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Chromosome Organization and Gene Control: It Is Difficult to See the Picture When You Are Inside the Frame

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Abstract The organization of the genome in the nucleus is related to its function. The functional compartmentalization of the genome is described at the nuclear, chromosomal, subchromosomal, nucleosomal, and DNA sequence level. These descriptions originate from the techniques that were used for analysis. The different levels of compartmentalization are not easily reconciled, because the techniques applied to identify genome compartmentalization generally cannot be performed in combination. We have obtained a large body of information on individual ''actors'' and ''scenes'' in the nucleus regarding genome compartmentalization, but we still do not understand how and by what pieces of equipment the ''actors'' play their game. The next challenge is to understand the combined operation of the various levels of functional genome organization in the nucleus, that is, how do the epigenetic and genetic levels act together. In this paper, I will highlight some of the general features and observations of functional organization of the eukaryotic genome in interphase nuclei and discuss the concepts and views based on observed correlations between genome organization and function. I will reflect on what is to be expected from this field of research when the functional levels of genome compartmentalization are integrated. In this context I will draw attention to what might be needed to improve our understanding. J. Cell. Biochem. 99: 24–35, 2006.

Key words: chromatin structure; nuclear organization; gene expression; epigenetics; systems approach

Each cell type uses a defined fraction of the available genes, whereas every cell of the organism contains the similar nucleotide sequence in their DNA. This stresses the point that proper regulation of gene expression profiles is indispensable. The genome contains at least two types of information: genetic information, that is, the nucleotide sequence in the DNA, and epigenetic information, which is encoded in a complex set of chemical modifications of histones and DNA. Epigenetic gene control systems decide about the genetic repertoire of the different differentiation states of cells in an organism and are stably transmitted during mitosis. Several studies point at perturbations of genome organization in diseased cells (i.e., several diseases have a clear link with changes in epigenetic gene control [Egger et al., 2004]). To be able to develop therapeutic strategies to treat such epigenetic diseases, we need to understand the integrated picture of the various observations on genome organization.

It is well established that a correlation exists between structural organization of the genome and gene expression control. There are several options to explain functional genome organization, and there is still a debate going on which option fits reality best. At present a major problem in this field is the difficulty to combine observations made at the various levels of genome organization. The techniques used to identify genome compartmentalization often cannot be performed in combination. For example, fixed cells cannot be analyzed in time and

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biochemical analysis is applied to populations of cells and does not give insight into the single cell situation. Therefore, mechanistic aspects of how genes and gene loci act together in an integrated way are not well understood yet.

One might expect that particular architectural components exist, but until now no unique structural components that determine functional chromosome organization have been described. Nuclear lamins are well-characterized intermediate filaments within nuclei, which play an important role to maintain nuclear shape and chromatin organization [Goldman et al., 2004]. Presently, studies on lamins and diseases associated with point mutations in lamin proteins as well as nuclear actin and actin-related proteins receive much attention, which encourages us to keep an open eye on this issue.

Genome organization is the result of interactions between the genome and other nuclear components. Centromeres and telomeres that bring chromatin from different chromosomes together and the association of defined chromosome regions with nuclear bodies are examples of large chromosome structures or compartments that contribute to genome organization. At the level of a particular stretch of chromatin, centromeric heterochromatin acting as a silencer might create stable interactions, thereby giving rise to a compartmentalized chromatin structure. Zooming into the DNA level, Aten et al. [2004] demonstrated that the introduction of double strand breaks causes clustering of chromatin domains. This chromatin clustering is not only dependent on the mobility of the chromatin in the nucleus, but also on an adhesive process. Most likely, proteins of the structural maintenance of chromosome (SMC) family containing the Mre11 DSB repair complex can specifically tether linear DNA molecules thereby providing a kind of molecular glue.

Macromolecular crowding is another concept to explain the formation of genomic compartments. In this concept the mechanisms of compartment formation depend on the characteristics of the intranuclear environment, rather than properties of individual molecules [Hancock, 2004]. Interestingly, compartments can be reformed by special stimuli such as viral infection, or disease (e.g., in certain neurodegenerative diseases typical aggregates or inclusions are formed in the nucleus [Bucciantini et al., 2002]). Furthermore, compartments are known to disassemble when nuclei are expanded by treatment with hypotonic buffer and to reassemble when inert macromolecules are added to expanded nuclei [Hancock, 2004]. Thus, macromolecular crowding or volume exclusion can also underlie the structured nuclear interior and the formation of genomic compartments.

Below, I will highlight some of the general features and observations of functional genome organization in interphase nuclei of higher eukaryotes (Fig. 1) and discuss the concepts and views based on the observed correlations between genome organization and function (Fig. 2). Along these lines I will reflect on the integrated functioning of the known levels of genome compartmentalization and what is needed to improve our understanding.

WHAT GENOMIC COMPARTMENTS CAN WE DEFINE?

There are various ways to describe compartments in the interphase cell nucleus (Fig. 1). The difficulty with these different descriptions is that since the descriptions are based on various ways of analysis, they are not easily combined in one overall picture.

Electron Dense Chromatin

Based on electron microscopy, the difference in electron density of chromatin regions was assumed to distinguish between functional chromatin regions in the interphase nucleus in fixed cells (Fig. 1A). Electron dense regions represented “inactive” and more compact chromatin, named heterochromatin, whereas less electron dense regions were supposed to represent “active” and more decondensed chromatin, named euchromatin [Fakan and Bernhard, 1971]. By now it is clear that a much wider range of chromatin “flavors” exists [Lachner and Jenuwein, 2002]. Basically, euchromatin regions are a mixture of actively transcribed chromatin and transiently silenced gene loci. Facultative heterochromatin describes a permissive chromatin environment that is subject to gene silencing but can potentially become active in response to a gene control trigger, for instance during differentiation. Constitutive heterochromatin is generally associated with pericentromeric regions of chromosomes. It consists predominantly of repetitive sequences,

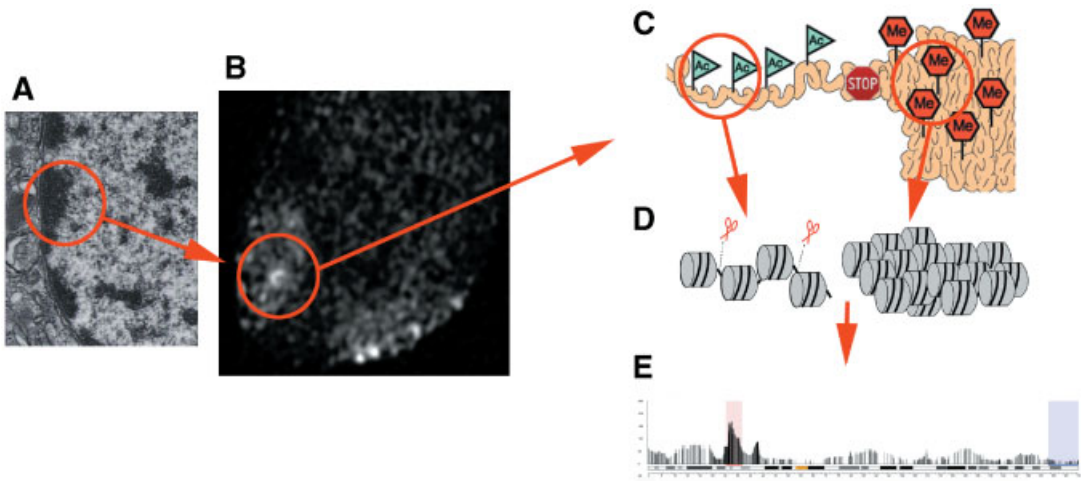

Fig. 1. Compartmentalization of the genome. There are various ways to describe genome compartments in the interphase cell nucleus. Encircled are the subsequent compartmentalization levels, whereas the arrows point how the represented compartmentalization continues in the next level of compartmentalization. **A**: Part of the nucleus at electron microscopy level. Electron dense regions and less electron dense regions are suggested to represent ''inactive'' and more compact chromatin and ''active'' more decondensed chromatin, respectively. Encircled is such an electron dense area of the nucleus. **B**: Part of the nucleus at light microscopy level, after fluorescence in situ hybridization of X-chromosomes. Individual interphase chromosomes form discrete entities in the nucleus predominantly occupying their own micrometer-scale territory. Encircled is the clear substructure within such chromosome territories. **C**: Cartoon representation of a chromatin domain. Compartmentalization in such chromatin domains is controlled by histone-modification states (e.g., histone acetylation is represented by the blue flags and histone methylation by the red hexagonals) that are bordered by boundary elements (represented by the red stop signal). Encircled is a decondensed chromatin domain defined by histone acetylation and a compacted chromatin domain defined by histone methylation. **D**: Cartoon representation of nucleosomal arrays. The right side shows a compact and regular spaced nucleosomal array and the left side shows an irregular and widely spaced nucleosomal array that can easily be digested with micrococcal nuclease. **E**: Representation of compartmentalization at the DNA sequence level by correlating gene expression profiles with the location of genes on the linear DNA of their respective chromosome. Y-axis represents the gene expression level (SAGEtag count at mm 49 scale of the data set), X-axis represents Mbp over chromosome 6. The horizontal bar represents the Giemsa banding pattern on chromosome 6. A cluster of genes with high-gene expression levels (RIDGE) is marked in red (at ~30–37 Mbp); a cluster of genes with low gene expression levels (anti-RIDGE) is marked in blue (at ~159–170 Mbp). The RIDGE on chromosome 6 is known to represent the *MHC* gene cluster.

related to transposable elements and retroviruses. Constitutive heterochromatin is typically gene-poor and condensed.

Nuclease Sensitivity

Based on biochemical in vitro analysis, heterochromatin differs from euchromatin on the basis of (i) a more regular spaced nucleosome array, (ii) a reduced length of the linker region between the nucleosomes, and (iii) an increased nucleosomal core region that is protected from micrococcal nuclease digestion [Weintraub and Groudine, 1976]. These characteristics are supposed to facilitate packaging of heterochromatin into a highly condensed configuration, whereas the transcriptionally active and accessible euchromatin is considered to involve selective disruption of the compacted nucleosome structure (Fig. 1D).

Chromosome Territories

Based on light microscopical analysis after fluorescence in situ hybridization of whole chromosomes (chromosome painting) in the interphase cell nucleus, it has been demonstrated that individual interphase chromosomes form discrete entities predominantly occupying their own micrometer-scale territory [Cremer and Cremer, 2001] (Fig. 1B). A striking substructure in such chromosome territories can be observed [Verschure et al., 1999]. Such chromosomal substructures might represent chromosome arms, mitotic chromosome bands, or even smaller chromatin domains. Chromosome territories are considered to have distinct borders with little intermingling of chromatin from adjacent territories [Visser and Aten, 1999]. An extensive analysis of chromosome paints on ultrathin cryosections in human

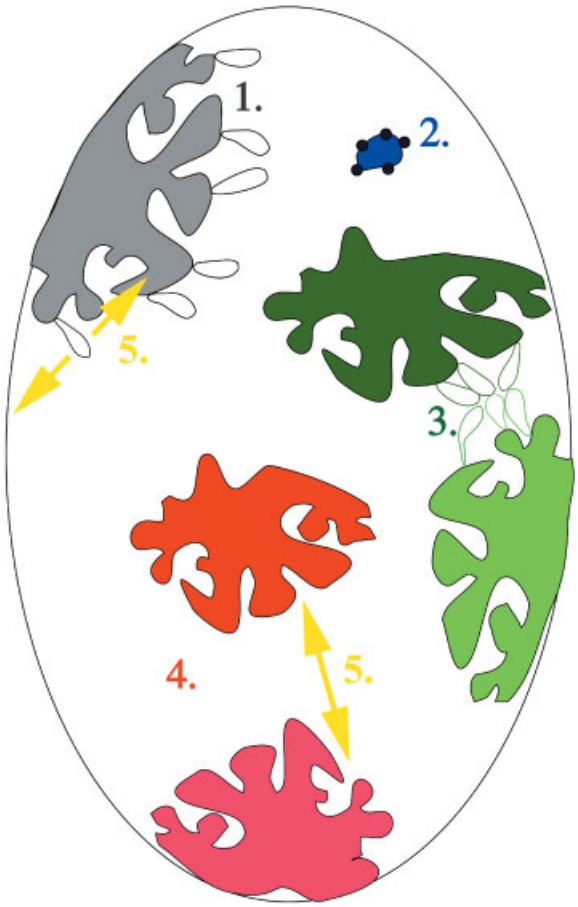

Fig. 2. Principles of genome organization and function. Several concepts and views of functional genome organization are cartoonwise presented. 1, sites of high-gene expression but also epigenetically silenced sites occur outside of chromosome territories or outside compact chromatin domains. Such sites might represent fine flexible chromatin loops emanating away from compact chromatin domains (gray representation). 2, several genes (dark blue dots) locate at defined nuclear protein compartments or bodies (lighter blue domains) in the nucleus. 3, particular chromosome regions or chromatin loci cluster together in the nucleus (green representation). 4, defined chromosome territories are preferentially located at particular places in the nucleus (gene density-related radial dependence of the chromosome position in the nucleus and location in the nucleus according to chromosome size) (red representation). 5, Mobility of defined chromosome domains or chromatin loci in the nucleus. Very rapid and short-scale movements over relatively small zones of the nucleus (0.2–0.5 μm) are represented by an arrow at 1; long-range movement of a large chromatin domain is represented by an arrows at 4 (yellow representation).

lymphocytes showed that actually 40% of the genome is intermingled, which means 20% of intermingling in terms of nuclear volume, as regions of intermingling contain mostly chromatin from two neighboring chromosomes (personal communication M.R. Branco and A. Pombo, MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College London, UK). Interestingly, the amount of overlap of different chromosome territories correlates well with the frequency of ionization radiation-induced translocations in the same cell type. This indicates that the nearness of different chromosomes can have a functional consequence. Whether the intermingling is functional or not is not yet clear. It may be that neighboring chromosomes come together at bordering zones for a particular purpose, for instance to collectively use factors or domains in the nucleus.

Epigenetically Defined Chromatin Domains

A recently described type of genome compartmentalization is the division in distinct functional chromatin domains flanked by boundary elements and controlled by epigenetic gene control systems (Fig. 1C). The functional state of chromatin domains, such as the histone-modification state (i.e., histone phosphorylation, acetylation, methylation, or ubiquitination) are thought to alter the interaction of histones with DNA (for instance by changing the charge) and to change interactions of chromatin with chromatin-associated proteins [Jenuwein and Allis, 2001]. The current concept is that a particular histone modification acts as a docking site for specific effector proteins that initiate distinct downstream events [Wang et al., 2004]. The combination of histone modifications and the degree of a histone modification (i.e., whether the modification occurs in a mono, di, or tri form or at a particular lysine) are related to gene activity and chromatin folding [Jenuwein and Allis, 2001]. Histone modifications also interact with other epigenetic signals (e.g., DNA methylation and small RNAs associated with the RNA interference pathway), thereby representing a complex gene regulatory system [Fuks et al., 2003; Martienssen, 2003]. The mating type region of *S. Pombe*, which is a typical example of a silent chromatin region, represents a clear example to illustrate the functional bordered chromatin principle [Hall et al., 2002]. Histone H3K9 methylation (a histone mark of chromatin that codes for transcriptional inactivity) takes place in a chromatin domain of the mating type region of 20 kbp that is flanked by inverted repeats, whereas histone H3K4 methylation (a histone mark of chromatin that codes for transcriptional activity) occurs

only outside that chromatin domain in flanking euchromatin regions. Mutation of the inverted repeats that flank the chromatin domain results in propagation of histone H3K9 methylation into the surrounding euchromatic regions.

Our present research focusses on epigenetic gene regulation at the chromatin domain level [Brink et al., 2005; Verschure et al., 2005]. To this end, we focused on the causal effects on gene expression control of targeting epigenetic regulatory proteins to a defined large 200 Mbp chromosome domain consisting of lac operator repeats in living cells. Such a large chromosomal domain consisting of lac operator repeats can be visualized and followed in time at the light microscopy level by means of lac repressor-GFP binding to the lac operator repeats. We analyzed targeting of heterochromatin protein 1 (HP1), a protein that is associated with heterochromatin, as a lac repressor-GFP fusion protein. Upon targeting of HP1 as well as upon targeting HP1 lacking its C-terminal chromo domain, which is essential for association of HP1 with heterochromatin via binding to methylated H3K9, we observed heterochromatin formation. We measured changes in compaction of the large chomosome domain, the change in the epigenetic coding of the domain, that is, tri-methylation of H3K9, and inrecruitment of heterochromatin-associated proteins (HP1 isoforms and histone methyltransferase SETDB1). Presently, we are systematically analyzing the causal effects of targeting various factors involved in epigenetic gene regulation on epigenetic gene control.

Regions of Increased Gene Expression (RIDGES)

Versteeg et al. [2003] developed the human transcriptome map, which correlates gene expression profiles with the position of genes on the linear DNA [Caron et al., 2001] (Fig. 1E). This linear mapping of gene expression profiles revealed that the human genome contains a number of chromosomal domains characterized by high-gene density and high-gene expression levels. Such domains of many megabase pairs, called regions of increased gene expression (RIDGES), may locate together forming a nuclear region of high-gene expression in the 3D-interphase nucleus. RIDGES might for instance represent large chromatin domains that loop out of their chromosome territories.

Integration of the Levels of Genome Compartmentalization

The link between the various concepts of genome compartmentalization is still unclear. Computer simulations have been performed to understand functional higher order chromosome structures. The existing models are not informative about chromosome functionality, they mainly enable an improved description or representation of the chromosomal structure [Wedemann and Langowski, 2002; Kreth et al., 2004]. To make testable predictions of functional chromatin organization we need better and more comprehensive computational models, taking recently found functional aspects of chromatin organization into account. Here, the challenge is to combine the data of genome compartmentalization at different levels. For example to find out how nuclease sensitivity correlates with chromosomal folding as observed using light and electron microscopy, or to compare how clustering of highly active genes at the linear DNA level relates with positioning of such chromatin stretches in the 3D-interphase nucleus. A recent study of Bickmore and colleagues is an elegant example of combining a biochemical approach and genome-wide microarray approach with single cell light microscopy analysis [Gilbert et al., 2004]. The authors analyzed the chromatin fiber structure in relation to its activity across the human genome. Compact and open chromatin fiber structures were separated by sucrose sedimentation and characterized by hybridization to metaphase chromosomes and microarrays, as well as analyzing their position relative to their chromosome territory. Strikingly, the authors demonstrated that there is no simple correlation between chromatin fiber condensation and gene expression, that is, many transcriptionally inactive genes are in open chromatin fiber domains and also active genes can reside within large domains of compact chromatin fibers.

WHAT GENOMIC ORGANIZATION PRINCIPLES CAN WE DISTINGUISH?

Based on the observed correlations between genome organization and function there are several concepts and views with respect to functional genome organization (Fig. 2). Here, I highlight various concepts that when integrated in one picture might give us insight in

what is needed to improve our understanding of this subject.

Chromatin Loops Outside Condensed Chromatin Domains

There is mounting evidence that sites of high-gene expression, but also Polycomb group binding sites (i.e., proteins involved in epigenetic gene silencing), occur outside chromosome territories or outside compact chromatin domains. Such sites might represent flexible chromatin loops emanating from compact chromatin domains (Fig. 2, representation 1). For instance, we have presented evidence, using light and electron microscopy, that transcription sites and also Polycomb group-silenced loci are concentrated at the surface of compact chromatin domains [Cmarko et al., 1999; Verschure et al., 1999; Cmarko et al., 2003]. Mahy et al. [2002] showed that a ubiquitously expressed gene often co-localizes with unlabeled or less intensely labeled areas of the chromosome territory, whereas the linked non-coding DNA is positioned frequently within intensely labeled compact subdomains of the chromosome territory. There are many observations that large chromatin loops with transcriptionally active genes can extent beyond their chromosome territory at a scale of several microns. For example, transcriptional upregulation of genes in the MHC class II complex led to an increase in the frequency with which this cluster relocates away from the main body of chromosome 6 upon gene induction [Volpi et al., 2000].

One might think that the positioning of actively transcribed gene loci outside chromosome territories or outside compact chromatin domains is related to accessibility of chromosome and chromatin domains. However, we have demonstrated that chromosome territories are accessible for the transcription complex, since transcription sites were found to occur throughout chromosome territories [Verschure et al., 1999]. What about the accessibility of chromatin domains? We have analyzed the accessibility of compact chromatin domains in nuclei of living cells for large inert molecules of various molecular sizes [Verschure et al., 2003]. Our results demonstrated that such compact chromatin domains are readily accessible for large macromolecules, including proteins with a molecular weight of several hundred kilodaltons. Apparently, other principles than simple steric exclusion are responsible for positioning of actively transcribed loci outside condensed chromatin and keeping non-expressed loci inside condensed chromatin.

In Arabidopsis nuclei, centromeric heterochromatin acts as an organizing center on which gene-rich chromosomal arms fold back forming multiple loops and creating a rosette-like structure [Fransz et al., 2002]. Mammalian chromosomes are much larger and have a more complex structure, containing more non-protein coding DNA. It is tempting to speculate that the in Arabidopsis observed phenomenon of heterochromatin organizing centers from where active chromatin loci loop out is a general organization principle in mammals and plants [van Driel and Fransz, 2004].

Association of Chromosome Regions With Nuclear Domains

Many examples exist of genes located at defined nuclear protein compartments or bodies within the nucleus. PML bodies associate with specific genes, Cajal bodies with histone and U2 snRNA genes and splicing speckles with gene-rich R-bands (reviewed in [Spector, 2001]). Such studies support the idea that specific chromosome regions have a defined location associated with nuclear protein compartments or bodies in the cell nucleus (Fig. 2, representation 2). Eskiw et al. [2004] proposed that the structure of nuclear bodies is regulated through direct contacts with chromatin. They demonstrated, that under normal conditions PML bodies and the surrounding chromatin contribute to each other's organization and stability. The authors found that chromatin retracts from the periphery of PML bodies following inhibition of transcription and an early stage of apoptosis. Chromatin retracts into numerous condensed chromatin domains, whereas a few residual 10 and 30 nm extended fibers remain physically anchored to protein-like structures, such as PML bodies. This chromatin compaction coincides with the formation of new PML-containing structures through fission of supramolecular PML-containing microbodies. Thus, the loss of chromatin contacts of nuclear bodies may lead to destabilization of protein accumulations.

Recently, Gorisch et al. [2005] proposed a "moving corral model" to define the relation between spatially restricted nuclear body movement and chromatin motion. This model is based on quantitative measurements of the

diffusion rates of nuclear bodies and chromatin in the nucleus. The authors depict that nuclear bodies are diffusing within a "corral" of mobile chromatin, whereas such corrals can translocate within the nucleus. Their model points out that the mobility of nuclear protein compartments or bodies is mostly determined by the fluctuations of chromatin density and movements of the chromatin.

Still, the extent to which nuclear compartments and bodies define functional compartments remains a matter of debate. The Cajal bodies illustrate this, as these structures can be disrupted in living cells or transgenic animals without obvious impairment to the host [Almeida et al., 1998; Tucker et al., 2001]. The structure of a nuclear body might not be determined by the functions that are performed by the components found within the bodies.

Clustering of Chromosome Regions

It is an intriguing hypothesis that clustering of highly expressed chromosome regions on different chromosomes (i.e., RIDGES) defines a functional organization principle in the cell nucleus. In fact, the nucleolus is an example of clustering of transcriptionally highly active regions of several chromosomes in the nucleus (Fig. 2, representation 3). Here tandemly repeated groups of ribosomal genes, located on several chromosomes, cluster. Osborne et al. [2004] illustrated that individual transcribed genes located more than 20 Mb apart on mouse chromosome 7 group together with high frequency in a single RNA polymerase II transcription domain, whereas the inactive alleles in the same cell are positioned away from such domains [Osborne et al., 2004]. This study suggests that active genes can migrate to a defined location in the nucleus, containing preassembled transcription sites. Brero et al. [2005] demonstrated that clustering of pericentromeric heterochromatin is a general feature of myogenenic differentiation that can be induced by Methyl-CpG binding protein 2 (MeCP2). The authors observed a striking fusion of compact chromatin domains throughout the interphase cell cycle after transfection with MeCP2, whereas extensive splitting of heterochromatin clusters occurred almost only in G_2.

Clustering of specific domains in the nucleus may represent an important organization principle. The major question remains: how is such clustering regulated; do loci just find each other by accident or do loci with similar functionality search for each other or are defined loci attached to a structure that keeps them together?

Positioning Within the Nucleus

In vertebrate nuclei, chromosomes with low gene density are described to reside preferentially towards the periphery of the nucleus, whereas chromosomes with high-gene density more frequently coincidence in the center of the nucleus [Croft et al., 1999] (Fig. 2, representation 4). The gene density-related radial dependence of the position of chromosomes is highly conserved during evolution, irrespective of major chromosomal rearrangements [Cremer et al., 2003]. Moreover, a limited number of studies, mainly in non-proliferating primary cells, chromosomes are found to position according to their size (i.e., small chromosomes towards the interior of the nucleus and large chromosomes towards the nuclear periphery) [Nagele et al., 1999; Bolzer et al., 2005]. It should be noted that due to huge cell-to-cell variation this preferred position of chromosomes in the nucleus is only established when scoring large numbers of cells.

There are many studies showing that subnuclear position influences gene activity or the other way around that gene expression status affects subnuclear location. As a clear example to illustrate this principle, a recent study of Zink et al. [2004] demonstrated the interdependence between gene positioning and activity of defined cell-type specific genes. It was shown that the *CFTR* gene, coding for a cyclic AMP-dependent chloride channel, locates more interior in Calu-3 adenocarcinoma cells where the gene is highly expressed, whereas the gene was relocated more to the perinuclear region after inhibition of gene expression. The other way around, the *CFTR* gene that is not expressed in neuroblastoma cells locates more perinuclearly but after TSA treatment inhibiting histone deacetylation the gene relocates significantly to the interior of the nucleus. Also in mouse lymphocytes individual silenced genes have been shown to undergo repositioning in the nucleus and become localized at pericentromeric heterochromatin [Brown et al., 2001] upon differentiation, whereas the β-globin locus is transcriptionally activated during erythroid differentiation and relocates away from the heterochromatin compartment.

There is a lot of data describing positioning of specific loci at particular positions in the

nucleus and also some studies point at a causal relationship between gene activity and positioning. It is fascinating that a high non-random correlation is found between the proximity of gene loci in the nucleus and their reciprocal translocation frequency suggesting that spatial proximity of potential translocation partners might significantly contribute to their likelihood of undergoing illegitimate rejoining once chromosome breaks have occurred [Roix et al., 2003]. Still, knowledge about underlying molecular mechanisms is lacking.

Chromatin Mobility

The mobility of genomic regions and nuclear proteins is likely to have a role in gene expression control (Fig. 2, representation 5). It is generally believed that restricted mobility is due to interactions of genes or gene loci with subnuclear structures. Several large components involved in transcription initiation, replication and repair diffuse rapidly inside the nucleus [Phair and Misteli, 2000]. Interestingly, also a protein known as a heterochromatin-associated component HP1, diffuses freely in both euchromatin and heterochromatin [Cheutin et al., 2003; Festenstein et al., 2003]. Larger nuclear structures, such as whole chromosomes and nuclear bodies, that is, Cajal bodies and PML bodies, have a restricted mobility in the nucleus. It is known that nuclear body mobility is partly dependent on a metabolic energy-dependent mechanism, that is, requirement of ATP and active transcription [Muratani et al., 2002; Platani et al., 2002]. At least two distinct types of chromatin mobility are described. One type of mobility encompasses very rapid, short-distance random movements (0.2–0.5 μm), although they are constrained to relatively small zones of the nucleus [Gasser, 2002]. Chubb et al. [2002] demonstrated that some genomic loci display a more constrained movement than others, depending on their location in the interphase nucleus. Loci located at the nucleoli or the nuclear periphery show significantly less mobility than loci that occur within the nucleoplasm. The second type of chromatin mobility that has been documented concerns long-range movement of a large chromatin domain [Zink et al., 1998; Edelmann et al., 2001]. This type of movement has a much lower diffusion constant and shows directionality. Belmont and colleagues showed that a lac operator-containing chromatin domain moved from the nuclear periphery to the nuclear interior and largely extended its configuration upon targeting of the transcription factor VP16 [Tumbar et al., 1999; Tumbar and Belmont, 2001]. In addition, progression through the cell cycle is known to be an important determinant of chromatin mobility [Csink and Henikoff, 1998]. It has been demonstrated that the early G_1 stage of the cell cycle represents a time window of increased chromatin mobility during which many aspects of nuclear architecture are established [Thomson et al., 2004].

Molecular mechanisms underlying active and constrained mobility of the genome are still unresolved. The mechanisms underlying an energy-dependent mobility are still largely unresolved. Concerning constrained mobility, we do not know whether the compaction state of a particular chromatin region defines its movement, or whether the tethering of a chromatin region to a nuclear compartment influences its mobility.

WHERE DO WE GO?

We can discriminate several levels of functional genome compartmentalization, that is, at the linear DNA level, at the nucleosomal level, at the large-scale chromatin level, and at the nuclear level. Several efforts have been taken to obtain information of a particular level of gene control and to develop a "blueprint" of such information, for example, the DNA sequence, the transcriptome map [Caron et al., 2001; Versteeg et al., 2003], a 3D map of the location of interphase chromosomes in the nucleus [Bolzer et al., 2005], or maps of histone modification pathways [Lachner et al., 2003]. However, we are now reaching the conclusion that these "blueprints" alone will not enable us to understand how different levels of gene control act together. A major challenge will be to integrate information obtained at different levels of functional genome compartmentalization to obtain knowledge of a more overall picture of gene control and genome organization.

To this end, we have to select a system that enables us (i) to portray several levels of gene control in a quantitative manner and (ii) to modulate and follow the transition of a functional situation (for example, transitions in chromatin compaction, in gene activity, in differentiation status etc.). We have used the

lac operator-lac repressor system to understand which mechanisms act together to induce transitions in large-scale chromatin compaction that are known to be linked to gene expression control [Verschure et al., 2005]. With this system we are currently studying the causal effects of various epigenetic regulatory proteins or enzymes when targeted to a defined chromosomal domain in the nucleus. There are several interacting pathways of epigenetic gene control (e.g., histone acetylation, histone methylation, DNA methylation) that determine transitions in chromatin compaction and thereby gene activity. By systematically analyzing the causal effects of targeting various regulatory proteins and/or enzymes on both transitions of chromatin compaction and on recruitment of proteins and enzymes involved in epigenetic control pathways, we aim to unravel the regulatory network (Fig. 3).

A next step is to use a system that provides the possibilities to study additional functional states, such as progression of cell differentiation or progression of disease. Differentiation is an intricate process of specialization where cells develop unique tissue-specific functions to become committed to particular cell lines. The sequential process of development is achieved through coordinated control of gene expression and nuclear chromatin remodeling. Therefore, differentiation is an ideal system to study the influence of genome organization on gene expression. For instance, in vitro differentiation systems using progenitor cells, such as embryonic or adult stem cells, when induced to specialize to particular lineages are ideal models to investigate chromatin organization and gene expression control. Also in vivo differentiation systems in sections of tissues and embryos represent ultimate model systems to understand the progression of overall functional genome organization.

Of course, there are several practical adventures to deal with. Within each cell type, changes in gene activity due to for instance differentiation stage or cell cycle status can

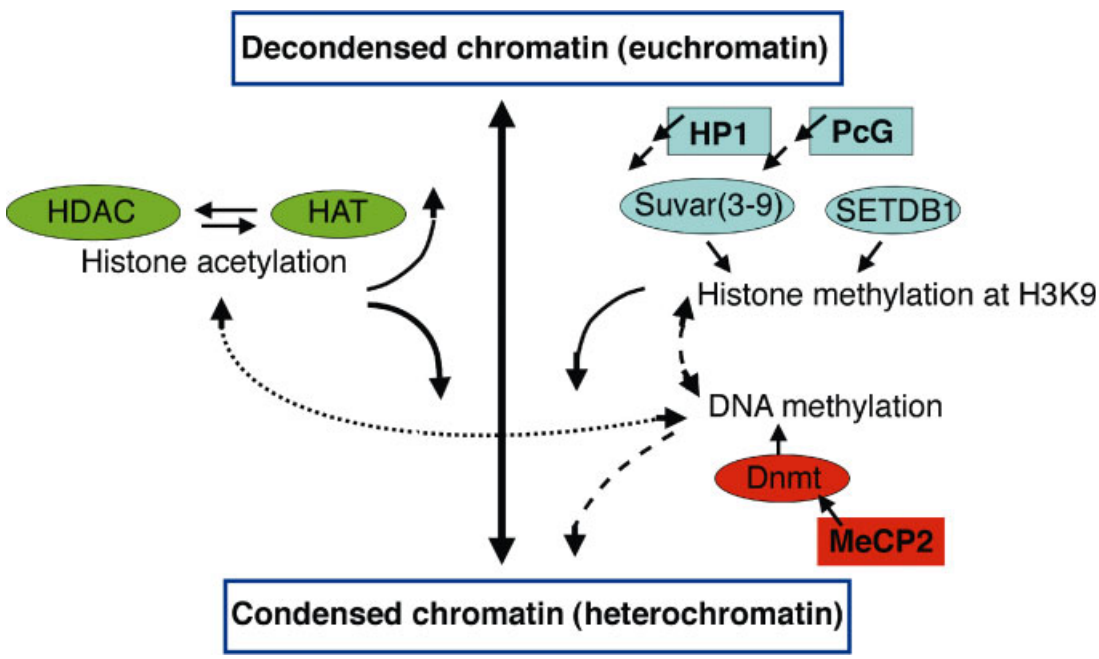

Fig. 3. The pathways involved: the causal effect of regulatory proteins on epigenetic gene control using a defined ''system.'' Using a defined system we analyzed the effect of targeting various regulatory proteins and/or enzymes on changes in chromatin compaction and changes in gene activity. Several pathways of epigenetic gene control act together (e.g., histone acetylation, histone methylation, DNA methylation). By systematically analyzing the causal effects of targeting various regulatory proteins and/or enzymes on both transitions of chromatin compaction and on recruitment of proteins and enzymes, we aim to unravel the regulatory network of interacting pathways. This schematic drawing represents a system of interacting pathways, showing in blue, red, and green some regulatory proteins and/or enzymes that control a particular control pathway (i.e., blue factors involved in histone H3K9 methylation, in green factors involved in histone acetylation and in red factors involved in DNA methylation). The network of interactions determines changes in functional chromatin compartmentalization. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

result in remarkable differences in nuclear architecture. Due to this large cell-to-cell variation, single cell analysis is indispensable. However, single cell analysis makes it extremely difficult to extrapolate conclusions drawn from any particular cell type to a general prediction. Another obstacle is the qualitative versus the quantitative impact of the gained data by studying a well-defined test system. A large part of the data obtained so far used light microscopy analyses, thereby providing a qualitative picture of a defined situation. Clearly, we have to design quantitative tools. When we are able to measure quantitative parameters, we can use such measurements to perform computational modeling to integrate various levels of functional genome organization.

Taken together, we have obtained a large body of information on individual "actors" and "scenes" in the nucleus regarding genome compartmentalization, but we still do not understand how and by what pieces of equipment the "actors" play their game. The next challenge is to understand the combined operation of the various levels of functional genome organization in the nucleus, that is, how do the epigenetic and genetic levels act together. We have to step away from our particular fragment of research on genome compartmentalization and try to understand the overall system.

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