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The Nucleus: A Black Box Being Opened

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Abstract Until recently our knowledge about the structural and functional organization of the cell nucleus was very limited. Recent technical developments in the field of ultrastructural analysis, combined with ongoing research on the properties of the nuclear matrix, give new insight into how the nucleus is structured. Two types of observations shape our ideas about nuclear organization. First, most nuclear functions (replication, transcription, RNA processing, and RNA transport) are highly localized within the nucleus, rather than diffusely distributed. Moreover, they are associated with the nuclear matrix. Second, chromatin is organized in discrete loops, bordered by nuclear matrix attachment sequences (MARs). Each loop may contain one or several genes. The arrangement of chromatin in loops has profound consequences for the regulation of gene expression.

Key words: nuclear matrix, MAR, chromatin loops, transcription, RNA processing, RNA transport

The cell nucleus combines several remarkable functions. First of all it organizes an immense amount of DNA in such a way that individual genes are readily accessible. Second, it harbours the complex machinery required for high fidelity replication of the genome and for selective and precisely controlled transcription, RNA processing, and RNA transport. Although most of us may intuitively feel that such intricate processes require a highly structured organelle, it is only recently that we are beginning to obtain insight into nuclear organization.

As we learn more about nuclear structure, we will inevitably discover important new control mechanisms related to gene expression and replication. An example is the discovery of a new type of cis-acting sequence elements, the MARs (matrix-associated regions), that define structural and functional chromatin domains. Another emerging view is that RNA synthesis, processing, and transport may be tightly coupled in time and space.

The nuclear matrix appears to play a central role in nuclear organization. This fibrous, scaffold type of structure has the same size and shape as the original nucleus and can be visualized after digestion and extraction of the chromatin. Replication, transcription, and RNA processing and transport are activities that are associated with the matrix. Despite its important function, our knowledge about this nuclear substructure is still very limited. Below we outline recent developments and ideas in the field of nuclear organization.

WHAT CAN BE VISUALIZED

Many Nuclear Components are Localized in Discrete Domains

Analysis of different tissues and cultured cells by immunofluorescense microscopy and immunogold electron microscopy has shown that the nucleoplasm is highly organized. Many nuclear components and activities are localized in discrete domains, rather than being diffusely distributed throughout the nucleus. Examples are (i) the localization in nucleoli of rRNA gene clusters and other components involved in ribosome biogenesis [1,2]; (ii) the presence of distinct clusters of replication sites during S-phase [3,4]; and (iii) the concentration in discrete nuclear domains of snRNPs and other factors involved in the processing of mRNA [5–7].

Transcription is also a highly localized nuclear process. Incorporation of [^{3}H]uridine in RNA, visualized by high resolution autoradiography, occurs mainly in the nucleolus, due to RNA polymerase I activity, and at the surface of

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euchromatin territories, due to RNA polymerases II and III activity [7,8]. Often these areas are in close contact with snRNP clusters [7]. Recent immunofluorescense studies, using a specific anti-RNA polymerase II antibody, support the notion that transcription is a highly localized nuclear process [D. Wansingk, unpublished]. Another approach to assessing the spatial distribution of active genes in the nucleus is by labeling by in situ nick-translation [9]. This method is based on the observation that active chromatin has an increased DNAse I sensitivity. After a brief digestion with DNAse I, the single-stranded gaps are filled by exogenous DNA polymerase, using biotinylated nucleotide analogues that can be visualized by specific binding of fluorescently labeled streptavidin. Labeling of discrete domains is observed [9,10]. These results indicate that domains exist in the nucleus in which active genes are clustered.

An intriguing, long-standing observation is that nuclei of many different cell types contain numerous, often electron dense, structures to which no functions have been ascribed so far [for a review see 11]. These so-called nuclear bodies do not seem to colocalize with any of the nuclear domains with known function. This suggests that additional nuclear substructures remain to be discovered. Recently, antibodies have become available against certain types of nuclear bodies [12–14; N. Stuurman, submitted] which will be helpful in resolving their function.

RNA Synthesis, Processing and Transport May Be Tightly Coupled Processes

A major activity of the nucleus is the production of specific mRNAs. Primary transcripts are processed to mature mRNAs, transported to the nuclear periphery, and exported to the cytoplasm via nucleopore complexes. RNA synthesis and the machinery for different steps in RNA processing are highly localized in the nucleus (see above). In addition, it has been shown by in situ hybridization that specific transcripts are concentrated in what seems to be tracks that run towards the nuclear periphery [15]. Evidently, RNA transcripts cannot move freely inside the nucleus. An attractive hypothesis is that mRNA synthesis, processing, and transport are activities that are tightly coupled in time and space. Thus, nascent RNA may be directly transferred from the place of synthesis to a nearby processing site (i.e., a cluster of snRNPs and other processing activities at the beginning of a transport track for RNA leading to a nucleopore complex). A word of caution here is appropriate. Several types of nuclear domains, like the snRNP clusters, are detected by immunological procedures. It still remains to be proven that RNA processing really takes place in these clusters. It cannot be excluded that we are looking at storage sites for snRNPs. A similar argument holds for the mRNA-containing tracks, for which it remains to be proven that they are related to RNA transport. In fact, our knowledge about the mechanism of RNA transport in the nucleus is still very limited. These questions can be answered by determining in same nucleus the position of a specific gene (i.e., the site of RNA synthesis), its primary transcript, and the processed mRNA product. Results will give detailed insight in the spatial organization of mRNA production.

Interestingly, the guided movement of transcripts in the nucleus agrees with the gene gating hypothesis proposed by Blobel [16]. In this theory it is assumed that each gene is connected to a particular nuclear pore complex, which translocates the mRNA to a particular sector of the cytoplasm where the gene product is required by the cell. An intriguing possibility that is raised by this hypothesis is that the putative RNA transport tracks in the nucleus are nonrandomly localized. If the localization of more mRNAs has been analyzed, we may be able to judge the validity of this hypothesis.

The Nuclear Matrix Forms a Structural Basis for Nuclear Compartmentation

What is the organization principle that determines the spatial distribution of nuclear domains with specific functions? An important finding is that domains are still present after most chromatin has been removed. Nuclear matrix preparations retain many of the nuclear domains mentioned in the previous sections. The spatial distribution in matrices is often similar to that in the intact nuclei. An example is the clustered arrangement of replication sites during S-phase of the cell cycle. The distribution in nuclei is the same as in nuclear matrix preparations [4]. In agreement with this observation it has been shown that DNA polymerase activity and nascent DNA are matrix-associated [17–20].

Importantly, nuclear matrices contain most of the activities required for mRNA synthesis (i.e., RNA polymerase II [21]) and splicing activ-

ity [22]. Matrices have been prepared that contain assembled spliceosomes and are capable to process exogenous substrates efficiently if provided with certain soluble protein factors [22]. In agreement with the idea that RNA processing occurs at the nuclear matrix, it has been shown that the nuclear matrix contains pre-mRNA in several stages of maturation [23]. Recently, Lawrence and coworkers [24] showed by in situ hybridization in a direct way that specific transcripts are tightly associated with the nuclear matrix. Finally, evidence is accumulating that transcription factors also co-isolate with the nuclear matrix. This has been shown extensively for steroid receptors [25] and more recently also for other proteins involved in regulation of transcription of specific genes [26]. These data are in excellent agreement with the observation that genes that are actively transcribed are associated with the nuclear matrix [26–29]. If a gene is inactivated, it is released from the matrix [27]. Together, these results strongly suggest that transcription complexes are tightly bound to nuclear matrix components. It will be important to unravel the molecular interactions that are involved.

In conclusion, most of the nuclear machinery required for replication, transcription, RNA processing, and RNA transport is localized in discrete domains in the nucleus, rather than being diffusely distributed throughout the nucleus. Since these domains appear to be associated with the nuclear matrix, it is likely that this scaffolding structure has a key function in nuclear compartmentation.

FUNCTIONAL INTERACTIONS BETWEEN CHROMATIN AND THE NUCLEAR MATRIX

MARs Define Individually Controlled Chromatin Domains

One important function of the nuclear matrix is organizing chromatin in loops. These loops seem to constitute discrete functional and structural chromatin units. Chromatin loops can be visualized by microscopic techniques on interphase nuclei [30,31] and metaphase chromosomes [32] after extraction of histones by treatment with, for instance, high ionic strength buffers. Loop sizes range from a few to several hundreds of kilobasepairs. The average length in animal cells is 80 to 90 Kbp [31,33,34].

Chromatin loops are at their bases firmly anchored to the nuclear matrix. This is concluded from the observation that the DNA in the loops is supercoiled [30,31]. DNA sequences have been identified that specifically interact with the nuclear matrix (reviewed by Gasser [35]). Recently, Strätling and coworkers [37] have isolated from chicken oviduct a 95 KDa matrix protein that has a high affinity for MAR DNA. MARs have been identified in many different cell types and organisms, including *Saccharomyces, Xenopus, Drosophila,* chicken, and mammals [35], and are probably also present in plants. Interactions between MARs and matrix are evolutionary strongly conserved, confirming the biological importance of chromatin loops. No striking sequence homologies have been found so far by comparing different MARs. They are all A+T rich, often contain several topoisomerase II consensus sequences and are located, at least in some cases, close to enhancer elements [38].

MARs have initially been defined as sequences that bind to the nuclear matrix under in vitro conditions. This has led to some scepticism about the physiological significance of MARs and chromatin loops. However, recent observations show that MARs play an important role in regulation of transcription. MARs have been found at the borders of chromatin domains that in some cases were identified on the basis of independent criteria (e.g., increased nuclease sensitivity). Examples are the chicken lysozyme domain [39], the human β-interferon domain [40], and the human apolipoprotein B domain [41]. In addition, MARs are located at the borders of certain gene clusters. Examples are the *Drosophila* histone gene cluster [42] and the human β-globin gene cluster [43] (the latter gene cluster also contains internal MARs). By constructing artificial chromatin domains, consisting of a reporter gene flanked by MAR sequences, several groups have shown that MARs dampen the well-documented variations in gene expression level due to position effects at the integration site in the genome, and increase transcription levels [36,44]. MARs exert these effects only after stable integration in the genome and not in transiently transfected cells [36,45]. Evidently, MARs shield genes from upstream and downstream cis-acting elements in the genome that affect transcription. Interestingly, like matrix binding, the shielding properties of MARs also appear to be evolutionary conserved [36,44]. Clearly, MARs combine important structural and functional properties.

The shielding effect of MARs is likely to be of key importance for a functional genome. MARs apparently define boundaries beyond which long

range cis-acting regulatory elements, like enhancers and silencers, cannot act. In this way MARs ensure the correct target specificity of long range regulatory elements. It is tempting to relate this shielding effect of MARs to their ability to bind to the nuclear matrix. This can be analyzed by dissecting MAR sequences and establishing which sequence elements are responsible for effects on gene expression and which are essential for matrix binding. Such studies are now becoming feasible. About the mechanism of gene shielding, we can only speculate. Conceivably, matrix attachment sites simply inhibit the transmission of superhelicity from one chromatin domain to another. Further investigations are important, because results will not only give information about the functional and structural properties of MARs, but they will also tell us about the mechanism of action of long range, cis-acting regulatory sequences.

Gene Activity May Be Controlled at Least at Two Levels

The picture that emerges is that of a nucleus in which chromatin is organized in individually controlled loop domains. Each domain may contain a single gene (e.g., the chicken lysozyme domain [39]) or several genes (e.g., the histone cluster of *Drosophila* [42]). The conformational state of the chromatin (e.g., supercoiling, compaction, accessibility for protein factors) is controlled for each loop individually. This implies that if several genes are located on one loop, these will be poised for transcription in a coordinated way. Actual transcriptional activity, however, is regulated on a second control level (i.e., the binding of transcription factors and the formation of an active transcription complex).

The mechanisms that control loop conformation have not been explored yet. The observation that all known MARs share topoisomerase II consensus sequences, combined with the fact that topoisomerase II is matrix-associated [46,47], suggests the involvement of this enzyme activity.

It is likely that additional, higher order control levels exist (i.e., acting on conglomerates of loops, rather than on a single loop). X-chromosome inactivation during early female development is an example. To assess such control levels requires insight in the packing of chromatin loops in the interphase chromosome structure. Several interesting models have been proposed [48,49]. Exploring such models in terms of higher order control mechanisms of gene expression may guide our thinking about these fascinating problems [50].

THE NUCLEAR MATRIX, FACTS AND ENIGMAS

The Nuclear Matrix is Still An Operationally Defined Structure

The nuclear matrix (see for a review [51,52]) has two major functions. First, it organizes chromatin in discrete loops. Second, it binds nuclear activities that are responsible for replication, transcription, and RNA processing and transport. Nuclear matrices are found throughout the eukaryotic world, ranging from yeast [53] to mammals [54] and plants [55,56]. Different isolation procedures have appeared in the literature. They have in common the solubilization of the nuclear membrane with detergents, digestion of chromatin with DNAse I or restriction nucleases, and extraction of chromatin fragments. The original procedure employs high salt concentrations to extract as many proteins as possible without destroying the matrix structure [54,57]. Penman and coworkers used buffers that appear to preserve the ultrastructure of the matrix as well as possible [58], whereas Laemmli and coworkers selected ionic conditions that result in optimal DNA binding properties of the matrix [42]. In addition, Jackson and Cook [21] developed a procedure that employs buffers with ionic conditions as close as possible to what is thought to be physiological.

All procedures yield similar nuclear matrix structures, which have the same size and shape as the original nucleus. Nuclear matrices consist of a nuclear lamina at the outside and an internal fibrogranular network. The lamina is a two-dimensional network of fibres of lamins (see [59] for a recent review). Lamins are proteins that belong to the family of the intermediate filaments. Evidence has been presented showing that the nuclear lamina and the cytoplasmic intermediate filament system form one continuous structure (see for a review [59,60]). Our knowledge about the components and structure of the internal, fibrogranular matrix structure is much more limited. No major structural components of this structure have been yet identified. A complication is that the internal nuclear matrix is much more labile than the nuclear lamina. Several nuclear matrix isolation procedures incorporate a special stabilization step [35]. Oxidation of SH-groups, resulting in interprotein S-S bridges, is effective. Many matrix

proteins form interprotein disulfide bridges after this treatment [57, N. Stuurman, submitted for publication]. Evidently, structural matrix proteins have juxtaposed SH-groups on their surface and crosslinking them stabilizes the internal matrix structure.

The Nuclear Matrix Has a Complex Protein Composition

The protein composition of the nuclear matrix is complex and distinct from that of chromatin and other cell fractions [61,62], indicating that we are dealing with a well-defined structure. Interestingly, a number of proteins are cell type specific, whereas another set is present in all cells of a given organism [62,63]. It has been claimed that matrix proteins may be good markers for cell and tissue typing, much better than proteins from other cell fractions [63]. The background of this observation may be that the machinery that is responsible for transcription is largely matrix-associated. This includes cell type-specific transcription factors [26], among them steroid receptors [25]. In fact, matrix preparations may turn out to be a rich source of proteins involved in the control of gene expression.

As might be expected, a more detailed analysis of nuclear matrix composition, structure, DNA binding properties, and associated enzyme activities reveals that matrices isolated by different protocols are not identical [64; N. Stuurman and B. Humbel, unpublished data]. This has resulted in considerable confusion in the field, but can be explained rather simply. It is likely that the nuclear matrix consists of two or more polymeric, fibrous structures, although a more complex type of organization cannot be excluded. One structure is the lamina, of which the major structural proteins are the lamins. The internal nuclear matrix may be formed by one or possibly more other polymeric systems. All isolation procedures obviously aim for integrity of these polymeric structures. However, accessory components, that interact with the basic skeleton structure, will be extracted to different extents, depending on the precise conditions. This once more stresses the importance of identifying the major structural components of the nuclear matrix.

Our lack of information about the molecular structure of the nuclear matrix hampers progress in understanding nuclear organization. Several approaches may be tried to solve this problem. Stripping matrix preparations of as many proteins and RNA components as possible without destroying the internal fibrous structure may be one way to narrow down on the basic structural components of the matrix. Setting up experiments assuming that some components of the matrix are self-polymerizing, as is found for cytoskeletal components, is another approach. Finally, one may look for acceptor proteins, that are part of the matrix and specifically bind matrix-associated components like RNA polymerase II, steroid receptors, or MARs. Which of these approaches will be most successful remains to be seen.

CONCLUSIONS

Understanding the spatial organization of chromatin and nuclear processes like replication, transcription, RNA processing, and RNA transport will reveal new cellular control mechanisms. These will be as important for the selective retrieval of genetic information as the well-known mechanisms involving promotors, enhancers, and transcription factors are. Evidently, we are only beginning to understand how the nucleus functions. Two notions are prominent. First, most nuclear processes are highly localized in the nucleus, rather than diffusely distributed, and are associated with a scaffolding structure, the nuclear matrix. Second, the organization of chromatin in discrete loops, bordered by nuclear matrix attachment sequences (MARs), has profound consequences for regulation of gene expression.

New technical developments that allow a precise analysis of the three-dimensional distribution of nuclear components and activities are combined with exquisitely sensitive in situ methods for detection of specific DNA and RNA sequences. The results are rapidly changing our view on how a nucleus is spatially organized. If we succeed in combining these ultrastructural techniques with biochemical and molecular genetic approaches, the black box that the nucleus has been for a long time may turn into a more transparent one.

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