_0 t}}{\sqrt{\pi}\lambda}\right) + D_\infty$$

On short length scales, particles showed free diffusive behavior with the microscopic diffusion coefficient D_0 in regions devoid of obstacles that would represent bona fide IC domains. However, when moving for a distance in the range of 1–2 μm (represented in the model by the correlation length λ), the GFP tracers with hydrodynamic diameters of 5–14 nm "sensed" the chromatin obstacle structure. Their mobility was largely slowed down leading to an increased local dwell time. It reached a plateau with a reduced macroscopic diffusion coefficient D_∞ measured at large time/length scales at which the particles mean squared displacement becomes linearly dependent on time again. In this type of nuclear organization the protein-chromatin interactions at the obstacle surface occur in a particle-size dependent manner. Such a linkage between particle size and chromatin organization might have important implications for the specificity and kinetics of enzymatic reactions that have chromatin as a substrate. According to the model depicted in Fig. 4, protein-chromatin interactions are dependent on variations in chromatin density and protein (complex) size. In this manner the perichromatin compartment, i.e. the chromatin surface surrounding lacunas with reduced chromatin density becomes a nuclear subcompartment in which genome interacting processes like transcription are largely facilitated (Figs. 2 and 3). These nuclear loci appear as accessible regions with reduced obstacle concentration from the perspective of the GFP multimers. In contrast, other chromatin

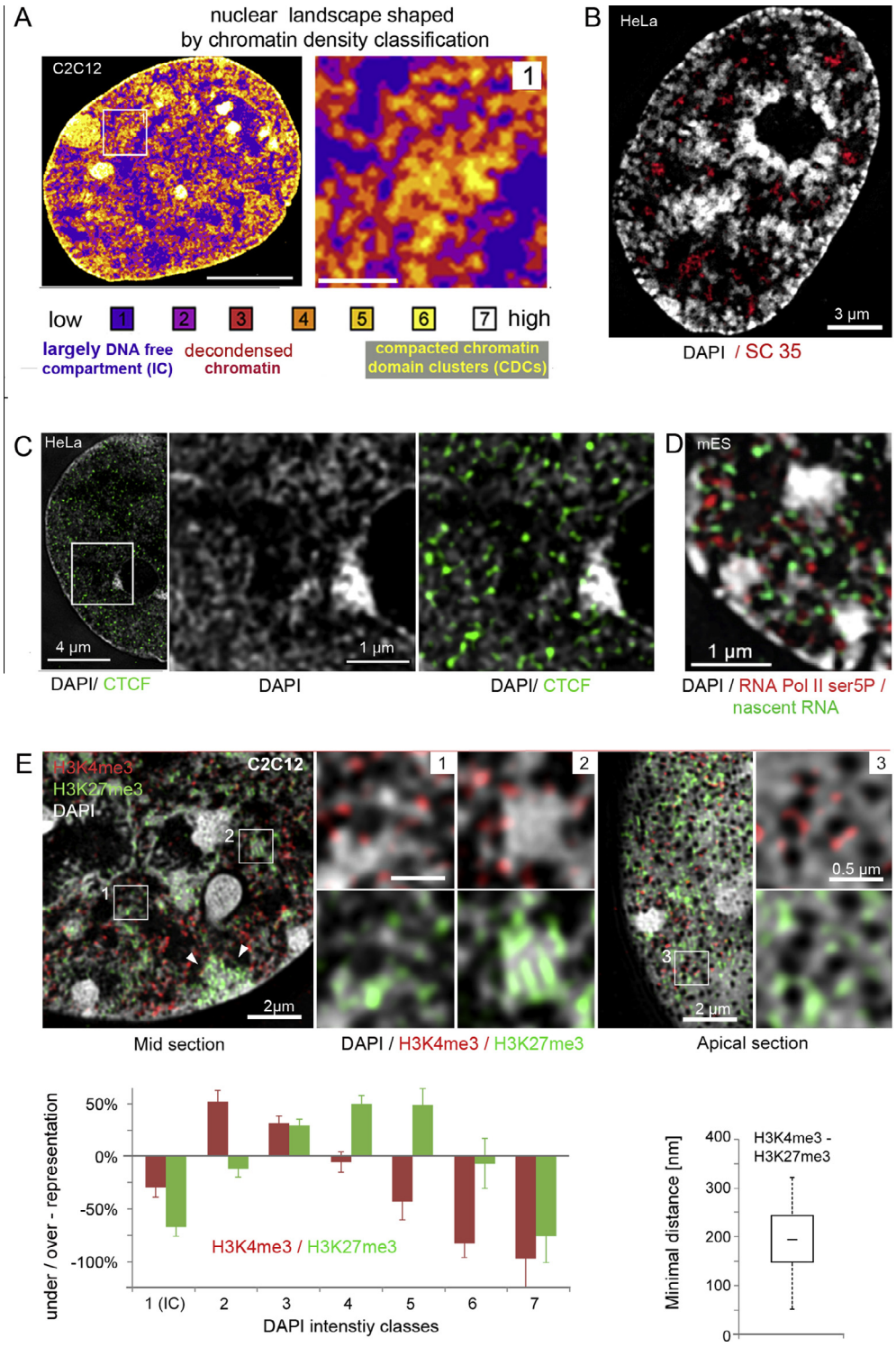

Fig. 3. Linking topological chromatin density maps with functionally relevant hallmarks using 3D-structured illumination microscopy (3D-SIM) (A) Nuclear landscape shaped by chromatin density in a C2C12 nucleus. Topological chromatin densities are based on seven classes with increasing DAPI intensity (blue = lowest DAPI intensities close to background; white = highest DAPI intensities). The inset magnification of a randomly selected area (1) reveals compacted chromatin domain clusters (CDCs, yellow) lined by a zone of low density chromatin (red/purple), permeated by an apparently DNA free interchromatin compartment (blue) (adapted from [43]). (B) Localization of immunodetected splicing speckles SC35 (red) in the interchromatin compartment (IC) of a HeLa cell nucleus (Barbara Hübner, unpublished; compare [39]). (C) Preferential positioning of the insulator binding factor CTCF at borders of compacted chromatin domains visualized in a HeLa cell nucleus. (D) Simultaneous visualization of nascent RNA (green) and immunostained RNA Pol II (red). The antibody detected the serine 5 phosphorylation that marks the initiating form of active RNA Pol II. The image reveals the preferential localization of these functional components in the active nuclear compartment comprising decondensed chromatin and the IC; compare supplementary movie 2. (E) Clear separation of H3K4me3- and H3K27me3-marked chromatin shown in an optical mid-section (left panels 1 and 2) and an apical z-section (right panel, 3) of a C2C12 nucleus (arrowheads delineate the inactive X chromosome). H3K27me3 is enriched within compacted CDCs representing the INC, whereas H3K4me3 is located mainly at the decondensed periphery of CDCs representing the ANC (insets 1 and 2; compare supplementary movie 3). In the apical z-section H3K4me3 enriched chromatin sites are largely restricted to the close vicinity of nuclear pores seen as DAPI voids whereas H3K27me3 is found also at more distant areas. The lower panels exemplify quantitative evaluations. *Left:* comparative mapping of H3K4me3 and H3K27me3 signals plotted as relative over/under-representation within the respective DAPI intensity classes; *right:* minimal distance distributions (nearest neighbor distances) for H3K27me3 and H3K4me3 (adapted from [43]).

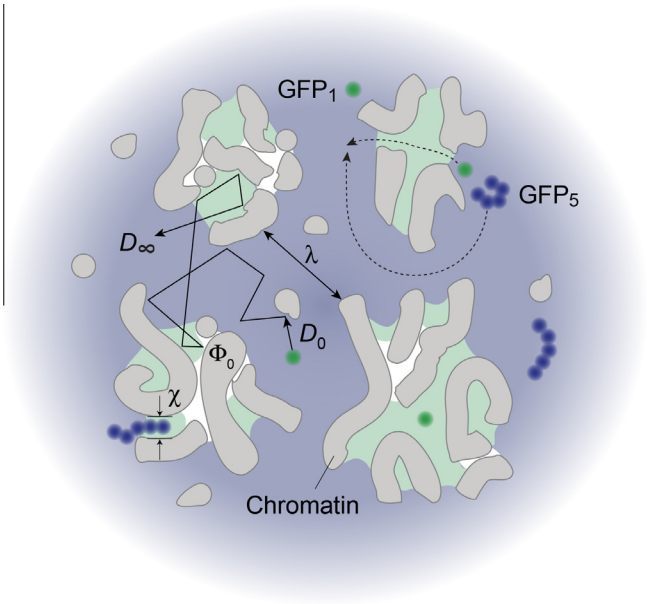

Fig. 4. The nuclear interior appears as a porous medium formed by randomly shaped chromatin obstacles. Nuclear organization as 'seen' by a protein according to a porous medium model that describes the particle mobility by its time-dependent diffusion coefficient $D(t)$ from three parameters as explained in the text (Baum et al. [103]): D_0, microscopic diffusion coefficient of particle mobility in the absence of collisions short time/length scales; λ, correlation length as a measure for the typical distance between obstacles; D_∞, reduced macroscopic diffusion coefficient at large time/length scales. These experimentally determined parameters depend on the particle size and can be used to derive additional important features of the chromatin obstacle structure as it is encountered by proteins in the nucleus: (i) The correlation length λ is inversely proportional to the surface-to-volume ratio S/V, which reflect the relative chromatin surface area that is accessible and searchable by a given protein. The scheme depicts that larger proteins have less accessible surface area on the obstacle clusters formed by chromatin. (ii) The dependence of the ratio of D_0/D_∞ on protein size yields a chromatin obstacles occupancy fraction of $\Phi_0 \sim 15\%$ of the nuclear space. (iii) The parameter $\chi \sim 15$ nm is the estimated throat size of small pores that confine regions in the nucleus where GFP_5 (hydrodynamic radius $r_H \approx 7.9$ nm) is trapped while GFP_3 ($r_H \approx 5.5$ nm) remains mobile (Figure adapted from [103]).

subcompartments with an increased chromatin density like lamina-associated domains [105] or pericentric heterochromatin ([106] and references therein) have a locally reduced surface to volume ratio and render a larger part of chromatin inactive for enzymatic processing. Notably, the heterogeneous nuclear distribution of the genome into chromatin domains with different density is dynamically regulated, for example by histone acetylation [107] or during differentiation of embryonic stem cells [108]. Interestingly, the Baum et al. study as well as previous work point to a throat size of nuclear channels in the range of 15–20 nm [107,109,110]. Accordingly, particles beyond this size are progressively excluded from dense chromatin regions. At a diameter of 100 nm particles show only locally confined movements restricted to distinct corralled regions surrounded by dense chromatin regions [111–113]. Thus, besides the protein size dependent access of chromatin surfaces discussed above, only particles $\leqslant$ 15 nm can explore the complete nuclear interior without becoming transiently trapped on the minute time scale.

The fraction of the nuclear volume occupied by chromatin domain clusters varies largely in different cell types. In a study of rat liver about 40% of the nuclear volume of endothelial cells belonged to the DNA-poor/-free nuclear space as compared to about 60% in hepatocyte nuclei [41]. The volume of nuclei and the width of channels changed drastically during bovine pre-implantation development [45]. The particularly large volumes and size of the IC in blastomere nuclei of bovine preimplantation embryos on their way toward major genome activation (MGA) can be explained by a necessity for the storage of factors, such as splicing factors, needed at the onset of MGA. This implies a necessity for the re-transport of such factors after mitosis from the cytoplasm via nuclear pores into newly formed daughter nuclei. Such a major shift of proteins and fluid may be compared with a flash flood entering a swamp like nuclear landscape (Daniel L. Hartl, personal discussion). A sudden, seasonal flood entering a swamp with its meandering and anastomosing channels will rapidly permeate throughout the channel system, but will much more slowly extent into or even be fully restricted from the land outside these channels. We expect that the patterns of normal and anomalous diffusion, which play a role in such a swamp, play also a role in the nuclear landscape.

7. Understanding the nuclear landscape in space and time

In summary, we propose a model of functional nuclear organization based on two spatially co-aligned, functionally interacting active and inactive nuclear compartments, called ANC and INC, respectively. In line with the concept of a Matryoshka doll-like 'globule in globule in globule' organization of higher order chromatin architecture [86], this ANC–INC network model argues for a higher order organization based on a network of chromatin domain clusters (CDCs). The INC is formed by the compact, transcriptionally inactive core of CDCs, whereas the ANC is formed by two components, the transcriptionally active periphery of CDCs, called the perichromatin region (PR), and a spatially co-aligned system of interchromatin compartment channels starting at nuclear pores, called the interchromatin compartment (IC). The IC channel system with its direct connections to nuclear pores serves three functional needs [49]: Firstly, it provides a means for the fast nuclear import of newly synthesized transcription factors and other functional proteins and protein complexes to chromatin target sites embedded in the nuclear interior. Secondly, it provides routes for the fast nuclear export of messenger RNPs. Thirdly, we assume rapid, normal diffusion of the corresponding factors within IC channels and into the decondensed chromatin of the PR facilitates contacts with target sequences exposed there, whereas a large fraction of the genome located in the compact interior of CDCs remains relatively, although not completely inaccessible. The paradigmatic shift from nuclear biochemistry to 4D nucleome research has resulted in a growing interest in fundamental problems of nuclear architecture and nuclear biophysics. Some of the open questions are illustrated below. The problems mentioned below illustrate our personal preferences and are not described with an intention to be complete.

7.1. Role of electrostatic effects for nuclear organization

Intracellular electric fields have been proposed to play a fundamental role in protein movement and localization within the cytoplasm [114]. New "Nanosized Voltmeter" approaches for cellular-wide electric field mapping, revealed that such E-fields may penetrate much deeper into the cytosol than previously estimated, indicating that, electrically, the cytoplasm cannot be described as a simple homogeneous solution, as often approximated, but should rather be thought of as a complex, heterogeneous hydrogel, with distinct microdomains [115]. Present experimental evidence shows that chromatin domains can function as "semipermeable barriers" for the movement of large charged particles like proteins. Microelectrode measurements [116] confirmed a negative intranuclear potential and showed that DNA has an inherent negative potential similar to that found in

intact cell nuclei. These observations support the view that the intranuclear potential originates from the composition and the functional state of the nuclear chromatin. DNA molecules can be considered as polyanions tightly organized in the cell nucleus and giving rise to a Gibbs-Donnan potential.

In line with electrophysiological observations and the structural evidence outlined above, we hypothesize that DNA, RNA and proteins display a complex spatial distribution of electrical charges, whose functional roles need to be considered in addition to physical contacts. Chromatin is not only a mechanically complex structure, but its behavior in nuclei of living cells depends on the complex spatial distribution of positive and negative electrical charges as well. Although the huge number of negative charges, which result from the phosphate groups in the backbone of genomic DNA, is neutralized to a large extent by positive charges resulting from histones and mobile ions, chromatin domains carry an excess of negative charges. Small and large molecules, which enter the nucleus through NPCs, also carry electrical charges. Accordingly, compact chromatin domains with negatively charged surfaces will attract positively charged molecules or molecule aggregates and repulse negatively charged ones. Such an effect in turn should influence the probability of mobile molecules and their aggregates to reside in the ANC or INC. Positively charged histones will interact with negatively charged DNA, while even a moderate enrichment of negatively charged, individual components of a macromolecule complex within the ANC compared to the INC could largely increase the probability that complexes involved in transcription, splicing, DNA replication and repair assemble within the ANC.

Nucleotides needed for both RNA and DNA synthesis and repair, as well as AMP, ADP, ATP and compounds like NAD, ADP-ribose and poly-ADP ribose all carry negatively charged phosphate groups. Molecules with a persistent negative net charge, which enter the IC channel system through NPCs, or are synthesized in the ANC may become enriched in the active nuclear compartment due to its strongly reduced density of chromatin [30,117]. In the context of the ANC–INC network model such an enrichment would help to fulfill the functional needs of the ANC as the nuclear compartment, where RNA and DNA synthesis, as well as DNA repair preferentially occurs. Considering the high concentration of mobile ions in a normotonic cell [38], it may, however, well be that negative electrical charges of such molecules are screened by counter-ions to an extent that their measurable enrichment within the ANC as compared to the INC is precluded. A definitive answer to this question cannot be based on intuition, but requires a combination of modeling and experiments. On the experimental side, it is important to note that poly-ADP ribose serves as a material source for intranuclear ATP synthesis [118]. It would be interesting to know, whether poly-ADP ribose is preferentially synthesized within the ANC with the consequence that the ANC would act as the major site for ADP ribose derived ATP synthesis. In addition or alternatively to effects of electrical charge and size on the enrichment of this compound within the ANC, preferential ATP synthesis in this compartment could result from a preferential location of enzymes and other factors needed for this synthesis.

Electrical charges may also play major roles for physical interactions within and possibly also between chromatin domains [119]. As an example, we consider large chromatin structures characterized by a local collapse of the IC channel system, such as chromocenters or the inactive X-territory [43]. The major difference of higher order chromatin organization between the inactive and the active X-territory, as well as other transcriptionally active autosomal territories may result from a major reduction or entire loss of macromolecular complexes carried within collapsed IC channels and a closing-up of CDCs. In addition, a major loss of transcriptional activity may be accompanied by an increased chromatin compaction in the perichromatin region (PR), whereas chromatin compaction in the interior of CDCs may be similar in both transcriptionally repressed and active CTs. We hypothesize that even highly compacted structures do not form an entangled chromatin mass, because the proper execution of structure–function interactions during DNA/chromatin replication, DNA repair and epigenetic modifications depends on the continued existence of separate chromatin domains pervaded by IC channels. The permanent existence of chromatin domains in both heterchromatic and euchromatic nuclear regions likely reflects the existence of macromolecular bridges, generated by architectural proteins and possibly also by non-coding RNAs between specific DNA segments within each domain [23,70,71,120,121]. In addition, repulsive forces between negatively charged chromatin domains may help to maintain very narrow IC channels between them and prevent an intermingling into larger chromatin masses in cases, where such domains come into close proximity (<10 nm) [29,30,118]. Mature chicken erythrocytes provide a model for nuclei in a predominantly heterochromatic state [122] where reactivation of such nuclei was observed in cell fusion experiments [123,124]. Although direct evidence for a role of electrical charges in maintaining the structural and functional integrity of both euchromatin and heterochromatin is lacking, we wish to emphasize the necessity to further explore biophysical fundamentals of nuclear architecture.

7.2. Potential architectural roles for RNAs and ‘junk’ DNA

4D nucleome research will further stimulate the search for architectural proteins, DNA and RNA sequences with effects on higher order chromatin organization. RNA in general shapes nuclear architecture in a variety of ways [125]. A recent example for such a function is the trans-chromosomal assembly of multiple gene loci mediated by the non-coding RNA Firre [126]. In general, the binding of a certain transcription factor at its DNA/chromatin-target site(s) may trigger a sequence of events resulting in functionally “open” (and nuclease sensitive) chromatin configurations as it has been shown for example for the high-mobility group protein HMGN5 [127]. This problem becomes even more complex, when one considers evidence for allele specific gene expression among non-imprinted autosomal genes [128]. Last not least, future studies of the potential implications of size, shape and charge of chromatin domains may shed light on the problem of the so-called junk DNA [129,130]. Junk DNA, by definition, includes evolutionary left-overs of DNA sequences, which lost any functional purpose or whose propagation serves only ‘egoistic’ roles without any advantage for the cell or organism, which carries them [131]. Yet, the purported loss of any functional significance may tell more about the focus of a researcher than about the true adaptive value or at least the selective neutrality of sequences. Repetitive sequences, for example, may play a role for the formation of architectural building blocks [132] and have been reported to produce highly abundant transcripts that are associated with euchromatin domains [133]. A comparison of the nucleus with a complex building may be useful in this respect. Certain pillars and walls may be critical for the stability of a building, others may be removed without an immediate consequence, but the removal of too many structural elements may still trigger a collapse. Integration of additional elements may help to improve the stability of the building, or at least have no negative consequences. In comparison with pillars and walls we argue that the size, shape and electric charge of chromatin domains may be essential for both a sufficiently stable and a sufficiently dynamic nuclear organization, where stability and dynamics depend on the actual circumstances. In case that the overall electrical charge of a chromatin domain matters, repetitive sequences may be of

adaptive value, since they can add to the actual size of the negative overall surface charge of chromatin domains.

7.3. Genome organization by a nuclear matrix

It is interesting to note that architectural proteins, which can be enriched in biochemical 'nuclear matrix' preparations, such as CTCF, SAF-A, Matrin 3, are enriched within the PR (Fig. 3; [43]). High salt conditions and/or the removal of fixed negative electric net charges of chromosomal domains by DNase I digestion, are two treatments commonly performed in nuclear matrix preparations. These reduce the repulsive forces, which normally exist between negatively-charged macromolecule aggregates and negatively charged chromatin surfaces and result in the precipitation of macromolecules within the crowded ANC without a need of a pre-existing filamentous network. Current experimental data and modeling provide evidence that higher order chromatin organization depends on the structural features of chromatin by itself [134]. While the early concept of a nuclear matrix, which organizes a cell type specific higher order chromatin organization like a nuclear skeleton has become outdated [135–137], an artificial aggregation of proteins under high-salt conditions does not rule out an important function of a subset of architectural proteins in the structural organization of the PR [138]. To what extent certain architectural proteins, possibly including non-coding RNAs, contribute to the formation of proteinaceous filaments with certain functional properties within the ANC, is still an open question. Independent of the outcome of future studies, we consider it as an important contribution of the nuclear matrix hypothesis that it emphasized the relevance of nuclear architecture for nuclear functions at a time, where many researchers seemed to be content with the assumption that studies of 3D and 4D genome organization were functionally irrelevant [139,140].

7.4. Dynamics of ANC–INC interactions

We do not know in which ways and to which extent the ANC and INC may dynamically interact with each other. With respect to the topography of regulatory and coding sequences we consider several scenarios: (a) Regulatory sequences of genes may be exposed within the ANC independent of whether genes are active or inactive. (b) Only regulatory sequences, which are in active use in a given cell, may be located within the ANC, whereas regulatory sequences of genes permanently shut off in this cell are retracted into the INC represented by the compact and largely inaccessible interior of CDCs. In this case shifts of regulatory sequences embedded within the INC into the ANC should occur prior to the activation of the respective genes, while repositioning of such sequences from the ANC into the INC should occur when these genes become inactive. (c) Similar considerations apply to coding sequences. It should be noted that the space–time topography of coding sequences may differ from the topography of regulatory sequences. The coding sequence of a non-transcribed gene may be embedded within the compact interior of a chromatin domain, while the related regulatory sequences are exposed at the periphery. We expect that future research will yield very dynamic interactions between co-aligned ANC and INC networks. Quantitative studies of the space–time 3D topography of epigenetic modifications within the ANC and INC during cell differentiation [43,45,49] will help to better define the structure–function relationships between the ANC and INC. In addition to epigenetic histone modifications, it is necessary to explore the space–time topography of epigenetic modifications at the DNA level, including 5-methylcytosine and its derivatives driven by TET enzymes, such as 5-hydroxymethylcytosine, 5-hydroxymethyluracil, 5-formylcytosine and 5-carboxylcytosine [141].

7.5. Nuclear envelope and lamina

Bas van Steensel and colleagues discovered that in cycling cells about 30% of lamina associated chromatin domains (LADs) were found in intermittent molecular contact with the lamina beneath the nuclear envelope [142]. These LADs remained constrained to the nuclear periphery. LAD positioning, however, was not detectably inherited from one cell cycle to the next but stochastically reshuffled. The mechanisms responsible for these phenomena are still not well understood [143,144]. While it is generally acknowledged that the nuclear envelope with its lamina exerts a major influence on higher order chromatin arrangements, its is not clear, whether cell cycle or cell type specific changes of this organization in the nuclear interior depend on other, currently unexplored mechanisms. Changes of higher order chromatin arrangements may necessitate chromatin movements over μm distances. Understanding the mechanisms involved in such large-scale chromatin movements is a major challenge of future 4D nucleome research.

7.6. Chromatin dynamics and DNA repair

In case that DNA repair is carried out in the ANC [60], sites of DNA damage in the INC would have to be relocated to the ANC prior to repair. Positional changes over distances of 100 nm may be required, when damaged DNA located within the compact interior of a chromatin domain cluster moves to its periphery. Alternatively, a decondensation process of a chromatin domain carrying DNA damage in its compact interior may be required to make a site of damage accessible for repair machineries. In combination with super-resolved fluorescence microscopy, the generation of local damage at selected nuclear sites in living cells with microbeam approaches can help to explore this question further [145–148].

8. Conclusions and outlook

The above questions represent only a selection of research areas that could be explored in future 4D nucleome studies to advance our understanding of functional nuclear architecture. While we are fully aware of the speculative nature of the ANC–INC network model described here, we consider it a helpful starting point. It integrates many current findings and, at the same time, helps to define open mechanistic questions and tests of model predictions.

We conclude this review with a renewed emphasis that dissecting nuclear structures in space and time at all length scales is indispensable for major progress toward understanding nuclear functions. Quantitative measurements of space–time relations between all structural components of the cell nucleus are essential to model biophysical properties of the cell nucleus, such as the diffusion and binding of factors for transcription, splicing, replication, repair, as well as the dynamics of chromatin condensation and decondensation. Studies at the single cell level are mandatory to dissect the heterogeneity and dynamics between cells in a given sample. The internal organization of individual TADs can be visualized by multicolor 3D FISH with sets of differentially labeled TAD specific probes in combination with super-resolved microscopy. The preservation of key characteristics of nuclear ultrastructures was shown in 3D-SIM experiments by a carefully adapted 3D-FISH protocol [44,149]. Yet, finest details may be destroyed by heat denaturation required to obtain single stranded target DNA. Methods, which avoid cell fixation and the necessity of single stranded target DNA need to be developed [150] for studies of TAD organization, boundary regions between them and the topography of machineries, which carry out nuclear functions. Genomic loci in

live cells have been visualized on the basis of transcription activator-like effectors (TALEs) [151,152] and with the CRISPR/dCas9 system [153,154]. Multicolor versions of the CRISPR/dCas9 system are currently implemented [155]. At the horizon of these developments we envisage the possibility to replace multicolor 3D FISH experiments of higher order nuclear organization in nuclei of fixed cells by super-resolved multicolor live cell imaging. The further development of super-resolved localization microscopy for multicolor, live cell technology for the 4D mapping of DNA together with its architectural and functional proteins constitutes a huge challenge. Exploitation of the entire set of available methods, including novel correlative fluorescence – electron-microscopic (CFEM) approaches [39,156–159], apparently provides the best safeguard against misleading interpretations resulting from artifacts of each individual method.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.05.037.

References

[1] Tashiro, S. and Lanctot, C. (2015) The international nucleome consortium. Nucleus 6, 89–92.
[2] Cremer, T. (1985) Von der Zellenlehre zur Chromosomentheorie, Naturwissenschaftliche Erkenntnis und Theorienwechsel in der frühen Zell- und Vererbungsforschung, Springer Verlag, Heidelberg.
[3] Cremer, T. and Cremer, C. (2006) Rise, fall and resurrection of chromosome territories: a historical perspective. Part I. The rise of chromosome territories. Eur. J. Histochem. 50, 161–176.
[4] Rabl, C. (1885) Über Zellteilung. Morphologisches Jahrbuch 10, 214–330.
[5] Boveri, T. (1909) Die Blastomerenkerne von Ascaris megalocephala und die Theorie der Chromosomenindividualität. Arch. Zellforsch. 3, 181–268.
[6] Cremer, T. and Cremer, C. (2006) 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. Eur. J. Histochem. 50, 223–272.
[7] Cremer, T., Cremer, C. and Lichter, P. (2014) Recollections of a scientific journey published in human genetics: from chromosome territories to interphase cytogenetics and comparative genome hybridization. Hum. Genet. 133, 403–416.
[8] Burgess, R.C., Burman, B., Kruhlak, M.J. and Misteli, T. (2014) Activation of DNA damage response signaling by condensed chromatin. Cell Rep. 9, 1703–1717.
[9] Therizols, P., Illingworth, R.S., Courilleau, C., Boyle, S., Wood, A.J., et al. (2014) Chromatin decondensation is sufficient to alter nuclear organization in embryonic stem cells. Science 346, 1238–1242.
[10] Giorgetti, L., Galupa, R., Nora, E.P., Piolot, T., Lam, F., et al. (2014) Predictive polymer modeling reveals coupled fluctuations in chromosome conformation and transcription. Cell 157, 950–963.
[11] Pederson, T. (2014) The nuclear physique. Int. Rev. Cell Mol. Biol. 307, 1–13.
[12] Hancock, R. and Jeon, K.W. (2014) Preface. New models of the cell nucleus: crowding, entropic forces, phase separation, and fractals. Int. Rev. Cell Mol. Biol. 307. xiii.
[13] Woringer, M., Darzacq, X. and Izeddin, I. (2014) Geometry of the nucleus: a perspective on gene expression regulation. Curr. Opin. Chem. Biol. 20, 112–119.
[14] Solovei, I., Kreysing, M., Lanctot, C., Kosem, S., Peichl, L., et al. (2009) Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution. Cell 137, 356–368.
[15] Cremer, T. (2013) Nuclear architecture and function studied in space and time: origin, current state and perspectives (Schleiden lecture at the DGZ meeting March 2013). Cell News 39, 6–13.
[16] Cremer, T. and Cremer, M. (2010) Chromosome territories. Cold Spring Harb. Perspect. Biol. 2, a003889.
[17] Amendola, M. and van Steensel, B. (2014) Mechanisms and dynamics of nuclear lamina-genome interactions. Curr. Opin. Cell Biol. 28, 61–68.
[18] Dekker, J., Marti-Renom, M.A. and Mirny, L.A. (2013) Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. Nat. Rev. Genet. 14, 390–403.
[19] Gerhold, C.B. and Gasser, S.M. (2014) INO80 and SWR complexes: relating structure to function in chromatin remodeling. Trends Cell Biol. 24, 619–631.
[20] Tark-Dame, M., Jerabek, H., Manders, E.M., Heermann, D.W. and van Driel, R. (2014) Depletion of the chromatin looping proteins CTCF and cohesin causes chromatin compaction: insight into chromatin folding by polymer modelling. PLoS Comput. Biol. 10, e1003877.
[21] Halverson, J.D., Smrek, J., Kremer, K. and Grosberg, A.Y. (2014) From a melt of rings to chromosome territories: the role of topological constraints in genome folding. Rep. Prog. Phys. 77, 022601.
[22] Sexton, T. and Cavalli, G. (2015) The role of chromosome domains in shaping the functional genome. Cell 160, 1049–1059.
[23] Nicodemi, M. and Pombo, A. (2014) Models of chromosome structure. Curr. Opin. Cell Biol. 28, 90–95.
[24] Maeshima, K., Imai, R., Tamura, S. and Nozaki, T. (2014) Chromatin as dynamic 10-nm fibers. Chromosoma 123, 225–237.
[25] Wendt, K.S. and Grosveld, F.G. (2014) Transcription in the context of the 3D nucleus. Curr. Opin. Genet. Dev. 25, 62–67.
[26] Cremer, T. and Cremer, C. (1988) Centennial of Wilhelm Waldeyer's introduction of the term "chromosome" in 1888. Cytogenet. Cell Genet. 48, 65–67.
[27] Blobel, G. (1985) Gene gating: a hypothesis. Proc. Natl. Acad. Sci. USA 82, 8527–8529.
[28] Chai, L.S. and Sandberg, A.A. (1988) Chromosomes and their relationship to nuclear components during the cell cycle in Chinese hamster cells. Cell Tissue Res. 251, 197–204.
[29] Cremer, T., Dietzel, S., Eils, R., Lichter, P. and Cremer, C. (1995) Chromosome territories, nuclear matrix filaments and inter-chromatin channels: a topological view on nuclear architecture and function in: (Brandham, P.E. and Bennet, M.D., Eds.), Royal Botanic Gardens, Kew. pp. 63–81.
[30] Cremer, T., Kurz, A., Zirbel, R., Dietzel, S., Rinke, B., et al. (1993) Role of chromosome territories in the functional compartmentalization of the cell nucleus. Cold Spring Harb. Symp. Quant. Biol. 58, 777–792.
[31] Razin, S.V. and Gromova, I.I. (1995) The channels model of nuclear matrix structure. BioEssays 17, 443–450.
[32] Razin, S.V., Gromova, I.I. and Iarovaia, O.V. (1995) Specificity and functional significance of DNA interaction with the nuclear matrix: new approaches to clarify the old questions. Int. Rev. Cytol. 162B, 405–448.
[33] Spector, D.L. (1990) Higher order nuclear organization: three-dimensional distribution of small nuclear ribonucleoprotein particles. Proc. Natl. Acad. Sci. USA 87, 147–151.
[34] Wansink, D.G., Manders, E.E., van der Kraan, I., Aten, J.A., van Driel, R., et al. (1994) RNA polymerase II transcription is concentrated outside replication domains throughout S-phase. J. Cell Sci. 107 (Pt 6), 1449–1456.
[35] Zachar, Z., Kramer, J., Mims, I.P. and Bingham, P.M. (1993) Evidence for channeled diffusion of pre-mRNAs during nuclear RNA transport in metazoans. J. Cell Biol. 121, 729–742.
[36] Zirbel, R.M., Mathieu, U.R., Kurz, A., Cremer, T. and Lichter, P. (1993) Evidence for a nuclear compartment of transcription and splicing located at chromosome domain boundaries. Chromosome Res. 1, 93–106.
[37] Crowley, K.S. and Brasch, K. (1987) Does the interchromatin compartment contain actin? Cell Biol. Int. Rep. 11, 537–546.
[38] Albiez, H., Cremer, M., Tiberi, C., Vecchio, L., Schermelleh, L., et al. (2006) Chromatin domains and the interchromatin compartment form structurally defined and functionally interacting nuclear networks. Chromosome Res. 14, 707–733.
[39] Hubner, B., Cremer, T. and Neumann, J. (2013) Correlative microscopy of individual cells: sequential application of microscopic systems with increasing resolution to study the nuclear landscape. Methods Mol. Biol. 1042, 299–336.
[40] Markaki, Y., Gunkel, M., Schermelleh, L., Beichmanis, S., Neumann, J., et al. (2010) Functional nuclear organization of transcription and DNA replication: a topographical marriage between chromatin domains and the interchromatin compartment. Cold Spring Harb. Symp. Quant. Biol. 75, 475–492.
[41] Rouquette, J., Genoud, C., Vazquez-Nin, G.H., Kraus, B., Cremer, T., et al. (2009) Revealing the high-resolution three-dimensional network of chromatin and interchromatin space: a novel electron-microscopic approach to reconstructing nuclear architecture. Chromosome Res. 17, 801–810.
[42] Schermelleh, L., Carlton, P.M., Haase, S., Shao, L., Winoto, L., et al. (2008) Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy. Science 320, 1332–1336.
[43] Smeets, D., Markaki, Y., Schmid, V.J., Kraus, F., Tattermusch, A., et al. (2014) Three-dimensional super-resolution microscopy of the inactive X chromosome territory reveals a collapse of its active nuclear compartment harboring distinct Xist RNA foci. Epigenet. Chromatin 7, 8.

[44] Markaki, Y., Smeets, D., Fiedler, S., Schmid, V.J., Schermelleh, L., et al. (2012) The potential of 3D-FISH and super-resolution structured illumination microscopy for studies of 3D nuclear architecture: 3D structured illumination microscopy of defined chromosomal structures visualized by 3D (immuno)-FISH opens new perspectives for studies of nuclear architecture. BioEssays 34, 412–426.

[45] Popken, J., Brero, A., Koehler, D., Schmid, V.J., Strauss, A., et al. (2014) Reprogramming of fibroblast nuclei in cloned bovine embryos involves major structural remodeling with both striking similarities and differences to nuclear phenotypes of in vitro fertilized embryos. Nucleus 5, 555–589.

[46] Bornfleth, H., Edelmann, P., Zink, D., Cremer, T. and Cremer, C. (1999) Quantitative motion analysis of subchromosomal foci in living cells using four-dimensional microscopy. Biophys. J. 77, 2871–2886.

[47] Strickfaden, H., Zunhammer, A., van Koningsbruggen, S., Kohler, D. and Cremer, T. (2010) 4D chromatin dynamics in cycling cells: Theodor Boveri's hypotheses revisited. Nucleus 1, 284–297.

[48] Walter, J., Schermelleh, L., Cremer, M., Tashiro, S. and Cremer, T. (2003) Chromosome order in HeLa cells changes during mitosis and early G1, but is stably maintained during subsequent interphase stages. J. Cell Biol. 160, 685–697.

[49] Popken, J., Graf, A., Krebs, S., Blum, H., Schmid, V.J., et al. (2015) Remodeling of the nuclear envelope and lamina during bovine preimplantation development and its functional implications. PLoS One 10, e0124619.

[50] Mayer, R., Brero, A., von Hase, J., Schroeder, T., Cremer, T., et al. (2005) Common themes and cell type specific variations of higher order chromatin arrangements in the mouse. BMC Cell Biol. 6, 44.

[51] Kizilyaprak, C., Spehner, D., Devys, D. and Schultz, P. (2010) In vivo chromatin organization of mouse rod photoreceptors correlates with histone modifications. PLoS One 5, e11039.

[52] Booth, A. and Doolittle, W.F. (2015) Eukaryogenesis, how special really? Proc. Natl. Acad. Sci. USA.

[53] Nelson-Sathi, S., Sousa, F.L., Roettger, M., Lozada-Chavez, N., Thiergart, T., et al. (2015) Origins of major archaeal clades correspond to gene acquisitions from bacteria. Nature 517, 77–80.

[54] Postberg, J., Lipps, H.J. and Cremer, T. (2010) Evolutionary origin of the cell nucleus and its functional architecture. Essays Biochem. 48, 1–24.

[55] Williams, T.A., Foster, P.G., Cox, C.J. and Embley, T.M. (2013) An archaeal origin of eukaryotes supports only two primary domains of life. Nature 504, 231–236.

[56] Fakan, S. and Bernhard, W. (1971) Localisation of rapidly and slowly labelled nuclear RNA as visualized by high resolution autoradiography. Exp. Cell Res. 67, 129–141.

[57] Monneron, A. and Bernhard, W. (1969) Fine structural organization of the interphase nucleus in some mammalian cells. J. Ultrastruct. Res. 27, 266–288.

[58] Olins, D.E., Olins, A.L., Levy, H.A., Durfee, R.C., Margle, S.M., et al. (1983) Electron microscope tomography: transcription in three dimensions. Science 220, 498–500.

[59] Smetana, K. and Hermansky, F. (1963) A contribution to the knowledge of the ultrastructure of leukaemic lymphocytes. Neoplasma 10, 405–411.

[60] Rouquette, J., Cremer, C., Cremer, T. and Fakan, S. (2010) Functional nuclear architecture studied by microscopy: present and future. Int. Rev. Cell Mol. Biol. 282, 1–90.

[61] Cmarko, D., Verschure, P.J., Martin, T.E., Dahmus, M.E., Krause, S., et al. (1999) Ultrastructural analysis of transcription and splicing in the cell nucleus after bromo-UTP microinjection. Mol. Biol. Cell 10, 211–223.

[62] Niedojadlo, J., Perret-Vivancos, C., Kalland, K.H., Cmarko, D., Cremer, T., et al. (2011) Transcribed DNA is preferentially located in the perichromatin region of mammalian cell nuclei. Exp. Cell Res. 317, 433–444.

[63] Jaunin, F., Visser, A.E., Cmarko, D., Aten, J.A. and Fakan, S. (2000) Fine structural in situ analysis of nascent DNA movement following DNA replication. Exp. Cell Res. 260, 313–323.

[64] Bazett-Jones, D.P., Li, R., Fussner, E., Nisman, R. and Dehghani, H. (2008) Elucidating chromatin and nuclear domain architecture with electron spectroscopic imaging. Chromosome Res. 16, 397–412.

[65] Schroeder-Reiter, E., Perez-Willard, F., Zeile, U. and Wanner, G. (2009) Focused ion beam (FIB) combined with high resolution scanning electron microscopy: a promising tool for 3D analysis of chromosome architecture. J. Struct. Biol. 165, 97–106.

[66] Orlov, I., Schertel, A., Zuber, G., Klaholz, B., Drillien, R., et al. (2015) Live cell immunogold labelling of RNA polymerase II. Sci. Rep. 5, 8324.

[67] Eltsov, M., Maclellan, K.M., Maeshima, K., Frangakis, A.S. and Dubochet, J. (2008) Analysis of cryo-electron microscopy images does not support the existence of 30-nm chromatin fibers in mitotic chromosomes in situ. Proc. Natl. Acad. Sci. USA 105, 19732–19737.

[68] Joti, Y., Hikima, T., Nishino, Y., Kamada, F., Hihara, S., et al. (2012) Chromosomes without a 30-nm chromatin fiber. Nucleus 3, 404–410.

[69] Maeshima, K., Hihara, S. and Eltsov, M. (2010) Chromatin structure: does the 30-nm fibre exist in vivo? Curr. Opin. Cell Biol. 22, 291–297.

[70] Bohn, M. and Heermann, D.W. (2010) Diffusion-driven looping provides a consistent framework for chromatin organization. PLoS One 5, e12218.

[71] Barbieri, M., Chotalia, M., Fraser, J., Lavitas, L.M., Dostie, J., et al. (2012) Complexity of chromatin folding is captured by the strings and binders switch model. Proc. Natl. Acad. Sci. USA 109, 16173–16178.

[72] Fornasiero, E.F. and Opazo, F. (2015) Super-resolution imaging for cell biologists: Concepts, applications, current challenges and developments. BioEssays 37, 436–451.

[73] Bechhoefer, J. (2015) What is superresolution microscopy? Am. J. Phys. 83, 22–29.

[74] Cremer, C. and Masters, B.R. (2013) Resolution enhancement techniques in microscopy. Eur. Phys. J. H 38, 281–344.

[75] Renz, M. (2013) Fluorescence microscopy -- a historical and technical perspective. Cytometry A 83, 767–779.

[76] Stelzer, E.H. (2014) Better imaging through chemistry. Cell 159, 1243–1246.

[77] Kaufmann, R., Cremer, C. and Gall, J.G. (2012) Superresolution imaging of transcription units on newt lampbrush chromosomes. Chromosome Res. 20, 1009–1015.

[78] Reymann, J., Baddeley, D., Gunkel, M., Lemmer, P., Stadter, W., et al. (2008) High-precision structural analysis of subnuclear complexes in fixed and live cells via spatially modulated illumination (SMI) microscopy. Chromosome Res. 16, 367–382.

[79] Szczurek, A.T., Prakash, K., Lee, H.K., Zurek-Biesiada, D.J., Best, G., et al. (2014) Single molecule localization microscopy of the distribution of chromatin using Hoechst and DAPI fluorescent probes. Nucleus 5, 331–340.

[80] Zessin, P.J., Finan, K. and Heilemann, M. (2012) Super-resolution fluorescence imaging of chromosomal DNA. J. Struct. Biol. 177, 344–348.

[81] Dekker, J., Rippe, K., Dekker, M. and Kleckner, N. (2002) Capturing chromosome conformation. Science 295, 1306–1311.

[82] Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., et al. (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485, 376–380.

[83] Gibcus, J.H. and Dekker, J. (2013) The hierarchy of the 3D genome. Mol. Cell 49, 773–782.

[84] Lieberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., et al. (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 326, 289–293.

[85] Nagano, T., Lubling, Y., Stevens, T.J., Schoenfelder, S., Yaffe, E., et al. (2013) Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature 502, 59–64.

[86] Rao, S.S., Huntley, M.H., Durand, N.C., Stamenova, E.K., Bochkov, I.D., et al. (2014) A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell 159, 1665–1680.

[87] Ong, C.T. and Corces, V.G. (2014) CTCF: an architectural protein bridging genome topology and function. Nat. Rev. Genet. 15, 234–246.

[88] Williamson, I., Berlivet, S., Eskeland, R., Boyle, S., Illingworth, R.S., et al. (2014) Spatial genome organization: contrasting views from chromosome conformation capture and fluorescence in situ hybridization. Genes Dev. 28, 2778–2791.

[89] Kupper, K., Kolbl, A., Biener, D., Dittrich, S., von Hase, J., et al. (2007) Radial chromatin positioning is shaped by local gene density, not by gene expression. Chromosoma 116, 285–306.

[90] Lanctot, C., Cheutin, T., Cremer, M., Cavalli, G. and Cremer, T. (2007) Dynamic genome architecture in the nuclear space: regulation of gene expression in three dimensions. Nat. Rev. Genet. 8, 104–115.

[91] Kosak, S.T. and Groudine, M. (2004) Form follows function: the genomic organization of cellular differentiation. Genes Dev. 18, 1371–1384.

[92] Naughton, C., Avlonitis, N., Corless, S., Prendergast, J.G., Mati, I.K., et al. (2013) Transcription forms and remodels supercoiling domains unfolding large-scale chromatin structures. Nat. Struct. Mol. Biol. 20, 387–395.

[93] Peric-Hupkes, D., Meuleman, W., Pagie, L., Bruggeman, S.W., Solovei, I., et al. (2010) Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. Mol. Cell 38, 603–613.

[94] Jackson, D.A. and Pombo, A. (1998) 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, 1285–1295.

[95] Ma, H., Samarabandu, J., Devdhar, R.S., Acharya, R., Cheng, P.C., et al. (1998) Spatial and temporal dynamics of DNA replication sites in mammalian cells. J. Cell Biol. 143, 1415–1425.

[96] Manders, E.M., Kimura, H. and Cook, P.R. (1999) Direct imaging of DNA in living cells reveals the dynamics of chromosome formation. J. Cell Biol. 144, 813–821.

[97] Zink, D. and Cremer, T. (1998) Cell nucleus: chromosome dynamics in nuclei of living cells. Curr. Biol. 8, R321–R324.

[98] Zink, D., Cremer, T., Saffrich, R., Fischer, R., Trendelenburg, M.F., et al. (1998) Structure and dynamics of human interphase chromosome territories in vivo. Hum. Genet. 102, 241–251.

[99] Khanna, N., Hu, Y. and Belmont, A.S. (2014) HSP70 transgene directed motion to nuclear speckles facilitates heat shock activation. Curr. Biol. 24, 1138–1144.

[100] Roukos, V., Voss, T.C., Schmidt, C.K., Lee, S., Wangsa, D., et al. (2013) Spatial dynamics of chromosome translocations in living cells. Science 341, 660–664.

[101] Mor, A., Suliman, S., Ben-Yishay, R., Yunger, S., Brody, Y., et al. (2010) Dynamics of single mRNP nucleocytoplasmic transport and export through the nuclear pore in living cells. Nat. Cell Biol. 12, 543–552.

[102] Daddysman, M.K. and Fecko, C.J. (2013) Revisiting point FRAP to quantitatively characterize anomalous diffusion in live cells. J. Phys. Chem. B 117, 1241–1251.

[103] Baum, M., Erdel, F., Wachsmuth, M. and Rippe, K. (2014) Retrieving the intracellular topology from multi-scale protein mobility mapping in living cells. Nat. Commun. 5, 4494.

[104] Erdel, F., Baum, M. and Rippe, K. (2015) The viscoelastic properties of chromatin and the nucleoplasm revealed by scale-dependent protein mobility. J. Phys. Condens. Matter 27, 064115.
[105] Kind, J. and van Steensel, B. (2010) Genome-nuclear lamina interactions and gene regulation. Curr. Opin. Cell Biol. 22, 320–325.
[106] Muller-Ott, K., Erdel, F., Matveeva, A., Mallm, J.P., Rademacher, A., et al. (2014) Specificity, propagation, and memory of pericentric heterochromatin. Mol. Syst. Biol. 10, 746.
[107] Gorisch, S.M., Wachsmuth, M., Toth, K.F., Lichter, P. and Rippe, K. (2005) Histone acetylation increases chromatin accessibility. J. Cell Sci. 118, 5825–5834.
[108] Gaspar-Maia, A., Alajem, A., Meshorer, E. and Ramalho-Santos, M. (2011) Open chromatin in pluripotency and reprogramming. Nat. Rev. Mol. Cell Biol. 12, 36–47.
[109] Pack, C., Saito, K., Tamura, M. and Kinjo, M. (2006) Microenvironment and effect of energy depletion in the nucleus analyzed by mobility of multiple oligomeric EGFPs. Biophys. J. 91, 3921–3936.
[110] Verschure, P.J., van der Kraan, I., Manders, E.M., Hoogstraten, D., Houtsmuller, A.B., et al. (2003) Condensed chromatin domains in the mammalian nucleus are accessible to large macromolecules. EMBO Rep. 4, 861–866.
[111] Bacher, C.P., Reichenzeller, M., Athale, C., Herrmann, H. and Eils, R. (2004) 4-D single particle tracking of synthetic and proteinaceous microspheres reveals preferential movement of nuclear particles along chromatin – poor tracks. BMC Cell Biol. 5, 45.
[112] Gorisch, S.M., Richter, K., Scheuermann, M.O., Herrmann, H. and Lichter, P. (2003) Diffusion-limited compartmentalization of mammalian cell nuclei assessed by microinjected macromolecules. Exp. Cell Res. 289, 282–294.
[113] Tseng, Y., Lee, J.S., Kole, T.P., Jiang, I. and Wirtz, D. (2004) Micro-organization and visco-elasticity of the interphase nucleus revealed by particle nanotracking. J. Cell Sci. 117, 2159–2167.
[114] Cunningham, J., Estrella, V., Lloyd, M., Gillies, R., Frieden, B.R., et al. (2012) Intracellular electric field and pH optimize protein localization and movement. PLoS One 7, e36894.
[115] Tyner, K.M., Kopelman, R. and Philbert, M.A. (2007) "Nanosized voltmeter" enables cellular-wide electric field mapping. Biophys. J. 93, 1163–1174.
[116] Mazzanti, M., Bustamante, J.O. and Oberleithner, H. (2001) Electrical dimension of the nuclear envelope. Physiol. Rev. 81, 1–19.
[117] Cremer, T., Kreth, G., Koester, H., Fink, R.H., Heintzmann, R., et al. (2000) Chromosome territories, interchromatin domain compartment, and nuclear matrix: an integrated view of the functional nuclear architecture. Crit. Rev. Eukaryot. Gene Expr. 10, 179–212.
[118] Wright, R.H.G., LeDily, F., Soronellas, D., Pohl, A., Bonet, J. et al. (2014) ADP-ribose derived nuclear ATP isrequired for chromatin remodeling and hormonal gene regulation. bioRxiv http://dx.doi.org/10.1101/006593.
[119] Cherstvy, A.G. and Teif, V.B. (2013) Structure-driven homology pairing of chromatin fibers: the role of electrostatics and protein-induced bridging. J. Biol. Phys. 39, 363–385.
[120] Sofueva, S., Yaffe, E., Chan, W.C., Georgopoulou, D., Vietri Rudan, M., et al. (2013) Cohesin-mediated interactions organize chromosomal domain architecture. EMBO J. 32, 3119–3129.
[121] Barbieri, M., Scialdone, A., Piccolo, A., Chiariello, A.M., di Lanno, C., et al. (2013) Polymer models of chromatin organization. Front. Genet. 4, 113.
[122] Zentgraf, H., Scheer, U. and Franke, W.W. (1975) Characterization and localization of the RNA synthesized in mature avian erythrocytes. Exp. Cell Res. 96, 81–95.
[123] Goto, S. and Ringertz, N.R. (1974) Preparation and characterization of chick erythrocyte nuclei from heterokaryons. Exp. Cell Res. 85, 173–181.
[124] Woodcock, C.L. and Woodcock, H. (1986) Nuclear matrix generation during reactivation of avian erythrocyte nuclei: an analysis of the protein traffic in cybrids. J. Cell Sci. 84, 105–127.
[125] Caudron-Herger, M. and Rippe, K. (2012) Nuclear architecture by RNA. Curr. Opin. Genet. Dev. 22, 179–187.
[126] Hacisuleyman, E., Goff, L.A., Trapnell, C., Williams, A., Henao-Mejia, J., et al. (2014) Topological organization of multichromosomal regions by the long intergenic non-coding RNA Firre. Nat. Struct. Mol. Biol. 21, 198–206.
[127] Malicet, C., Rochman, M., Postnikov, Y. and Bustin, M. (2011) Distinct properties of human HMGN5 reveal a rapidly evolving but functionally conserved nucleosome binding protein. Mol. Cell. Biol. 31, 2742–2755.
[128] Lo, H.S., Wang, Z., Hu, Y., Yang, H.H., Gere, S., et al. (2003) Allelic variation in gene expression is common in the human genome. Genome Res. 13, 1855–1862.
[129] Ohno, S. (1972) So much "junk" DNA in our genome. Brookhaven Symp. Biol. 23, 366–370.
[130] Palazzo, A.F. and Gregory, T.R. (2014) The case for junk DNA. PLoS Genet. 10, e1004351.
[131] Kidwell, M.G. and Lisch, D.R. (2001) Perspective: transposable elements, parasitic DNA, and genome evolution. Evolution 55, 1–24.
[132] Olins, A.L., Ishaque, N., Chotewutmontri, S., Langowski, J. and Olins, D.E. (2014) Retrotransposon Alu is enriched in the epichromatin of HL-60 cells. Nucleus 5, 237–246.
[133] Hall, L.L., Carone, D.M., Gomez, A.V., Kolpa, H.J., Byron, M., et al. (2014) Stable C0T-1 repeat RNA is abundant and is associated with euchromatic interphase chromosomes. Cell 156, 907–919.
[134] Razin, S.V., Borunova, V.V., Iarovaia, O.V. and Vassetzky, Y.S. (2014) Nuclear matrix and structural and functional compartmentalization of the eucaryotic cell nucleus. Biochemistry (Mosc) 79, 608–618.
[135] Razin, S.V., Iarovaia, O.V. and Vassetzky, Y.S. (2014) A requiem to the nuclear matrix: from a controversial concept to 3D organization of the nucleus. Chromosoma 123, 217–224.
[136] Pederson, T. (2000) Half a century of "the nuclear matrix". Mol. Biol. Cell 11, 799–805.
[137] Hancock, R. (2004) Internal organisation of the nucleus: assembly of compartments by macromolecular crowding and the nuclear matrix model. Biol. Cell 96, 595–601.
[138] Zeitz, M.J., Malyavantham, K.S., Seifert, B. and Berezney, R. (2009) Matrin 3: chromosomal distribution and protein interactions. J. Cell. Biochem. 108, 125–133.
[139] Zbarskii, I.B. and Debov, S.S. (1948) On the proteins of the cell nucleus. Dokl. Akad. Nauk SSSR 63.
[140] Berezney, R. and Coffey, D.S. (1977) Nuclear matrix. Isolation and characterization of a framework structure from rat liver nuclei. J. Cell Biol. 73, 616–637.
[141] Kohli, R.M. and Zhang, Y. (2013) TET enzymes, TDG and the dynamics of DNA demethylation. Nature 502, 472–479.
[142] Kind, J., Pagie, L., Ortabozkoyun, H., Boyle, S., de Vries, S.S., et al. (2013) Single-cell dynamics of genome-nuclear lamina interactions. Cell 153, 178–192.
[143] Solovei, I., Wang, A.S., Thanisch, K., Schmidt, C.S., Krebs, S., et al. (2013) LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. Cell 152, 584–598.
[144] Amendola, M. and van Steensel, B. (2015) Nuclear lamins are not required for lamina-associated domain organization in mouse embryonic stem cells. EMBO Rep. 16, 610–617.
[145] Greubel, C., Hable, V., Drexler, G.A., Hauptner, A., Dietzel, S., et al. (2008) Competition effect in DNA damage response. Radiat. Environ. Biophys. 47, 423–429.
[146] Seiler, D.M., Rouquette, J., Schmid, V.J., Strickfaden, H., Ottmann, C., et al. (2011) Double-strand break-induced transcriptional silencing is associated with loss of tri-methylation at H3K4. Chromosome Res. 19, 883–899.
[147] Hable, V., Drexler, G.A., Bruning, T., Burgdorf, C., Greubel, C., et al. (2012) Recruitment kinetics of DNA repair proteins Mdc1 and Rad52 but not 53BP1 depend on damage complexity. PLoS One 7, e41943.
[148] Muller, I., Merk, B., Voss, K.O., Averbeck, N., Jakob, B., et al. (2013) Species conserved DNA damage response at the inactive human X chromosome. Mutat. Res. 756, 30–36.
[149] Markaki, Y., Smeets, D., Cremer, M. and Schermelleh, L. (2013) Fluorescence in situ hybridization applications for super-resolution 3D structured illumination microscopy. Methods Mol. Biol. 950, 43–64.
[150] Schmitt, E., Wagner, J. and Hausmann, M. (2012) Combinatorial selection of short triplex forming oligonucleotides for fluorescence in situ hybridisation combo-fish. J. Comput. Sci. 3, 328–334.
[151] Miyanari, Y., Ziegler-Birling, C. and Torres-Padilla, M.E. (2013) Live visualization of chromatin dynamics with fluorescent TALEs. Nat. Struct. Mol. Biol. 20, 1321–1324.
[152] Thanisch, K., Schneider, K., Morbitzer, R., Solovei, I., Lahaye, T., et al. (2014) Targeting and tracing of specific DNA sequences with dTALEs in living cells. Nucleic Acids Res. 42, e38.
[153] Anton, T., Bultmann, S., Leonhardt, H. and Markaki, Y. (2014) Visualization of specific DNA sequences in living mouse embryonic stem cells with a programmable fluorescent CRISPR/Cas system. Nucleus 5, 163–172.
[154] Chen, B., Gilbert, L.A., Cimini, B.A., Schnitzbauer, J., Zhang, W., et al. (2013) Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. Cell 155, 1479–1491.
[155] Ma, H., Naseri, A., Reyes-Gutierrez, P., Wolfe, S.A., Zhang, S., et al. (2015) Multicolor CRISPR labeling of chromosomal loci in human cells. Proc. Natl. Acad. Sci. USA 112, 3002–3007.
[156] Vicidomini, G., Gagliani, M.C., Canfora, M., Cortese, K., Frosi, F., et al. (2008) High data output and automated 3D correlative light-electron microscopy method. Traffic 9, 1828–1838.
[157] Smith, E.A., Cinquin, B.P., McDermott, G., Le Gros, M.A., Parkinson, D.Y., et al. (2013) Correlative microscopy methods that maximize specimen fidelity and data completeness, and improve molecular localization capabilities. J Struct Biol 184, 12–20.
[158] Gong, Z., Chen, B.K., Liu, R., Anchel, D., Li, X., et al. (2014) Fluorescence and SEM correlative microscopy for nanomanipulation of subcellular structures. Light Sci Appl. e224.
[159] Hagen, C., Werner, S., Carregal-Romero, S., NM, A., GK, B., et al. (2014) Multimodal nanoparticles as alignment and correlation markers in fluorescence/soft X-ray cryo-microscopy/tomography of nucleoplasmic reticulum and apoptosis in mammalian cells. Ultramicroscopy 146, 46–54.