Chromatin Higher Order Structure and Chromosomal Positioning in the Nucleus

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Abstract

While it has been known for some time that chromatin is highly packaged, it has only recently been discovered that chromatin is attached to the nuclear matrix. It has also been recently shown that interphase chromosomes remain separate from one another in so called, “chromosome domains.” This author suggests a functional relationship between chromatin attachment to the nuclear matrix and chromosomal domains in the interphase nucleus. Herein, the evidence for the presence of matrix attachment sites and chromosomal domains is presented, and it is proposed that chromatin attachment to the nuclear matrix provides the physical foundation for interphase chromosomes to be localized to chromosome domains.

Introduction

It has long been known, based on electron microscopy studies, that chromatin is packaged into nucleosomes (Olins and Olins, 1974), then into 10 nm fibers (Cameron et al., 1979), and then into 30 nm solenoids (Finch and Klug, 1976; Felsenfeld and McGhee, 1986). This knowledge raised, among other issues, the question of how chromatin gets packaged into metaphase chromosomes. Beyond the issue of chromatin packaging is the organization of the interphase nucleus and where chromatin, as linear uncondensed chromosomes, fits in. This question was first addressed by David Comings in 1968 when he described, “The Rationale for an Ordered Arrangement of Chromatin in the Interphase Nucleus” (Comings, 1968). In this paper, Comings described the evidence at that time for the non-random localization of chromatin in the nucleus. This includes some of the first evidence that DNA replication occurs, to a greater extent, at specific points on the nuclear membrane and along the edge of the nucleolus, as well as some suggestive observations that chromatin is ordered in a specific way in the nucleus. Since then we have learned a great deal about the internal structure of the nucleus and how it is compartmentalized. We now have evidence for rRNA processing in the nucleolus (Sheer and Benavente, 1990) and for RNA Polymerase II transcription at certain locations in the nucleus, but not the nucleolus (van Driel, 1995). It is also known that the nucleus has an internal skeleton, called the nuclear matrix, where the machinery for DNA replication and transcription is anchored (Nelson, 1986). The nuclear matrix also seems to be a site for chromatin attachment, which may provide a foundation for the higher order structure of the chromatin (Nelson, 1986; van Driel, 1995). This concept of nuclear compartmentalization alluded to the hypothesis that whole chromosomes or parts of chromosomes may occupy certain regions of the nucleus (Manuelidis, 1985, 1990).

The existence of chromatin loops and chromosome domains lend support to the contention that the nucleus is a highly structured organelle. The first level of chromatin organization is chromatin loops. The chromatin of uncondensed chromosomes in interphase nuclei is attached to the nuclear matrix at regular intervals making loops of 5 to 100 kb in length (Cook and Brazell, 1976; Paulson and Leammli, 1977). The second level of chromatin organization is the location of the uncondensed chromosomes in the nucleus. Chromosomes occupy unique domains in the nucleus such that individual chromosomes do not overlap (Manuelidis, 1985, 1990). This author would like to suggest that these two levels of organization are linked together, such that chromatin loops, which attach to the nuclear matrix provide a foundation for the localization of interphase chromosomes to unique domains.

Chromatin Loops and SARs/MARs

The chromatin of interphase nuclei is attached to the nuclear matrix at specific sites called matrix association regions (MARs) or scaffold attachment regions (SARs). These names refer to the same sequences that are associated with the nuclear matrix after high salt treatment or ionic detergent treatment (Razin and Gromova, 1995) and will be hereafter referred to as MARs only (see Fig. 1). The purpose of the high salt treatment is to remove the histones from chromatin, so that the DNA will be more accessible to nuclease treatment. Unfortunately, this preparation method leaves behind actively transcribing and replicating DNA as well as nuclear matrix-associated DNA sequences (Razin and Gromova, 1995). This makes it difficult to map specific sequences, but is sufficient for the initial visualization of the chromatin loops themselves (Paulson and Laemmli, 1977; Hancock and Hughes, 1982).

The presence of chromatin loops was also suggested based on ultra-centrifugation of high salt-extracted nuclei (Cook and Brazell, 1976). Based on those initial studies, a model was proposed where the chromatin loops are composed of the 30 nm solenoid, which is constricted between MARs (see Figure 2) (Marsden and
Laemmli, 1979). Firm evidence for the existence of these loops came from the identification of the MAR sequences. These sequences were identified using Exonuclease III (Exo III) sensitivity assays followed by restriction mapping to identify the location of fragments that are resistant to Exo III digestion. Blasquez et al. (1989) used this technique to identify MAR sequences in the mouse light chain immunoglobulin gene (see Figure 3). The numbered bands (1-11) in the matrix lanes indicate sequences that were protected from Exo III digestion, because they were bound to the nuclear matrix. It is important to note the periodicity of the attachment sites. It is this periodicity that leads to the loop structures. Other groups used similar approaches to determine the MAR sites within the Drosophila histone gene cluster (Gasser and Laemmli, 1986) and the Drosophila hsp70 gene (Mirkovitch et al., 1984). A fortuitous observation was that MARs often contain topoisomerase II binding sites (Cockerill and Garrard, 1986a). Razin et al. (1993) took advantage of the presence of topoisomerase II binding sites as a way to map MAR sequences in the chicken β-globin domain (see Figure 4 for a schematic of the procedure). These data showing MAR sequences in different genes of one organism and in genes from different organisms provide strong evidence for the evolutionary conservation of chromatin loops (see Cockerill and Garrard, 1986b for further discussion).

What is the significance of these loops? Do they have any function other than as a means of packaging DNA in the nucleus? The observation that the nuclear matrix and replication origins are bound to each other was first made almost thirty years ago by David Comings (1968) and was demonstrated more recently by Razin et al. (1986). These chromatin loops and their attachment to the nuclear matrix through MARs may be important for initiating replication or for facilitating the process of replication by bringing the DNA to the replication machinery. They may also be important for localizing certain sequences to specific compartments in the nucleus in order to silence them or bring them near the transcriptional machinery, but this is only speculation.
Evidence for DNA localization to certain domains for the purpose of silencing or activating stretches of the DNA is present more at the level of chromosome domains.

**Chromosome domains**

Uncondensed chromosomes in the interphase nucleus do not unravel and then randomly associate with each other, but remain segregated from each other in their own chromosomal domains (Manuelidis, 1990). The domain organization of interphase chromosomes has been observed in Drosophila (Mathog and Sedat, 1989), plants (Leitch et al., 1990), and mammals (Zink et al., 1998; Manuelidis and Borden, 1988). This demonstrates that these domains have been conserved throughout evolution in eukaryotic organisms. During metaphase, the condensed chromosomes adopt what has become known as the Rabl orientation, where the centromeres are collected at one side of the cell and the telomeres are at the other side (Rabl, 1885; Manuelidis and Borden, 1988). It has been suggested that during interphase, the uncondensed chromosomes continue to adopt this orientation leaving a gap in the middle of the nucleus for the nucleolus (Avivi and Feldman, 1980). Manuelidis and Borden (1988) proposed that this may not be a ubiquitous feature. Their studies of human central nervous system cells using biotin labeled probes, in situ hybridization, and three-dimensional reconstruction of nuclei indicate that some interphase chromosomes do not adopt this Rabl orientation. Regardless of their orientation in the nucleus, it is clear that interphase chromosomes remain separate entities and the domains they form remain exclusive of the nucleolus.

The most popular techniques for determining chromosomal domains are in situ hybridization with biotin labeled probes followed by electron microscopy, fluorescent in situ hybridization (FISH), pulse labeling of cells with BrdU (or similar analog) followed by confocal microscopy (see Figure 5), or injection of a fluorescent nucleotide analog (Cy3-AP3-dUTP) followed by video microscopy or confocal microscopy (Leitch et al., 1990; Sadoni et al., 1999; Zink et al., 1998). The in situ hybridization techniques are very powerful techniques for localizing DNA in the nucleus, but they require fixation of the cells at some point during the procedure, which could introduce artifacts. These artifacts could distort the actual chromosomal positioning in vivo. The technique developed by Zink et al. (1998) provides a much better approach, because labeled chromatin can be observed in live cells, which effectively removes the artifacts introduced from fixation. This technique provides the added benefit of video recording labeled chromatin in live nuclei to determine, in real time, if chromosome domains move through the nucleus or remain fixed in place. In fact, their results demonstrate that there is some shifting of chromatin within domains, but the domains themselves remain fairly stationary (see Figure 6). In contrast to this, Brown et al. (1999) and Bridger et al. (2000) demonstrate that when cells change from a proliferative state to a...
quiescent state, either certain sets of genes or whole chromosomes change their position in the nucleus. Brown et al. (1999) showed that a set of transcriptionally inactive genes associate with a centromeric heterochromatin region in the nucleus in dividing lymphocytes, but not in non-dividing lymphocytes. They also demonstrated that when non-dividing cells are stimulated to divide, there is a shift of this set of genes back to the centromeric heterochromatin. This demonstration of a shift in nuclear chromosome positioning is at the gene level; Bridger et al. (2000) demonstrated a similar phenomenon at the level of whole chromosomes. They looked at the positions of chromosomes 18 and 19 in proliferative and quiescent human fibroblasts. In proliferative fibroblasts, chromosome 18 is associated with the nuclear periphery, and chromosome 19 is associated with the nucleolus. As cells exit the cell cycle and enter a quiescent state after serum starvation, chromosomes 18 and 19 lose their associations with the nuclear periphery and the nucleolus, respectively. Surprisingly, when serum is added back followed by several passages, chromosomes 18 and 19 reestablish their associations with the nuclear periphery and nucleolus, respectively. This suggests that chromosomes may actively move around the nucleus, and that this movement has a functional relationship to the proliferative state of the cell.

Comings (1968) suggested that chromosomes may be localized to certain locations in the nucleus. There seems to be some evidence that heterochromatic regions of chromosomes are localized to the nuclear membrane or the outer periphery of the nucleolus while euchromatic regions are localized internally (Avivi and Feldman, 1980). The best example of a whole chromosome being localized to a reproducible location in the nucleus is the inactivated X chromosome in mammals. Dyer et al. (1989) did in situ hybridization studies of human female cell nuclei and showed that the inactive X chromosome almost always associates with the heterochromatic sex chromosome body (SCB). They also showed that in hybrids between human and mouse nuclei, this close association of the X chromosome with the SCB is lost. This seems to suggest a breakdown of the heterochromatic region in these hybrids. Sanchez et al. (1997) did localization studies of certain chromosomes, including the X and Y chromosomes, in human neutrophil nuclei. They determined that the X and Y chromosomes seemed to localize to protrusions of the neutrophil nucleus (see

Figure 3: Association of the X gene MAR with the nuclear matrix occurs through multiple specific binding sites. A 341-bp fragment encompassing the X gene MAR was 5’ end labeled, in either the upper or lower strand, opposite to a 3’ overhang. Exonuclease III digestion was performed after specific binding to plasmacytoma matrices (prepared using micrococcal nuclease instead of Dnase I digestion), and matrix-bound DNA was purified for electrophoretic separation on a sequencing gel. Naked DNA was similarly digested as a control. Barriers toward the 5’ side (1-5) and the 3’ side (6-11) are indicated. Four pairwise combinations of these barriers are shown, each of which leads to approximately 76 bp segments (Adapted from Blasquez et al., 1989).
Chromatin Higher Order Structure

Figure 7), while somatic chromosomes seem to localize in a more random pattern. This argues against a general theory of specific localization of chromosomes in the nucleus. An interesting observation made by Borden and Manuelidis (1988) was that in cells derived from human females with epilepsy, the inactive X chromosome loses its association with the heterochromatic region. Somatic chromosomes and the Y chromosome were also tested, and their heterochromatic regions remain associated with the periphery of the nucleolus or the nuclear membrane. This seems to suggest a connection between the disease state and the localization of certain chromosomes in the nucleus.

Certain domains within chromosomes seem to localize in a non-random way within the nucleus. Even if the whole chromosome is not specifically positioned, certain portions of it may be localized. Satellite sequences, which cluster in certain portions of the chromosome, including the centromere, non-randomly associate with certain portions of the nucleus. Manuelidis (1984) demonstrated this using biotin-labeled, centromere-specific satellite DNA that was hybridized in situ to fixed tissue from mouse cerebellum. She showed that individual neuronal cell types display reproducible patterns of centromere localization to the nuclear membrane or to areas adjacent to the nucleolus. However, no consistent pattern of centromere localization was seen in all of the cell types that were studied.

Ferreira et al. (1997) and Sadoni et al. (1999) did studies of chromosomal replication patterns in multiple cell types. They employed the use of BrdU replication-labeling alone, Cy3-dUTP replication-labeling alone, or a unique double labeling procedure where they pulse labeled during S phase with Cy3-dUTP and then labeled in the next S phase with BrdU. The double labeling procedure allowed them to distinguish between DNA
Figure 5: Metaphase spread with two differently sized (non-homologous), replication-labeled chromosomes. Note that only one of the two sister chromatids contains the label. Chromosomes were counterstained with DAPI (blue). Interphase nuclei in B counterstained with DAPI from the same slide as the metaphase shown in A depict replication-labeled and segregated chromatid territories. Small patches of replication-labeled chromatin (arrow) most likely result from sister chromatid exchanges (Adapted from Zink et al., 1998).

Figure 6: Time-lapse recording of one nucleus from the neuroblastoma cell line. Cy3-AP3-dUTP-microinjected cells were grown for several cell cycles. Each panel shows a projection of optical sections. 3D stacks were recorded every 20 minutes for 5 hours. The nucleus performed rotational and translational movements during the entire period. The framed segregated territory is clearly separated from the rest of the labeled territories. After a major initial rearrangement (indicated by large arrowheads), this territory maintained its overall shape. The inset (upper right) within the first six panels shows an enlargement (factor 2.3) of subdomains (arrow). The subdomains display changing patterns of foldings and extensions (some examples are indicated by arrowheads) (Adapted from Zink et al., 1998).
Figure 7: Deconvolved volumetric views of neutrophil nuclei from healthy females (top two panel pairs) and males (bottom two panel pairs). The leftmost panel of each corresponds to a volumetric view of a nucleus stained for total DNA with DAPI (negative image). The rightmost panel of each pair corresponds to a volumetric view of a nucleus hybridized for the X chromosome and the Y chromosome centromere sequences. In these panels, the outline of the nuclei is indicated by a white dotted line and the asterisks mark the position of the sex-specific appendages. Notice the presence of an X chromosome in the appendage of the female nucleus, a Y chromosome in the appendage of the male nucleus, and the absence of chromosome pairing when the sex chromosomes reside in the same lobe. Bar represents 5 μm (Adapted from Sanchez et al., 1997).

Labeled at a first S phase and DNA labeled at the next S phase. Using these labeling techniques, Ferreira et al. (1997) demonstrated that early replicating portions of chromosomes (R bands that are identified by a light Giemsa staining on chromosomes) preferentially localize to interior regions of the nucleus, while late replicating portions (G/Q bands that are identified as dark Giemsa staining on chromosomes) preferentially localize to peripheral regions. Sadoni et al. (1999) verified these results (see Figure 8) and further showed, using their double labeling technique, that S phase labeling patterns are maintained from division to division. So, not only are regions of chromosomes that replicate at different times in S phase localized to different portions of the nucleus, but that pattern is maintained in a cell type-specific manner with every cell division. These results provide a potential structural explanation for the tissue-specific expression patterns of different genes. It is noteworthy that housekeeping genes are mostly localized in R bands, whereas tissue specific genes are almost exclusively located in G/Q bands (Craig and Bickmore, 1993, 1994).

Conclusion

Two levels of chromatin structure have been presented, that of chromatin loops and chromosome domains. Marsden and Laemmli (1979) proposed that the 30 nm solenoid chromatin filaments were attached at regular intervals to the nuclear matrix forming chromatin loops. The sequences that mediate the attachment of the DNA to the nuclear matrix (MARs) have been extensively studied (Blasquez et al., 1989; Gasser and Laemmli, 1986; Mirkovitch et al., 1984; Razin et al., 1993). The attachment of chromatin to the nuclear matrix has also been
Figure 8: S phase replication labeling patterns are preserved. Cells ([a and b] Hv, human diploid fibroblasts; [c and d] SH-EP N14 human neuroblastoma cells; [e and f] HeLa cells; [g and h] CHO cells; and [i and j] C2C12 mouse myoblasts) were replication-labeled with BrdU ([a-d, i, and j; 30-min pulses) or Cy3-dUTP (e-h). Cells were fixed immediately after BrdU labeling or 30 minutes after microinjection of Cy3-dUTP to obtain labeled S phase cells (left, a,c,e,g, and i). S phase cells display the typical replication labeling patterns (indicated on the left): (a) type I, (c) type II, (e) type III, (g) type IV, and (i) type V. Similarly labeled cells were grown for one to five hours after labeling and fixed after this growth period (right, b,d,f,h, and j). The presence of similar patterns (types indicated on the right) several days after S phase labeling suggests that DNA replicating at a particular time during S phase always occupies similar nuclear positions. Arrowheads mark the nucleoli that are always unlabeled. Arrowheads in c-e mark perinucleolar label. Arrows in b and d indicate unlabeled chromosome territories. The presence of unlabeled territories demonstrates that cells do not display similar patterns because they stopped cycling, but rather that they went through at least two mitoses after labeling. Note also the two very close nuclei in f displaying similar type III patterns, suggesting that similar patterns were conserved in sister nuclei after cell division. All pictures display single light optical sections through midnuclear planes except i and j, which show epifluorescence images. Bar in a is similar for all images (Adapted from Sadoni et al., 1999).
implicated in the process of replication initiation (Razin et al., 1986). The suggestion that interphase chromosomes are specifically positioned in the nucleus was first made by Comings in 1968. It has since been shown that chromosomes occupy specific domains in the nucleus and do not overlap in the interphase nucleus (Manuelidis, 1990; Leitch et al., 1990). There is also a great deal of evidence to suggest that certain portions of chromosomes are non-randomly arranged in the nucleus (Manuelidis, 1984; Ferreira et al., 1997; Sadoni et al., 1999).

How do these two levels of chromatin organization connect with one another? What is their functional connection, if any? This author believes that the chromatin attachments to the nuclear matrix in the form of chromatin loops may provide the "foundation" for the localization of, at least, specific domains of chromosomes if not whole chromosomes. Some of the most striking evidence for a structural basis to this model comes from the work of Ma et al. (1999). When they did a classical extraction for nuclear matrix in the absence of DNase I followed by FISH, they showed that chromosome domains remain intact. In contrast to this, when they did a complete matrix extraction by doing RNase A treatment, to remove interactions with RNAs or RNPs, followed by 2.0 M NaCl, the chromosome domains are disrupted. This suggests that chromosome domains are attached to the nuclear matrix most likely through the attachment of chromatin loops to the matrix. This model is also functionally supported by the observation that chromatin is attached to the nuclear envelope at the site of replication origins (Razin, 1986), which would bring these regions of chromosomes near the nuclear pore complexes. The proximity of the DNA and replication complexes near the nuclear pore complexes means that these complexes have ready access to nucleotide precursors and other cytoplasmic factors. Razin and Gromova’s (1995) “channels model” of nuclear matrix structure incorporates some of this idea. Their model postulates that the nuclear matrix forms channels near the nuclear pore complexes. These channels have large lateral extensions coming off of the main channel. The chromatin loops are then attached to the matrix along these channels. The model provides a good explanation for the differential sensitivity of loop attachment sites to nuclease treatment after the different extraction methods. It also supports the observation that newly synthesized mRNAs seem to track toward the nuclear pore complex and presumably out of the nucleus (Lawrence et al., 1989; Kramer et al., 1994). These nuclear matrix channels may be the means by which mRNAs exit the nucleus; this remains to be seen. These channels may exist within chromosome territories or, as Cremer et al. (1993) suggest, they may exist between chromosome territories. They suggested that the interchromosomal spaces may provide channels for newly synthesized mRNAs and other precursor molecules to move in and out of the nucleus. Both of these models provide a framework under which the two levels of chromatin organization can be understood. Undoubtedly, more work needs to be done in order to fully understand the functional organization of the nucleus and the functional relationships between chromatin loops, chromosome territories, and the replication and transcription machinery.

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References


