CHROMOSOME TERRITORIES, NUCLEAR ARCHITECTURE AND GENE REGULATION IN MAMMALIAN CELLS

T. Cremer‡ and C. Cremer‡§*

The expression of genes is regulated at many levels. Perhaps the area in which least is known is how nuclear organization influences gene expression. Studies of higher-order chromatin arrangements and their dynamic interactions with other nuclear components have been boosted by recent technical advances. The emerging view is that chromosomes are compartmentalized into discrete territories. The location of a gene within a chromosome territory seems to influence its access to the machinery responsible for specific nuclear functions, such as transcription and splicing. This view is consistent with a topological model for gene regulation.

EPIGENETICS
Any heritable influence (in the progeny of cells or of individuals) on gene activity, unaccompanied by a change in DNA sequence.

**Institute of Anthropology and Human Genetics, Ludwig Maximilians University, Richard Wagner Strasse 10, D-80333 Munich, Germany. ‡Interdisciplinary Centre for Scientific Computing, Ruprecht Karls University, D-69120 Heidelberg, Germany. §Applied Optics and Information Processing, Kirchhoff-Institute of Physics, Ruprecht Karls University, Albert Ueberle Strasse 3–5, D-69120 Heidelberg, Germany. Correspondence to T.C. and C.C. e-mails: Thomas.Cremer@lrz.uni-muenchen.de; Christoph.Cremer@kip.uni-heidelberg.de*

Despite all the celebrations associated with the sequencing of the human genome, and the genomes of other model organisms, our abilities to interpret genome sequences are quite limited. For example, we cannot understand the orchestrated activity — and the silencing — of many thousands of genes in any given cell just on the basis of DNA sequences, such as promoter and enhancer elements. How are the profound differences in gene activities established and maintained in a large number of cell types to ensure the development and functioning of a complex multicellular organism? To answer this question fully, we need to understand how genomes are organized in the nucleus, the basic principles of nuclear architecture and the changes in nuclear organization that occur during cellular differentiation.

During recent years, EPIGENETIC mechanisms of gene regulation, such as DNA methylation and histone modification, have entered the centre stage of chromatin research[1]. Modifications of DNA and nucleosomes, however, as well as boundaries and insulators[2], that affect gene regulation at the chromatin level are not the focus of this article. Instead, we review experimental data and models for a higher level of the regulation of gene expression and other nuclear functions — namely the architecture of the nucleus as a whole[3–14]. In particular, we describe evidence for a compartmentalized nuclear architecture in the mammalian cell nucleus based on chromosome territories (CTs) and an interchromatin compartment (IC) that contains macromolecular complexes that are required for replication, transcription, splicing and repair[12] (summarized in FIG. 1). Other nuclear components, such as the nucleolus, nuclear lamina and pores, are not reviewed here (for reviews, see REFS 15,16), and although the focus of this review is the mammalian nucleus, the nuclear architecture of other organisms will be mentioned where appropriate.

During the past two decades, various new methods have expanded the cell biologist's 'toolkit' for the study of nuclear architecture and function (BOX 1). These methods have provided the basis for detailed studies of CTs, as well as for studies of the topology and dynamics of non-chromatin domains in the nucleus of fixed and, more recently, living cells. Computer simulations of CTs and nuclear architecture are also being used to make quantitative predictions that can be tested experimentally. On the basis of

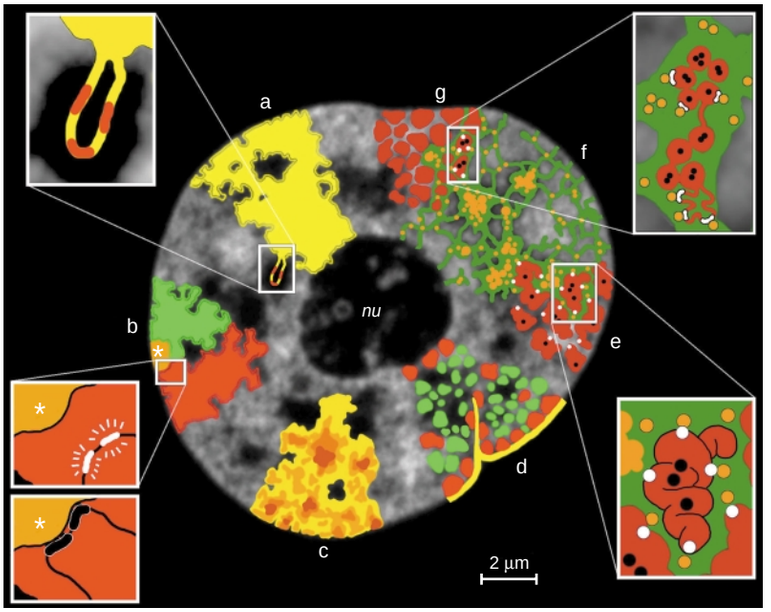

Figure 1 | **Model of functional nuclear architecture.** Structural features that support the chromosome-territory–interchromatin-compartment (CT–IC) model are shown. These features are drawn roughly to scale on an optical section taken from the nucleus of a living HeLa cell. Although experimental evidence is available to support these features, the overall model of functional nuclear architecture is speculative (see text). **a** | CTs have complex folded surfaces. Inset: topological model of gene regulation[23]. A giant chromatin loop with several active genes (red) expands from the CT surface into the IC space. **b** | CTs contain separate arm domains for the short (p) and long chromosome arms (q), and a centromeric domain (asterisks). Inset: topological model of gene regulation[78,79]. Top, actively transcribed genes (white) are located on a chromatin loop that is remote from centromeric heterochromatin. Bottom, recruitment of the same genes (black) to the centromeric heterochromatin leads to their silencing. **c** | CTs have variable chromatin density (dark brown, high density; light yellow, low density). Loose chromatin expands into the IC, whereas the most dense chromatin is remote from the IC. **d** | CT showing early-replicating chromatin domains (green) and mid-to-late-replicating chromatin domains (red). Each domain comprises ~1 Mb. Gene-poor chromatin (red), is preferentially located at the nuclear periphery and in close contact with the nuclear lamina (yellow), as well as with infoldings of the lamina and around the nucleolus (nu). Gene-rich chromatin (green) is located between the gene-poor compartments. **e** | Higher-order chromatin structures built up from a hierarchy of chromatin fibres[88]. Inset: this topological view of gene regulation[27,68] indicates that active genes (white dots) are at the surface of convoluted chromatin fibres. Silenced genes (black dots) may be located towards the interior of the chromatin structure. **f** | The CT–IC model predicts that the IC (green) contains complexes (orange dots) and larger non-chromatin domains (aggregations of orange dots) for transcription, splicing, DNA replication and repair. **g** | CT with ~1-Mb chromatin domains (red) and IC (green) expanding between these domains. Inset: the topological relationships between the IC, and active and inactive genes[72]. The finest branches of the IC end between ~100-kb chromatin domains. Top: active genes (white dots) are located at the surface of these domains, whereas silenced genes (black dots) are located in the interior. Bottom: alternatively, closed ~100-kb chromatin domains with silenced genes are transformed into an open configuration before transcriptional activation.

CHROMOSOME PAINTING
Visualization of individual, whole chromosomes by fluorescence *in situ* hybridization (FISH).

CENTROMERIC HETEROCHROMATIN
Comprises the genetically inert, constitutive heterochromatin of the centromere and is built up from tandem repetitive DNA sequences.

these developments, we discuss a refined model of nuclear architecture — the CT–IC model — and its implications for understanding gene regulation at the topological level.

Chromosome territories

During the past 20 years, fluorescence *in situ* hybridization (FISH) techniques have been developed to detect specific DNA (or RNA) sequences in single cells. Combinations of fluorochromes can be used to distinguish numerous differently coloured chromosomal targets simultaneously in a single cell (for review, see REF. 17), and CHROMOSOME PAINTING has unequivocally confirmed circumstantial evidence from the 1970s and early 1980s[18,19] that chromosomes occupy discrete territories in the cell nucleus (for review, see REF. 5, BOX 1, FIG. 2). The FISH experiments further showed that CTs are composed of distinct chromosome-arm domains and chromosome-band domains[20] and allowed determination of the three-dimensional positions of individual active and inactive genes[21–24] (FIG. 3).

More recently, an entirely different approach has been introduced to study CTs, in which nuclear DNA is labelled in living cells during the S phase. Labelled cells are followed through several cell cycles, resulting in the segregation of labelled and non-labelled sister chromatids into daughter nuclei during the second and subsequent mitotic events. This labelling/segregation (L/S) approach yields nuclei with distinct, replication-labelled patches. Each patch reflects the territory of a single chromatid and, occasionally, a partial chromatid owing to a sister chromatid exchange[25,26]. More detailed light- and electron-microscopic studies indicate that the three-dimensional CT structure might be reminiscent of a 'sponge': invaginating non-chromatin spaces or interchromatin channels extend from the CT periphery throughout the interior of the territory[27–29] (supplementary figure 1 online).

In combination with nucleotides that are directly conjugated to fluorochromes, the L/S approach allowed the first direct observation of individual CTs during the cell cycle of living cells[30,31]. Territories showed only small movements during an observation period of several hours[32]. However, this finding might be true only for the few cultured cell types studied so far and should not be generalized. In neuronal cells, large chromatin movements have been noted during differentiation[4,33] or in pathological situations[34]. Large-scale chromatin movements were also observed during the interphase of *Drosophila* cells[35].

Chromosome painting has been used to examine whether reproducible arrangements of chromosomes exist in cells. The conclusions are conflicting and range from the claim that chromosomes show highly ordered arrangements[36], to the conclusion that they barely show any order[37] or that the degree of order depends on the cell type[38] (for a review of the older literature, see REF. 39). Recently, a correlation between CT location and human chromosome size was described, in which smaller chromosomes are generally situated towards the interior and larger chromosomes towards the periphery of the nucleus[40]. However, the finding that CTs with similar DNA content, but with very different gene densities, occupy distinct exterior and interior nuclear positions, indicates that gene content is a key determinant of CT positioning[41]. The distribution of human CTs 18 and 19 provides a striking example. Although both chromosomes have a similar DNA content (85 and 67 Mb, respectively), the gene-poor chromosome 18 territories were typically found at the nuclear periphery, whereas the gene-rich chromosome 19 territories were located in the nuclear interior[42] (FIG. 4).

Box 1 | The evolving toolkit for studies of nuclear architecture

The following chronology indicates when specific techniques were first introduced. In subsequent years, the techniques have been developed and improved, and continue to be used in studies of nuclear organization and function. References to original publications in this box should be regarded as examples.

1950s: Electron microscopy allowed studies of chromatin and other nuclear structures at ultrahigh resolution[55,58,94–96].

1970s: Microbeam irradiation of chromatin in living-cell nuclei showed the presence of chromosome territories (CTs), and allowed the first studies of their arrangements and of post-irradiation dynamics in living cells[97]. Laser micro-irradiation was also used to manipulate chromosomes in living cells[98]. Recently, fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) have allowed the study of rapid movements of nuclear proteins tagged with green fluorescence protein (GFP) throughout the nuclear space[60,99].

1980s: In 1983, EPIFLUORESCENCE MICROSCOPY was combined with image reconstruction techniques to study the three-dimensional chromosome topography in polytene nuclei of *Drosophila melanogaster*[100]. In the mid-1980s, the first fluorescence-laser scanning microscopes were ready to use[101]. Since then, important efforts have been made to develop advanced types of laser microscope with resolution beyond the ABBE LIMIT of conventional light microscopy[102,103]. Submicrometre particle tracking has been used to directly measure the motion of interphase chromatin[104]. Spectral-precision distance microscopy (SPDM)[105] allows precise three-dimensional distance measurements down to the nanometre scale. The development of fluorescence-resonance energy-transfer (FRET) microscopy has allowed studies of macromolecule interactions in living-cell nuclei[106]. *In vivo* fluorescence correlation spectroscopy (FCS) showed that nuclear poly(A) RNA moves by a diffusion-like process in the interchromatin space[107]. Fluorescence lifetime imaging (FLIM), based on the use of fluorochromes with different fluorescence lifetimes, has increased the number of targets that can be simultaneously discriminated in single-cell studies[108].

1980s: Fluorescence *in situ* hybridization (FISH)[17] provided a tool for visualizing specific chromatin structures, from CTs, to chromosome arms and bands, down to the level of individual genes. Concomitantly, RNA *in situ* hybridization protocols were developed for studies of gene expression in single cells[109].

1980s: Labelling of DNA using base analogues (BrdU, CldU, IdU) allowed visualization of early- and mid-to-late-replicating chromatin in the nuclei of fixed cells[110,111]. Replication labelling of DNA, followed by culture of cells for several additional cell cycles, provided another tool for visualizing individual CTs[25]. DNA-labelling protocols with fluorochrome-linked nucleotides allowed studies of CTs and higher-order chromatin domains in living-cell nuclei[30,31].

1994: DNA fusion constructs for the expression of proteins tagged with GFP[112] allowed the visualization of specific proteins in living cells. Artificial chromatin structures that contain repeated *lac* operons were studied in living cells using GFP-tagged lac repressor protein[84]. Recently, GFP colour variants (for example, yellow and red fluorescent protein)[113] have opened the door to multicolour, living-cell studies of nuclear proteins. In combination with the visualization of chromatin, these advances will lead to rapid advances in our understanding of the topology, movements and dynamic interactions of chromatin and non-chromatin domains within living-cell nuclei.

1995: A first quantitative simulation of CTs was published[90]. Since then, several other mathematical and computer models have been developed to simulate higher-order chromatin structure and arrangements[91,114]. Computer models that allow the simulation of dynamic aspects of nuclear architecture are now being developed.

EPIFLUORESCENCE MICROSCOPY
The entire cell is illuminated and fluorescence is recorded through the same objective from an entire focal plane.

ABBE LIMIT
Theoretical limit of light-microscopic resolution defined in 1873 by Ernst Abbe. This limit holds for conventional light microscopy but can be overcome by new laser microscopic approaches (BOX 1).

Another question is whether non-random associations exist between homologous CTs. Once again, there have been different answers, depending on the species and cell type. In *Drosophila* and other dipterans, associations between homologous chromosomes are a typical finding in diploid somatic cells[43], whereas such associations — at least with regard to the larger chromosomes — occur infrequently and probably as random events in the nuclei of human lymphocytes and fibroblasts. We have observed more frequent homologous association of CTs for gene-dense, smaller chromosomes, such as chromosome 19 (FIG. 4), but this finding can probably be explained by their preferential location in the nuclear interior[41,42]. In human T lymphocytes, a temporal and spatial association between chromosomes 15 was observed during late S phase, specifically at the 15q11–q13 region[44]. These regions contain the Prader–Willi syndrome (PWS) and the Angelman syndrome (AS) loci, which are subject to parental imprinting. Notably, cells from PWS and AS patients were deficient in association, and it was concluded that normal imprinting might involve mutual recognition and preferential association of chromosomes 15 during each cell cycle.

The enormous amount of shuffling of chromosome segments that has occurred during evolution is also relevant to this discussion. For example, a minimum number of 160 rearrangments distinguishes the mouse from the human karyotype[45]. It therefore seems highly unlikely that a functional nuclear architecture should require strict ordering of entire sets of CTs. Nevertheless, it is still possible that conserved arrangements of higher-order chromatin do exist — a proposition that can be tested by comparing chromatin arrangements in several cell types from different species.

Chromosome bands

The banded pattern of mitotic mammalian chromosomes provides a well-known example of genome compartmentalization. The arms of mitotic chromosomes are composed of early-replicating bands, called Giemsa-light (G-light) bands or R-bands, which alternate with mid-to-late-replicating G-dark bands. G-light bands have a high gene density and contain housekeeping and tissue-specific genes, whereas G-dark bands are gene poor and contain only tissue-specific genes. The highest gene density is noted in a fraction of R-bands, called the T-bands[46]. Late-replicating C-bands , which probably do not contain any genes at all, include CENTROMERIC HETEROCHROMATIN and some other segments of constitutive heterochromatin. Chromatin that reflects these bands in CTs is also organized in discrete domains[47] (FIG. 3b). It is a commonly held view that replication begins at hundreds of dispersed nuclear sites in R/T-chromatin that is located in an interior nuclear compartment[48]. However, a recent study carried out in primary fibroblasts has contradicted this view and has shown that replication starts in a limited number of foci that surround the nucleolus[49]. The reason for this discrepancy is not clear at present. Mid-replicating chromatin comprises mostly G-dark chromatin and is predominantly observed in nuclear compartments that are located at the nuclear and perinucleolar periphery, as well as at invaginations of the nuclear lamina (FIG. 5)[48]. Late-replicating chromatin comprises heterochromatic regions and is located both at the nuclear periphery and in the nuclear interior.

In several cell lines, the formation of nascent RNA was observed in the interior nuclear compartment, which comprises the R/T-chromatin[48]. In another study, DNase-sensitive, and most probably transcriptionally active, chromatin domains were found at the nuclear periphery,

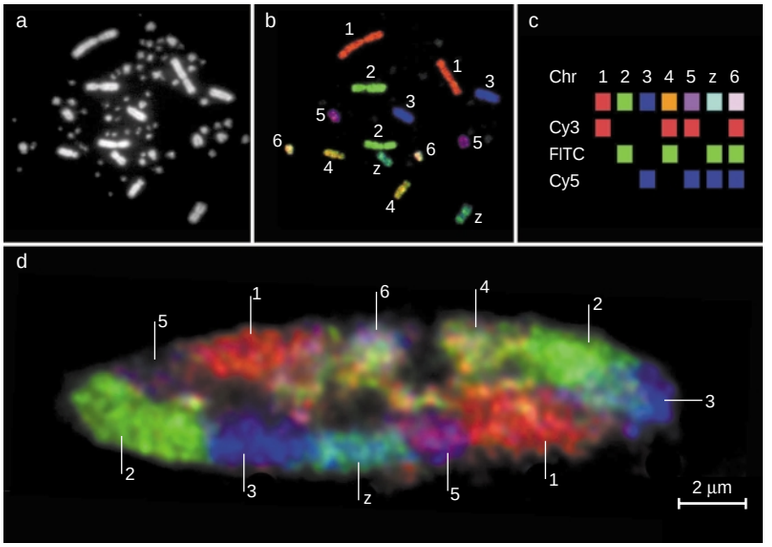

Figure 2 | **Chromosome territories in the chicken. a** | 4,6-diamidino-2-phenylindole (DAPI)-stained, diploid, chicken metaphase spread with macro- and microchromosomes. **b** | The same metaphase spread after multicolour fluorescence *in situ* hybridization with pseudocoloured chromosomes. Chicken chromosome paint probes (image courtesy of Johannes Wienberg) were labelled by a combinatorial scheme with oestradiol (1, 4, 5, 6), digoxigenin (2, 4, 6, Z) and biotin (3, 5, 6, Z). **c** | Oestradiol- and digoxigenin-labelled probes were detected using secondary antibodies labelled with Cy3 and fluorescein isothiocyanate (FITC); biotinylated probes were detected with Cy5-conjugated streptavidin. **d** | Mid-plane light optical section through a chicken fibroblast nucleus shows mutually exclusive chromosome territories (CTs) with homologous chromosomes seen in separate locations. (Note that only one of the two CTs for each of 4 and 6 is displayed in this section.) (Image courtesy of F. Habermann.)

which indicated that the nuclear periphery might function as a compartment for the spatial coupling of transcription and nucleocytoplasmic transport[50]. These discrepancies indicate that our understanding of the dynamics of higher-order chromatin is incomplete and that we should refrain from any attempts to generalize findings from one or a few cell types only.

Focal chromatin aggregates

Labelling of DNA with thymidine analogues in various living mammalian cells has provided compelling evidence for chromatin aggregates of DNA synthesis, termed replication foci (FIG. 5). Each replication focus consists of a cluster of active replicons together with replication factors, and has a DNA content of ~1 Mb[51,52]. During S phase, the replication machinery is assembled with a given 'replication focus' for the period that is necessary to complete its replication (~1 h)[51,52]. For clarity, we restrict the use of the term 'replication focus' to this period of continuing DNA replication and use the term '~1-Mb chromatin domain' to describe focal chromatin aggregates on the order of several hundred kilobases to several megabases, irrespective of their involvement in specific nuclear functions.

The important point is that labelled ~1-Mb chromatin domains remain visible after the completion of replication and are observed throughout several subsequent cell cycles, independent of the cell-cycle stage[26,53,54]. This finding indicates that these domains provide a high level of chromatin organization that might assemble different sets of factors (for replication, transcription or repair) at different times during the cell cycle[7]. It is not known whether each domain persists as an individual entity (composed of a continuous DNA segment or of several, non-contiguous segments) throughout the lifespan of a cell, or whether the DNA-sequence composition of ~1-Mb domains changes with time, supporting a more dynamic view of chromatin-domain assembly and disassembly. Space–time observations of fluorescently labelled ~1-Mb domains in the nuclei of living cells indicate that these domains have constrained Brownian motions, as well as occasional directed movements[32].

Interchromatin compartment

Apart from the higher-order chromatin compartments discussed so far, the nucleus also contains a largely chromatin-free space lined by chromatin-domain surfaces. A ribonucleoprotein network located in this space was first noted in electron-microscopic studies[55]. Recently, using fusion proteins of histone 2B tagged with green fluorescent protein (GFP), the entire chromatin complement was visualized in the nucleus of living HeLa cells together with a non-stained interchromatin space[56] (FIG. 6). At its most expanded sites, this space forms lacunas with diameters of up to several micrometres; at its finest branches, its width might be as small as a few nanometres and might be maintained by repulsive electrostatic forces between the apposed chromatin surfaces that line the branch[12]. Chromatin loops apparently can expand into this space[28] (FIGS 1f and 6). More expanded sections of the interchromatin space contain non-chromatin domains[57], such as SPECKLES, CAJAL (OR COILED) BODIES and PML BODIES (for a review of early electron-microscopic evidence of nuclear bodies, see REF. 58). It is not clear whether non-chromatin domains represent storage sites of proteins or protein complexes, which are released to sites of action, or whether these domains might directly serve as functional sites[59].

The existence of an interchromatin space is not very intriguing news: any meshwork of CHROMATIN FIBRES, by necessity, is embedded within an interchromatin space. Consider a model in which non-chromatin domains of increasing size push the surrounding chromatin fibres aside and locally expand the interchromatin space into lacunas (FIG. 6). Transcription and splicing factors freely roam the entire interchromatin space[60]. They might form functional complexes directly at the sites of genes. Alternatively, (sub-) complexes are built up at distant sites and subsequently reach the genes by diffusion. In this model, the existence of an interchromatin space that contains non-chromatin domains would not require any pre-existing higher-order nuclear architecture above the level of the 10- and 30-nm chromatin fibres[61].

However, we argue for the existence of a 3D interconnected IC (synonym: interchromatin domain (ICD) compartment[12]) with distinct structural and functional properties that have co-evolved with a dynamic chromatin-domain architecture (see also below). The IC starts at nuclear pores[28], expands between CTs and into their interior, and possibly ends with its smallest

SPECKLES
Irregularly shaped regions that contain splicing factors. At the electron-microscopic level they correspond to interchromatin granule clusters (IGCs), which function in the storage and supply of components of the pre-mRNA splicing machinery, and perichromatin fibrils located in the vicinity of IGCs.

CAJAL BODIES
(also known as coiled bodies). Nuclear organelles of unknown function named in honour of Ramón y Cajal. Cajal bodies are possibly sites of assembly or modification of the transcription machinery of the nucleus.

PML BODIES
Contain wild-type promyelocytic leukaemia (PML) protein and other proteins. Their function remains elusive, but might be related to transcription control.

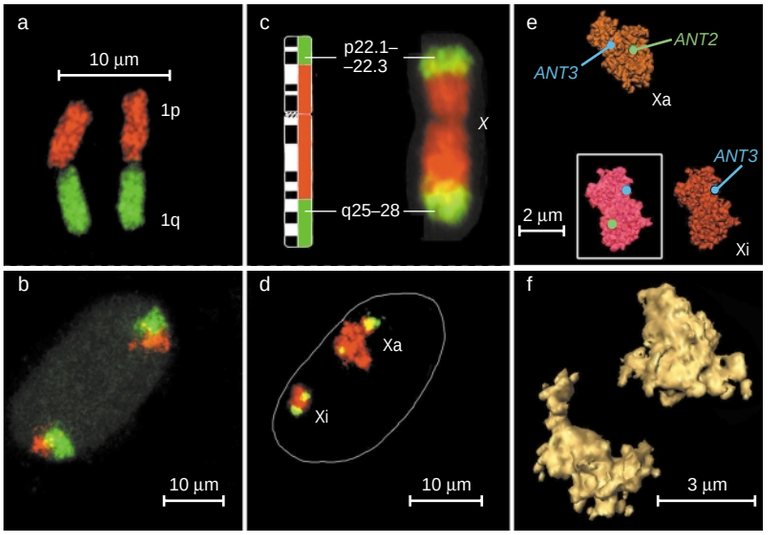

Figure 3 | **Features of human chromosome territories. a** | Two-colour painting of the p-arm (red) and the q-arm (green) of human chromosome 1 in a lymphocyte metaphase spread. **b** | Visualization of the two arms in a light optical section through a human diploid fibroblast nucleus (bottom) shows two distinct, mutually exclusive arm domains[20]. (Image courtesy of Steffen Dietzel). **c** | Painting of the human X chromosome (red) and several distal bands of its p-arm and q-arm (green) using MICRODISSECTION PROBES[20]. **d** | Visualization of the active and inactive X-chromosome territories (Xa and Xi, respectively) together with the respective distal-band domains in a light optical section through a female human fibroblast nucleus. (Image courtesy of Joachim Karpf and Irina Solovei). **e** | Three-dimensional reconstructions of the Xa and Xi territories from a human female fibroblast nucleus (Reproduced with permission from REF. 22). The three-dimensional positions of the *ANT2* and *ANT3* (adenosine nucleotide translocase) genes are noted as green and blue spheres, respectively. Note that active *ANT* genes can be seen at the territory surface (two on Xa and one on Xi). The white box provides a transparent view of the Xi territory (pink), indicating the location of the inactive *ANT2* gene in the territory interior. **f** | Three-dimensonal reconstructions of two chromosome-17 territories, established from light optical serial sections through a human diploid fibroblast nucleus, show complex territory surfaces. (Image courtesy of Irina Solovei.)

branches between ~1-Mb and ~100-kb chromatin-loop domains (FIG. 1 and see below). We propose that surfaces of compact chromatin domains provide a functionally relevant barrier, which can be penetrated by single proteins or small protein aggregates, but not by larger macromolecular complexes above a certain threshold size. The IC (by definition) does not comprise the additional interchromatin space present between chromatin fibres in the interior of compact chromatin domains (FIG. 7c, see below). We further propose that spliced RNA can be complexed with proteins and exported to the nuclear pores in the IC space, thus preventing the entangling of RNA that is produced in the interior of compact chromatin domains.

A critical evaluation of the IC concept requires a detailed analysis of the movements of macromolecules and complexes in the nucleus. The kinetic and thermodynamic aspects of these studies support passive diffusion as the decisive mechanism that is responsible for the movement of factors and factor complexes[60,62]. The conditions that influence these movements (such as transient binding to immobile obstacles) have not yet been fully determined[63,64]. Microinjection of size-fractionated fluorescein isothiocyanate (FITC) dextrans into HeLa cell nuclei showed that 70- and 580-kDa dextrans (equivalent to DNA sizes of 106 and 878 bp, respectively) freely diffused in the nucleus, whereas 2,000-kDa dextrans were essentially immobile[65]. Fluorescence recovery after photobleaching (FRAP) experiments (BOX 1) indicate 'homogeneous' movement of proteins at all nuclear sites, including bleached CTs[60]. This finding clearly indicates that proteins can move 'through' CTs. The IC concept requires that individual nuclear proteins or small protein complexes roam the entire interchromatin space (IC plus the interior of compact chromatin domains). By contrast, diffusion of larger (sub-) complexes should be constrained to the IC. Interchromatin channels that expand through CTs[28] should even allow channelled movements of such complexes through the CTs. Experiments based on fluorescence microscopy at present lack the resolution to support or disprove the IC concept.

The CT–IC model

Chromosome territories and the IC provide the fundamental components of the CT–IC model of a functional nuclear architecture. We first consider the essential features of this model and then (circumstantial) supporting evidence. The hypothesis that partial transcription complexes are pre-established in, and that their diffusion is restricted to, the IC has an important consequence: to fulfil its role as a functionally defined compartment, the IC requires a specific topology of transcriptionally active genes. Regulatory and coding sequences of these active genes can interact with the transcription machinery only when they are positioned at the surface of chromatin domains that line the IC, or on chromatin loops that extend into the IC (FIG. 1e,f). The argument can be extended to genes that are subject to short-term inactivation, the expression of which needs to be rapidly upregulated. By contrast, long-term or permanently silenced genes should be located within the interior of compact chromatin domains that are inaccessible to the transcription machinery, according to this model. In more general terms, genes that require long-term silencing should be physically separated from permanently active genes to an extent that allows their positioning in different chromatin compartments. Chromatin remodelling events that result in the positioning of genes into proper nuclear compartments are considered an essential part of gene-activation and gene-silencing mechanisms.

So much for the predictions of the CT–IC model. What about experimental evidence to support the model? In a first version of the CT–IC model, CTs were considered as compact objects with a smooth enveloping surface and it was assumed that an interchromatin-domain compartment expanded between these smooth CT surfaces and was excluded from the entire CT interior[66]. Accordingly, it was predicted that genes could only be transcribed when they were located at the CT periphery in contact with the IC. However, contrary to this prediction, transcription and splicing was observed not only at the periphery but also in the interior of CTs[27,67,68]. Concomitantly, more detailed experimental studies of CT architecture showed that CTs have a complex, folded structure that results in a largely expanded surface with IC channels that penetrate into the CT

CHROMATIN FIBRES
These 30-nm fibres are produced by the compaction of 10-nm nucleosome fibres. Nucleosome fibres are visible under the electron microscope after treatments that unfold higher-order chromatin packaging into a 'beads-on-a-string' 10-nm diameter form.

MICRODISSECTION PROBES
DNA probes established from microdissected chromosomal subregions. The probes are useful for the labelling of chromosome arms and bands.

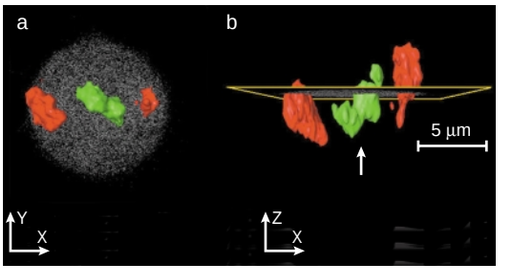

Figure 4 | **Gene-rich and gene-poor chromosome territories.** Three-dimensional reconstructions of chromosome 18 (red; gene-poor) and 19 (green; gene-rich) territories painted in the nucleus of a non-stimulated human lymphocyte. (Image courtesy of Marion Cremer and Irina Solovei.) Chromosome 18 territories were typically found at the nuclear periphery, whereas chromosome 19 territories were located in the nuclear interior[42]. **a** | X,Y view: a mid-plane section of the nucleus is shown as a grey shade. Only the parts of the territories below this section can be seen. **b** | X,Z view: the arrow marks the side from which the section in part **a** is viewed.

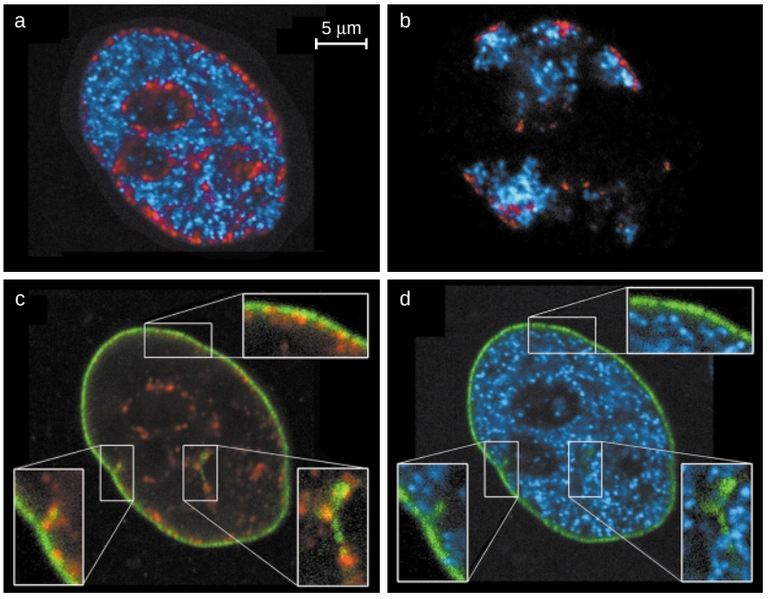

Figure 5 | **Early- and mid-to-late-replicating chromatin domains. a** | Mid-plane light optical section through the nucleus of an SH-EP N14 neuroblastoma cell fixed 20 h after direct two-colour labelling of DNA with Cy3- and Cy5-conjugated nucleotides at early and mid-S-phase, respectively, shows typical early- (blue) and mid-replicated chromatin (red)[115]. The cell shown is a daughter of the labelled cell produced after one cell division. The experiment shows that the arrangement of early- and mid-replicating chromatin domains is maintained from one cell cycle to the next. **b** | Light optical mid-section through the nucleus of an SH-EP N14 cell fixed three days after two-colour DNA labelling at early- and mid-S-phase shows several chromosome territories (CTs) with typical early- (blue) and mid-(red) replicated ~1-Mb chromatin domains[115]. Only a minority of CTs is labelled, which indicates that fixation was carried out after at least three post-labelling cell cycles. Note that the topology of mid-replicating chromatin (at the nuclear periphery and around the nucleoli) and early-replicating chromatin (in the interior nuclear compartment) was maintained through several post-labelling cell cycles. **c, d** | Mid-plane light optical section through the same cell nucleus shown in **a** immunostained with lamin B (green). A comparison of **c** and **d** shows that mid-replicating ~1-Mb chromatin domains (**c**, red) are closely associated with the lamina, in contrast to the early-replicating domains (**d**, blue). (Images courtesy of Lothar Schermelleh.) (Adapted with permission from REF. 115.)

interior[12,27,29]. Therefore, a transcribed gene can be located within the CT interior but still at a chromatin surface with direct access to the IC (FIG. 1c,d). The refined model prediction that nascent RNA is synthesized and spliced at chromatin-domain surfaces is supported by evidence that RNA synthesis and co-transcriptional splicing occurs in perichromatin fibrils that are located at chromatin-domain surfaces[27,59,68,69]. Further tests of the CT–IC model will require experimental procedures that allow the precise four-dimensional (space–time) mapping of active and silenced genes in relation to CTs, chromatin domains and the IC (see below).

The question of whether a nuclear matrix is an essential component of the *in vivo* nuclear architecture is still debated[70,73].The CT–IC model is compatible with a three-dimensional continuous network of nuclear matrix core filaments[70] that branch within chromatin domains and/or within the IC[71]. However, a nuclear matrix in the sense of a continuous nuclear skeleton that organizes the nuclear chromatin is not a necessary condition of this model[12]. Computer modelling (see below) shows that CTs and nuclear organization consistent with experimentally available evidence can be simulated without such an assumption[72]. (For a thorough discussion of the nuclear matrix and its relevance to the CT–IC model see REF. 12.) The IC might be functionally equivalent to a dynamic *in situ* nuclear matrix[7], which provides attachment sites, for example, for transcription and replication complexes[74]. The best-defined part of the nuclear matrix is the nuclear lamina, and its role in gene regulation has been studied most thoroughly in budding yeast (see, for example, REF. 75).

Gene topology and activity

An important unsolved question concerns the three-dimensional positions of active and inactive genes in relation to CTs, chromatin domains and the IC. Several groups have studied this and although the results are sometimes conflicting, they tend to support a non-random 3D organization of CTs. Peter Lichter and co-workers[21] noted that several active and inactive genes were preferentially located in the periphery of CTs. In another study[22], the 3D positions of the adenine nucleotide translocase genes, *ANT2* and *ANT3*, were determined in the CTs of the active and inactive X chromosomes (Xa and Xi, respectively) of female human cell nuclei. *ANT2* is transcriptionally active on Xa, but is inactive on Xi, whereas *ANT3* is located in the pseudoautosomal region and escapes X inactivation. Active *ANT2* and *ANT3* genes were positioned towards the exterior of both X-chromosome CTs. By contrast, the inactive *ANT2* gene on Xi showed a shift towards the territory's interior (FIG. 3e). Denise Sheer and co-workers[23] described the 3D large-scale chromatin organization of the major histocompatibility complex locus on human chromosome 6. Large chromatin loops that contain several megabases of DNA were observed to extend outwards from the surface of the chromosome 6 territory and possibly into the IC (FIG. 1a). Transcriptional upregulation led to an increase in the frequency with which active genes (but not inactive genes) were found on an external chromatin loop.

CONFOCAL LASER SCANNING MICROSCOPES
(CLSM). A three-dimensional cell is illuminated and the fluorescence is recorded point by point.

SC-35 DOMAINS
The essential non-snRNP (small nuclear ribonucleoprotein particles) splicing factor SC-35 shows a speckled distribution in the nucleus that co-localizes with snRNPs in speckles.

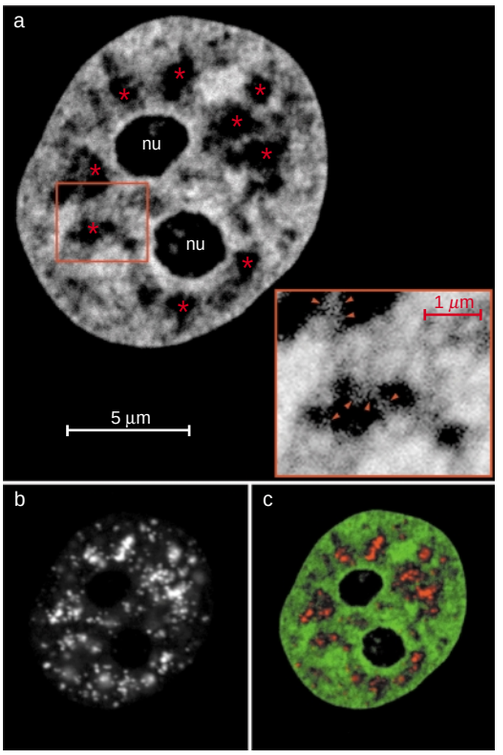

Figure 6 | **The interchromatin compartment.** LASER CONFOCAL sections through a HeLa cell nucleus with green-fluorescent-protein (GFP)-tagged H2B (cells kindly supplied by Ken Sullivan)[56], and staining of speckles (image courtesy of Irina Solovei). **a** | Section showing GFP-tagged chromatin (high density, white; low density, grey), two nucleoli (nu) and the interchromatin compartment (IC) space (black). Note the variability in the width of this space with examples of IC lacunas (asterisks). The inset shows expansions of less-condensed chromatin into the IC space at higher magnification. **b** | Speckles visualized in the same section using antibodies to the non-snRNP splicing factor SC-35. **c** | Overlay of sections (chromatin, green; speckles, red) shows that speckles form clusters in IC lacunas. These lacunas are only partially filled by the speckles, leaving space for other non-chromatin domains.

A spatial association of several actively transcribed genes, but not inactive genes, has also been observed with splicing-factor-rich speckles (FIG. 6)[76]. These domains were found preferentially at the periphery of CTs[66]. A specific topology of active genes with regard to speckles was further emphasized by David Spector and co-workers[75] in living-cell studies using GFP-tagged splicing factors. On activation of a nearby gene, speckles formed finger-like protrusions towards the new site of transcription[59,75]. Whether genes can also move towards speckles is not yet known.

Several studies lend support to the hypothesis that the transcriptional status of genes is affected by their nuclear topology. An important role of heterochromatin in gene silencing has been well established in *Drosophila* cells[35,77], which indicates that heritable gene silencing might require gene relocation events from a transcriptionally competent into a transcriptionally silent compartment. In support of this, Amanda Fisher and co-workers[78,79] showed a dynamic repositioning of certain genes in mouse B and T cells that depends on their transcriptional status. The transcriptionally inactive genes were localized at centromeric heterochromatin clusters in contrast to transcriptionally active genes, which were positioned away from them (FIG. 1b). In a study of chromatin opening and transcriptional activation of the human β-globin locus, it was suggested that localization of this locus away from centromeric heterochromatin is required for general hyperacetylation and an open chromatin structure of this locus, but is not sufficient for high-level transcription[80]. Furthermore, several studies indicate a role for DNA motifs in gene positioning. A functional enhancer was shown to antagonize silencing of a transgene by preventing localization of a gene close to centromeric heterochromatin[81]. In another case, a Dnase-I-hypersensitive site was shown to relocate a transgene to the outside of a pericentromeric heterochromatin complex. Transcription, however, required a specific transcription factor. Reducing the dosage of this factor resulted in a reduced frequency of localization of the transgene to the outside of the heterochromatin complex and lower levels of transcription[82]. Finally, chromatin insulators can affect the nuclear localization and transcriptional status of genes[83].

So far, *in situ* studies of gene positioning and changes in higher-order chromatin architecture have been carried out using FISH. However, this method can produce artefacts because it requires the fixation and permeabilization of cells and the denaturing of chromatin. Recently, an approach was developed that allows the visualization of transgenes and the monitoring of the gene product in living cells[84,85]. This methodological breakthrough promises great advances in our understanding of gene positioning and activity. For example, Tumbar and Belmont[86] showed that chromosome sites that contain such a transgene can show a significant change in position at specific times during the cell cycle, and that the timing of these movements can be changed by transcriptional activation.

Computer models

The unfolding story of CTs and gene topology is complex. Current views of higher-order chromatin architecture, its dynamics and function are probably oversimplified or incorrect. Chromatin loops of different sizes have become a common textbook scheme, which purports to explain how chromatin packaging is achieved from the DNA level to the level of entire metaphase chromosomes[87], but strong evidence for such schemes does not exist. A hierarchy of chromatin fibres[88] possibly coexists with, or is to some extent equivalent to, a hierarchy of chromatin domains (FIG. 1e,g). For example, it is possible that chromatin domains visualized by pulse labelling (see above) represent simply short segments of a higher-order chromatin fibre. Further experimental progress in understanding functional nuclear architecture will strongly depend on the development of models that make quantitative and experimentally testable predictions. In this section, we consider how computer simulations can help to inform the issue of nuclear organization.

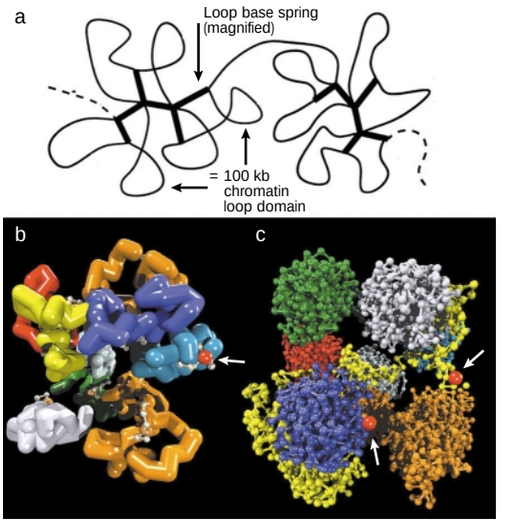

Figure 7 | **The multiloop subcompartment model. a** | Two ~1-Mb chromatin domains or 'subcompartments' are shown linked by a chromatin fibre (redrawn with permission from REF. 72). Each ~1-Mb chromatin domain is built up as a rosette of looped ~100-kb chromatin fibres. At the centre, loops are held together by a magnified LOOP BASE SPRING, which simulates the function of CHROMOSOME TERRITORY ANCHOR PROTEINS[116]. **b, c** | Two three-dimensional models of the internal ultrastructure of a ~1-Mb chromatin domain (image courtesy of Gregor Kreth; models redrawn with permission from REF. 12 © Begell House, Inc. (2000)). **b** | The nucleosome chain is compacted into a 30-nm chromatin fibre (visualized by cylinder segments) and folded into ten 100-kb-sized loop domains according to the multiloop subcompartment model. Occasionally, 30-nm fibres are interrupted by short regions of individual nucleosomes (small white dots). The arrow points to a red sphere, with a diameter of 30 nm, that represents a transcription factor complex. **c** | Each of the ten 100-kb chromatin domains was modelled under the assumption of a restricted random walk (zig-zag) nucleosome chain. Each dot represents an individual nucleosome. Nine 100-kb chromatin domains are shown in a closed configuration and one in an open chromatin configuration with a relaxed chain structure that expands at the periphery of the 1-Mb domain. The open domain will have enhanced accessibility to partial transcription complexes preformed in the interchromatin compartment. By contrast, most of the chromatin in the nine closed domains remains inaccessible to larger factor complexes (arrows).

Several computer models have been proposed to model higher-order chromatin structure up to the structure of entire CTs. Backfolding of chromatin fibres at some level is indispensable to obtain CTs: in models that dismiss such backfolding, chromatin fibres expand throughout much of the nuclear space, which results in a non-territorial interphase chromosome organization[89]. In the random-walk/giant-loop (RW/GL) model, chromatin loops with a size of several megabases are backfolded to an underlying structure, but otherwise each giant loop is folded randomly[90]. Another model, the multi-loop subcompartment (MLS) model, assumes that ~1-Mb chromatin domains are built up like a rosette from a series of chromatin-loop domains with sizes of ~100 kb, again assuming a random organization for each loop[72] (FIG. 7). These two models are useful to simulate CTs and both assume a RW folding of chromatin loops. Accordingly, these models are compatible with the assumption that specific positions of genes inside or outside a chromatin-loop domain are not required for activation or silencing. However, they do make different predictions: first, about the extent of intermingling of different giant chromatin loops and chromatin-loop rosettes; and second, about the interphase distances between genes and other DNA segments that are located along a given chromosome. The available experimental data, in our view, are more compatible with the MLS model than with the RW/GL model[72] (T.C. and C.C., unpublished data). Recently, the spherical ~1-Mb chromatin domain (SCD) model was introduced as a modified version of the MLS model. The SCD model does not make any assumptions about the internal structure of ~1-Mb chromatin domains, and it is possible to simulate, on a single PC, entire sets of CTs in human diploid cell nuclei together with the IC[91] (supplementary figure 2 online). This and other models have already been used to predict translocation frequencies after irradiation[89,91–93].

A global view of gene regulation

The concept of CTs can be traced back to the early work of Carl Rabl, Theodor Boveri and Eduard Strasburger[5]. Models belong to the evolving toolkit for studies of functional nuclear architecture (BOX 1), and will be improved or replaced just as other outdated parts of the toolkit have been. Their value should be judged by their ability to stimulate further experiments that test their predictions. The essentials of a topological model for gene regulation can be summarized as follows: long-term changes in gene-expression patterns require a higher-order chromatin remodelling that reflects the repositioning of genes in open or closed higher-order chromatin compartments; and differentiating cells establish a cell-specific pattern of gene locations with respect to certain nuclear compartments, such as heterochromatin, the IC or the nuclear lamina. Compelling evidence for the predicted space–time structure and functions of the IC is at present lacking. Nevertheless, we believe that the proposal of the CT–IC model is timely and that it would be useful to put the following predictions to the test: long-term active genes, as well as genes that need to be turned on and off rapidly, are exposed at the chromatin-domain surfaces that line the IC, or are located on loops that extend into the IC (FIG. 1); long-term silenced genes are located in the interior of chromatin domains that are remote from the IC.

When considering different levels of gene regulation, we should keep in mind that evolution does not work like a designer who is keen to pursue a long-term plan for the most straightforward systems of gene regulation. A designer might have implemented a regulatory hierarchy that comprised many elements: regulatory DNA elements[81,83], DNA methylation[1], histone modifications[1,80], and possible influences of chromosome territory and nuclear architecture. The different levels of the designer's

LOOP BASE SPRINGS
In the multiloop subcompartment model of chromosome territory architecture, stiff springs were assumed to exist at the loop bases for a simulation of chromosome territory anchor proteins.

CHROMOSOME TERRITORY ANCHOR PROTEINS
Proteins that are essential for the maintenance of chromosome territories.

hierarchy might show little dependence on each other and so we could safely study one level while neglecting the others. However, our knowledge of Darwinian evolution gives us good reason to expect complex, sometimes messy, interactions between all possible levels of gene regulation. A comprehensive model for these interactions does not yet exist, but we are in an age when genome sequencing, microarray technologies and proteomics offer high hopes for understanding not just the regulation of groups of genes, but also, one day, entire genomes. We wish to emphasize our view that studies of the genome in the context of nuclear architecture are indispensable for a better understanding of the cell-specific orchestration of gene activity.

Links

DATABASE LINKS Prader–Willi syndrome | Angelman syndrome | *ANT2* | *ANT3*

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