

Review

Dynamic View of the Nuclear Matrix

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The nuclear matrix is an operationally defined nuclear skeletal structure that is believed to be involved in many nuclear functions including DNA replication, transcription, repair, and pre-mRNA processing/transport. Until relatively recently, the nuclear matrix was thought to be a rigid and static structure, but it is now thought to be dynamic. This paradigm shift was based in part on the tracking of the intranuclear movement of proteins tagged with fluorochromes. In this review, we attempt to redefine the nuclear matrix in light of recent findings and describe some useful techniques for the dynamic analysis of nuclear function.

Key words: nuclear matrix, MAR, chromatin, histone modification, topoisomerase

Not everything that changes continuously is alive, but all living things continue to change until they die. Naturally, therefore, we cannot truly understand life without considering the dimension of time. Changes in the position of objects can be easily traced by light-mediated observations. More than just movement can be monitored, however. Methods based on electromagnetic or sonic waves have been used to analyze molecular interactions, and may one day be used to monitor every event occurring in living organisms. At present, however, real-time analysis is not always possible. Instead, one must reconstitute a dynamic picture, just as in stop motion animation, from the data collected at multiple time points. This is a laborious method for making a high time-resolution picture, but it is essential for a crucial understanding of living processes.

The nuclear matrix is a dynamic nuclear compartment

Presence of some substrate or skeletal structure inside the cell nucleus had long been postulated without solid evidence. In 1974, Berezney and Coffey biochemically isolated a nuclear fraction having the appearance of such a structure and named it the *nuclear matrix* [1]. In addition to the nuclear matrix, other similar structures have since been isolated using different procedures and named the *nuclear scaffold* [2], *nucleoskeleton* [3], *karyoskeleton* [4], and *nuclear endoskeleton* [5]. There have been critical discussions, however, as to whether the nuclear matrix is a physiological entity [6]. The most serious argument is that the nuclear matrix may contain an artifactual precipitation of nuclear proteins caused by unphysiological conditions applied to nuclei [7].

The nuclear space demarcated by nuclear lamina can be divided into 2 compartments, the *chromosome territory* and the remaining space, or *interchromatin domain* (ICD) [8]. The chromosome territory is either highly conden-

sed (heterochromatin) or largely dispersed (euchromatin). Heterochromatin is usually found in the peripheral area of the nucleus and transcriptionally inactive, whereas actively transcribed genes are located in the euchromatic region. In a modern interpretation, the ICD is an equivalent of the nuclear matrix when observed in unfractionated nuclei or in living cells (Fig. 1). Biochemical purification of chromatin from isolated nuclei removes most of the proteins contained in the ICD compartment. In contrast, the preparative procedure of the nuclear matrix involves nuclease and high salt treatments that remove chromatin and other soluble nuclear proteins [9]. An important fact worth mentioning here is that even proteins soluble in nature become insoluble to varying degrees by the factors applied to nuclei during the matrix preparation, resulting in the presence of proteins in the nuclear matrix preparations. These factors include mild heat (37–42 °C) [2, 10], Cu^{2+} [2, 11], sulfhydryl cross-linker [12], and highly concentrated monovalent salts such as 2 M NaCl [13], which otherwise seems unlikely to induce protein precipitation. Similarly, salting-out effects may not be ignored with 0.25 M ammonium sulfate, which is used frequently to extract chromatin [14]. Thus, it is not surprising that the use of different preparation procedures results in nuclear matrixes of different compositions. In an over simplified view, all the nuclear proteins other than those associated with chromatin can be considered nuclear matrix proteins. Copurification of a certain protein with the nuclear matrix may not be significant in itself, unless accompanied by other findings. Whether or not the nuclear matrix contains a stable filamentous structure,

such as a cytoskeleton, remains to be established (however, see [15, 16]). It is tempting to assume a network-like structure that fills the entire nucleus and serves as a scaffold for the functional assembly of proteins.

The nuclear compartment corresponding to the nuclear matrix is inevitably a crowded space (Fig. 1) in which RNA is transcribed, spliced, and transported into the cytoplasm, and DNA is replicated and repaired. It is also a passenger concourse for proteins going in and out of the nucleus through the nuclear pore complex. In addition, this compartment contains so-called *nuclear bodies*, such as promyelocytic leukemia (PML) bodies, Cajal bodies, nuclear speckles, and transient foci or factories in that functional proteins become assembled depending on cellular physiological states, e.g., growth, differentiation, or stress [17, 18]. This view is consistent with a number of previous reports showing plausible connections between the nuclear matrix and multiple nuclear functions [9].

It is already common knowledge that macromolecules such as protein or RNA can move around within the nuclear space by simple thermal diffusion at an unexpectedly high rate [19]. The time required for traveling from the center to the periphery of a nucleus is several seconds for an average-sized monomeric protein and only several minutes even for a large complex such as a spliceosome or ribosome. Although molecules interacting with chromatin move more slowly, the nuclear lamina and core histones are the only nuclear structures that are known to be almost immobile. A logical consequence here is that most of the proteins in the matrix compartment are not

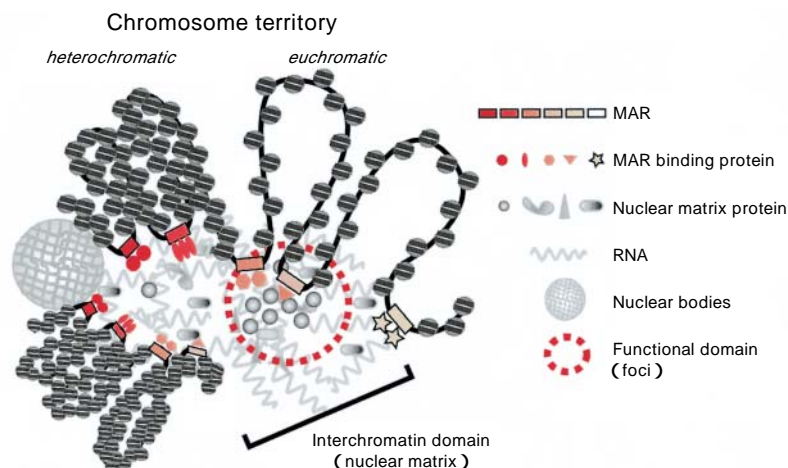


Fig. 1 Schematic representation of nuclear compartments.

firmly restrained. DNA topoisomerase II [20], a protein once believed to be a structural component of the nuclear matrix [21], is no exception, as it has been shown recently by fluorescence recovery after photobleaching (FRAP) of green fluorescent protein (GFP)-fused topoisomerase II [22]. The 2 isoforms of vertebrate topoisomerase II, α and β , both showed a relatively high diffusion rate in interphase nuclei, and a rapid exchange was observed between the molecules localized in the nucleolus and nucleoplasm. As reported previously [23], only topoisomerase II α was associated with metaphase chromosomes, but it showed a rapid movement, as in interphase, distributing over the entire chromosome without localization to the chromosome axial region where the *chromosome scaffold* is located. These results are not compatible with the previous model in which topoisomerase II, as a stable resident of the nuclear skeletal structure, binds to the scaffold attachment region (SAR) or matrix attachment region (MAR) to organize nuclear DNA into looped chromatin domains [24]. Furthermore, *in vivo* observation of the cells containing an exogenous DHFR gene attached to a tandem repeat of the lac operator sequence (used for visualization by a fluorochrome-labeled repressor) flanked by 2 MARs showed that the distribution of MARs in the metaphase chromosome was surprisingly homogeneous [25], suggesting that the chromatid is formed through hierarchical folding of subunit fibers. Therefore, the situation is more complex than in the *radial loop model* [26], in which chromatin loops stick out from the chromosome axis where SARs are clustered. A simplified scheme for these models is shown in Fig. 2.

MAR as a localization signal

If the concept of a *nuclear compartment* is to be substituted for that of a *nuclear skeleton*, then the statement "MARs mediate the binding of chromatin loops with the nuclear matrix" would be better expressed as "MARs and associated proteins retain the loop base in the matrix compartment". Since association between MARs and MAR-binding proteins is a reversible reaction, MARs are assumed to shuttle between the chromatin compartment and the matrix compartment. Thus, MARs can serve as a signal for partitioning a particular segment of chromatin between these compartments.

MARs are generally AT-rich noncoding sequences longer than 300 bp [27]. Tens of thousands of sequences

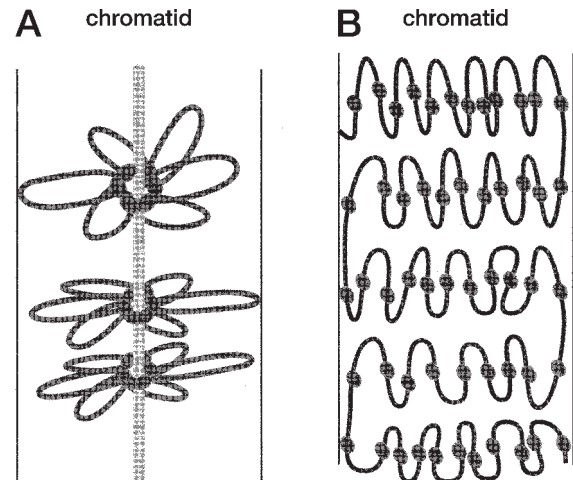


Fig. 2 Models for the structural organization of chromatid. A, Simple radial loop model. B, Hierarchical folding model. Filled circles show MARs.

per genome, without sequence similarities, are estimated to operate as MARs. In terms of sequences *per se*, MARs are not conserved between species, but their genomic locations with respect to genes are relatively well conserved [28]. Varieties of proteins are known to bind MARs. MAR-binding proteins have been identified by competitive binding between labeled MAR probes and unlabeled nonspecific DNA such as *E. coli* DNA. These proteins usually show less affinity to DNA (MAR) than do DNA-binding proteins with high sequence specificity [29]. It also appears that highly conserved orthologues are not present in lower eukaryotes. The most characteristic feature of the interaction between MARs and their binding proteins is that the low affinity of individual proteins is greatly enhanced through the cooperative interaction between the proteins bound to multiple sites within MARs. This effect is also called *mass binding* [30], and it plays a fundamental role in the formation of nuclear architectural components, such as chromosome bands and epigenetic-silencing domains.

MAR-binding proteins in gene regulation

MAR-binding proteins, when complexed with MARs, may recruit and retain the flanking DNA segments in the matrix compartment. They can also interact with other proteins to form larger complexes, serving as a nucleation center for the formation of specific functional domains (Fig. 1). We describe here 2 examples of gene regulation

in which the interplay between MARs and their binding proteins appears to be important.

SAF-A/SP120/hnRNP U, first recognized as one of the components of the heterogeneous nuclear RNA-protein (hnRNP) complex [31], was later shown to bind with MARs selectively [32, 33]. It is a multifunctional protein with a MAR-binding region at the N-terminus and an RNA-binding region enriched with Arg and Gly (RGG box) at the C-terminus (Fig. 3). It binds cooperatively with typical AT-rich MARs by recognizing the minor groove of AT tracts, which is narrower than average B form DNA [34]. A sequence motif consisting of 31 residues with a characteristic Leu repeat in the MAR-binding domain of SAF-A/SP120 is called a *SAF box* [35]. A similar region known as the *SAP domain* has been identified in several nuclear proteins (SAP stands for SAF-A, acinus, and PIAS1) [36]. Under forced expression, SAF-A/SP120 colocalizes with glucocorticoid receptor in the nucleus and suppresses the dexamethasone-dependent expression of a reporter gene [37]. SAF-A/SP120 associated with the MAR of [the] transcriptionally inactive topoisomerase I gene was shown to have a physical contact with the coactivator/acetyltransferase p300, and this contact is probably responsible for the observed hyperacetylation of histone H3 adjacent to the MAR [38]. When active transcription begins, the histone acetylation propagates to other regions of the topoisomerase I gene, implying that the initial acetylation around the MAR may be a preparatory step for the transcriptional initiation. An unexpected finding is that SAF-A/SP120 is concentrated on the inactivated X chromosome [39], which is covered with a noncoding RNA transcribed from the *XIST* gene locus on the same chromosome [40]. The RNA-binding domain (RGG box) of SAF-A/SP120 was shown to be involved in the binding with *XIST* RNA and required for this pattern of localization. The C-terminal region contain-

ing the RGG box is also essential for the binding to glucocorticoid receptor and p300. SAF-A/SP120 probably has multiple competitive binding partners, including undiscovered ones, and thus performs a variety of regulatory functions by recruiting MARs to different subregions of the matrix compartment.

While SAF-A/SP120 is a ubiquitous protein expressed in most tissues, SATB1 is a typical tissue-specific MAR-binding protein mainly expressed in the thymus [41]. The nucleotide sequence recognized by SATB1 is also enriched with A and T, GC-skewed (1 strand contains C but not G), and easily dissociated into single strands [42]. In mice with a disrupted SATB1 gene, the temporal and spatial expression patterns of many genes were found to be abnormal [43]. Analysis of the *IL-2R α* gene, which is ectopically transcribed in SATB1-deficient mice, revealed that, in normal mice, SATB1 bound to its binding sequence in the *IL-2R α* gene locus represses the transcription of the *IL-2R α* gene by recruiting the histone deacetylase (HDAC1) in a chromatin remodeling complex called NURD, and thereby induces a hypoacetylated state over a large genomic region [44]. In another gene locus, about 10kb of the genomic region flanking a SATB1-binding site shows the histone code pattern associated with active transcription (acetylated histone H3 K9/K14 and methylated histone H3 K4), whereas in SATB1-knockout mice, K9 of histone H3 is methylated, indicating that the region is under a repressed state of transcription [45]. Thus, SATB1 appears to control the chromatin structure of considerably large genomic regions by acting as a platform to which different remodeling complexes that either activate or suppress gene expression are recruited.

Quantitative determination of the partition ratio of genomic segments between matrix and chromatin compartments

After extraction of chromatin proteins with a high concentration of a salt (such as 2 M NaCl), the nuclei adopt a so-called *nuclear halo* configuration, in which nuclear DNA sticks out from the residual structure (nuclear matrix) in the form of negatively supercoiled loops (Fig. 4A). In this form, nuclear DNA can be fractionated into matrix-associated DNA and loop DNA by treating the halo with restriction enzymes. The conventional procedure for identifying MARs has been Southern blotting after gel-separation of these DNA



Fig. 3 Domain structure of SAF-A/SP120/hnRNP U. Domains designated: LR, Leu repeat with MAR-binding activity; EDA, Glu-Asp-Ala rich; NBF, nucleotide binding fold (putative ATPase domain); RGG, Arg-Gly rich domain with RNA-binding activity. The positions of the nuclear localization signal (NLS) and caspase-target sites (arrowheads) are also indicated.

fractions for comparing the relative hybridization signal intensities between these fractions, using radiolabeled probes complementary to the genomic region of interest. Once the genomic DNA sequence has been determined, it should be possible, using real-time PCR technology, to quickly obtain more quantitative results on any genomic portions using smaller amounts of sample.

In a typical scheme employing this approach, an optimal restriction enzyme is first selected by considering the genomic range to be analyzed, and then primer pairs are set between the restriction sites (Fig. 4B). The partition ratio R_M/R_L can be calculated from the initial target concentrations in the matrix DNA and loop DNA fractions that are determined by quantitative PCR (qPCR). When this type of analysis is performed at multiple time points in the cells under various conditions, such as cell division or differentiation, a dynamic picture of the association between a particular genomic region and the matrix compartment will emerge. We have used this procedure together with other qPCR-based methods for probing the higher-order structure of chromatin to analyze the role of topoisomerase II β in the transcriptional induction of differentiation-related genes [46]. We found a topoisomerase II β -dependent enrichment of the genomic segment containing the induced genes in the matrix compartment (unpublished results).

The higher partition ratio R_M/R_L denotes the higher affinity of the genomic segment in question towards the matrix compartment. More quantitative analysis becomes possible when a simple binding equilibrium is assumed between free MAR (M), its binding protein (B), and their complex (MB) (see equation 1 in Fig. 4 C). The ratio R_M/R_L is equal to the product of the binding constant K and the concentration of the free binding protein [B] under these conditions (equation 4 in Fig. 4 C). This relationship appears to roughly hold if 10^5 - 10^6 molecules of the MAR-binding protein are present per nucleus and the mass binding effect is operating. Temporal changes in the partition ratio are caused either by cooperative recruitment of MAR-binding proteins at the locus or by some structural modifications occurring on MAR or on the protein.

Detection of physical interaction between distant chromosomal loci

MARs at the base of chromatin loops can be placed close to each other (Fig. 1). For quantitative estimation

of the physical distance between remote sites on the primary sequence, a subtle method called *chromosome conformation capture* (3 C) was developed [47]. Adjacent chromatin regions in isolated nuclei are first cross-linked with formaldehyde through proteins in close contact (Fig. 5A). Nuclei are then digested with appropriate restriction enzymes followed by partial solubilization of chromatin fragments and dispersion by dilution. After ligation of DNA fragments held in proximity (equivalent to intramolecular ligation reaction), cross-links are reversed by heat treatment, and cross-linking frequencies between different sites are deduced from the amplification efficiency of qPCR with various combinations of primers on the genomic region to be analyzed.

Dekker *et al.* used *S. cerevisiae* as a model organism and applied this method to detect the disappearance of centromere clustering in meiosis and the differential interaction between homologous and nonhomologous chromosomes, demonstrating that 3 C can be used for quantitative analysis of nuclear dynamics that is well reflected by cytological observations. They set PCR primers at 13 sites on chromosome III (about 320kb in length, shown by a simplified scheme in Fig. 5B) of haploid cells arrested at G1 phase and performed PCR with every primer combination (78 pairs) to generate a matrix of cross-linking frequency between the sites (Fig. 5 C). The matrix was then transformed into a matrix of spatial distance between the sites, and finally, a three dimensional model of chromosome III could be constructed (Fig. 5D). Although the resulting image represents an averaged structure in time, it clearly shows the skewed appearance of the chromosome, with telomeres at the ends being positioned close to each other. In addition, when parameters were calculated separately for subregions of the chromosome (they used a mathematical model in which chromosomes were simulated by elastic sticks), the relatively AT-rich central portion was shown to be straighter than the outer portions that are rich in G and C.

The following study utilized the 3 C method effectively. On the 87A7 heat shock locus of *Drosophila melanogaster*, 2 *hsp70* genes are placed head-to-head and are embraced by boundary DNA elements, *scs* and *scs'*, about 15kb apart from the *hsp70* genes [48]. The 2 elements have been shown to bind to specific proteins, Zw5 to *scs* and BEAF to *scs'* [49, 50]. Chromatin immunoprecipitation with antibody against Zw5 showed that not only the *scs* but also the *scs'* sequence was

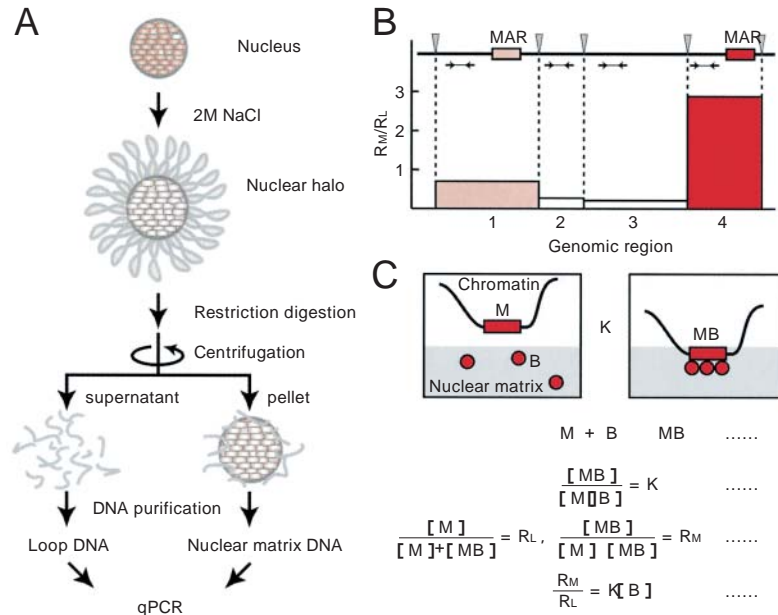


Fig. 4 Interaction between MARs and the matrix compartment. **A**, Outline of the experimental procedure for separating nuclear DNA into matrix-associated and loop DNA fractions. **B**, A typical result. Restriction sites and PCR primers are depicted by arrowheads and paired arrows, respectively. R_M/R_L represents the partition ratio of the PCR targets between the matrix (M) and loop (L) DNA fractions. R_M/R_L is equivalent to the product of the following: C_M/C_L , the ratio of copy numbers in the matrix and loop DNA when the same amounts of these fractions were used for amplification; F_M/F_L , the ratio of DNA yields in these fractions. **C**, An interpretation of R_M/R_L . When a binding equilibrium is postulated between MARs and MAR-binding proteins (1), the equation of equilibrium (2) and the relationship shown in (3) give the relationship between R_M/R_L and the binding constant K (4).

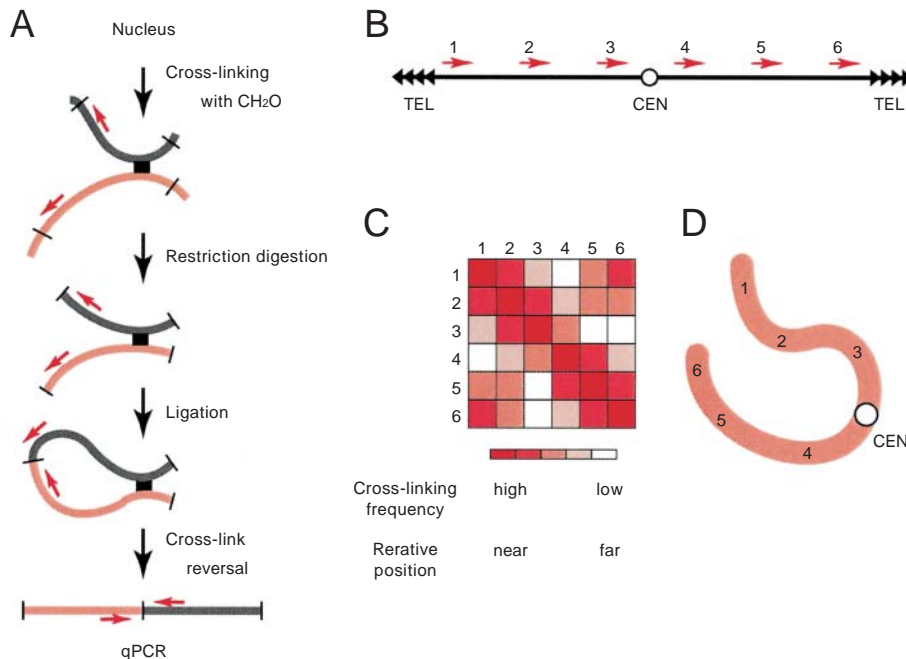


Fig. 5 Probing the interaction of distant sites on chromosome (3C). **A**, Outline of the experimental procedure. **B**, An example with yeast chromosome. PCR primer sites (shown by arrows) were designed considering the positions relative to restriction sites (not shown). **C**, Matrix of cross-linking frequency derived from the amplification efficiency on qPCR. These frequencies can be translated into spatial distances by using a mechanical model of DNA. **D**, A 3D model of the chromosome generated after further mathematical processing.

detectable in the immunoprecipitate, suggesting that the Zw5 and BEAF bound to these elements were associated with each other to make the DNA region between the sites a looped-out chromatin. Since this result alone does not rule out the possible contribution of the Zw5-BEAF complex unbound to *scs*, the 3 C technique was used here and the results were consistent with the looping model [51]. There should be many loop structures in the nucleus that are formed on the same principle. Therefore, this technique would be useful in various applications, such as for clarifying the molecular mechanisms of insulators, enhancers, and MARs.

Conclusions

As described in this review, real-time *in vivo* observation of nuclei clearly suggests that the nuclear matrix, as a biochemical entity, resembles a dynamic nuclear compartment. Recently, it seems a general tendency among researchers in this field to avoid the term *nuclear matrix*. This is unfortunate, because the word “matrix” itself is perfectly acceptable in this case, which is compatible with its original meaning, “something within which something else originates, develops, or takes form”. We do not think the term should be abandoned, but rather that other terms such as *nucleoskeleton* or *nuclear scaffold* that evoke a more static image may be discouraged unless an interchromatin component as immobile as nuclear lamina is identified *in vivo*. Similarly, the words *attachment* or *tethering* may not be particularly suitable for describing the connection between nuclear DNA and the matrix, and MAR might come to be considered an acronym for *matrix-addressed region* rather than *matrix attachment region*. It is our hope that more dynamic interpretation of the nuclear matrix reloaded with new ideas will soon gain universal acceptance.

As the sequence information becomes available, it will be possible to analyze the temporal changes in the local chromatin structure of any genomic region by using qPCR. Higher throughput analysis is feasible by substituting DNA microarrays for qPCR analysis. Real-time visualization techniques for living cells are increasingly being applied to molecular interactions and visualization of a single molecule. Clearly, the world of nuclear dynamics is expanding rapidly.

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References

1. Berezney R and Coffey DS: Identification of a nuclear protein matrix. *Biochem Biophys Res Commun* (1974) 60: 1410–1417.
2. Mirkovitch J, Mirault ME and Laemmli UK: Organization of the higher-order chromatin loop: Specific DNA attachment sites on nuclear scaffold. *Cell* (1984) 39: 223–232.
3. Jackson DA, Dickinson P and Cook PR: Attachment of DNA to the nucleoskeleton of hela cells examined using physiological conditions. *Nucleic Acids Res* (1990) 18: 4385–4393.
4. Fisher PA: Chromosomes and chromatin structure: The extra-chromosomal karyoskeleton. *Curr Opin Cell Biol* (1989) 1: 447–453.
5. Wasser M and Chia W: The EAST protein of *Drosophila* controls an expandable nuclear endoskeleton. *Nat Cell Biol* (2000) 2: 268–275.
6. Pederson T: Thinking about a nuclear matrix. *J Mol Biol* (1998) 277: 147–159.
7. Hancock R: A new look at the nuclear matrix. *Chromosoma* (2000) 109: 219–225.
8. Cremer T, Kreth G, Koester H, Fink RH, Heintzmann R, Cremer M, Solovei I, Zink D and Cremer C: Chromosome territories, inter-chromatin domain compartment, and nuclear matrix: An integrated view of the functional nuclear architecture. *Crit Rev Eukaryot Gene Expr* (2000) 10: 179–212.
9. Nickerson J: Experimental observations of a nuclear matrix. *J Cell Sci* (2001) 114: 463–474.
10. Martelli AM, Falcieri E, Gobbi P, Manzoli L, Gilmour RS and Cocco L: Heat-induced stabilization of the nuclear matrix: A morphological and biochemical analysis in murine erythroleukemia cells. *Exp Cell Res* (1991) 196: 216–225.
11. Neri LM, Fackelmayer FO, Zweyer M, Kohwi-Shigematsu T and Martelli AM: Subnuclear localization of s/mar-binding proteins is differently affected by *in vitro* stabilization with heat or Cu²⁺. *Chromosoma* (1997) 106: 81–93.
12. Kaufmann SH and Shaper JH: A subset of non-histone nuclear proteins reversibly stabilized by the sulfhydryl cross-linking reagent tetrathionate. *Polypeptides of the internal nuclear matrix. Exp Cell Res* (1984) 155: 477–495.
13. Berezney R and Coffey DS: Nuclear matrix. Isolation and characterization of a framework structure from rat liver nuclei. *J Cell Biol* (1977) 73: 616–637.
14. Fey EG, Krochmalnic G and Penman S: The nonchromatin substructures of the nucleus: The ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionation and resin-section electron microscopy. *J Cell Biol* (1986) 102: 1654–1665.
15. Brinkley BR: Elusive nucleoskeleton visualized in live cells. *Cell Motil Cytoskeleton* (2004) 59: 119.
16. Nalepa G and Harper JW: Visualization of a highly organized intranuclear network of filaments in living mammalian cells. *Cell Motil Cytoskeleton* (2004) 59: 94–108.
17. Cremer T and Cremer C: Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat Rev Genet* (2001) 2: 292–301.
18. Stein G, Zaidi S, Braastad C, Montecino M, van WA, Choi J, Stein J, Lian J and Javed A: Functional architecture of the nucleus: Organizing the regulatory machinery for gene expression, replication and repair. *Trends Cell Biol* (2003) 13: 584–592.
19. Misteli T: Protein dynamics: Implications for nuclear architecture and

- gene expression. *Science* (2001) 291: 843-847.
20. Wang J: DNA topoisomerases. *Annu Rev Biochem* (1996) 65: 635-692.
 21. Berrios M, Osheroff N and Fisher PA: In situ localization of DNA topoisomerase II, a major polypeptide component of the *Drosophila* nuclear matrix fraction. *Proc Natl Acad Sci U S A* (1985) 82: 4142-46.
 22. Christensen M, Larsen M, Barthelmes H, Hock R, Andersen C, Kjeldsen E, Knudsen B, Westergaard O, Boege F and Mielke C: Dynamics of human DNA topoisomerases II α and II β in living cells. *J Cell Biol* (2002) 157: 31-44.
 23. Sumner A: The distribution of topoisomerase II on mammalian chromosomes. *Chromosome Res* (1996) 4: 5-14.
 24. Cockerill PN and Garrard WT: Chromosomal loop anchorage of the κ immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. *Cell* (1986) 44: 273-282.
 25. Strukov YG, Wang Y and Belmont AS: Engineered chromosome regions with altered sequence composition demonstrate hierarchical large-scale folding within metaphase chromosomes. *J Cell Biol* (2003) 162: 23-35.
 26. Earnshaw W and Heck M: Localization of topoisomerase II in mitotic chromosomes. *J Cell Biol* (1985) 100: 1716-1725.
 27. Boulikas T: Chromatin domains and prediction of MAR sequences. *Int Rev Cytol* (1995) 162A: 279-388.
 28. Glazko GV, Koonin EV, Rogozin IB and Shabalina SA: A significant fraction of conserved noncoding DNA in human and mouse consists of predicted matrix attachment regions. *Trends Genet* (2003) 19: 119-124.
 29. Kay V and Bode J: Binding specificity of a nuclear scaffold: Supercoiled, single-stranded, and scaffold-attached-region DNA. *Biochemistry* (1994) 33: 367-374.
 30. Zuckerkandl E and Villet R: Generation of high specificity of effect through low-specificity binding of proteins to DNA. *FEBS Lett* (1988) 231: 291-298.
 31. Kiledjian M and Dreyfuss G: Primary structure and binding activity of the hnRNP U protein: Binding RNA through RGG box. *Embo J* (1992) 11: 2655-2664.
 32. Romig H, Fackelmayer FO, Renz A, Ramsperger U and Richter A: Characterization of SAF-A, a novel nuclear DNA binding protein from hela cells with high affinity for nuclear matrix/scaffold attachment DNA elements. *Embo J* (1992) 11: 3431-3440.
 33. Tsutsui K, Tsutsui KM, Okada S, Watarai S, Seki S, Yasuda T and Shohmori T: Identification and characterization of a nuclear scaffold protein that binds the matrix attachment region DNA. *J Biol Chem* (1993) 268: 12886-12894.
 34. Kas E, Izaurralde E and Laemmli UK: Specific inhibition of DNA binding to nuclear scaffolds and histone H1 by distamycin. The role of oligo(dA): Oligo(dT) tracts. *J Mol Biol* (1989) 210: 587-599.
 35. Kipp M, Gohring F, Ostendorp T, van DC, van DR, Przybylski M and Fackelmayer FO: SAF-box, a conserved protein domain that specifically recognizes scaffold attachment region DNA. *Mol Cell Biol* (2000) 20: 7480-7489.
 36. Aravind L and Koonin E: SAP - a putative DNA-binding motif involved in chromosomal organization. *Trends Biochem Sci* (2000) 25: 112-114.
 37. Eggert M, Michel J, Schneider S, Bornfleth H, Baniahmad A, Fackelmayer FO, Schmidt S and Renkawitz R: The glucocorticoid receptor is associated with the RNA-binding nuclear matrix protein hnRNP U. *J Biol Chem* (1997) 272: 28471-28478.
 38. Martens J, Verlaan M, Kalkhoven E, Dorsman J and Zantema A: Scaffold/matrix attachment region elements interact with a p300-scaffold attachment factor a complex and are bound by acetylated nucleosomes. *Mol Cell Biol* (2002) 22: 2598-2606.
 39. Helbig R and Fackelmayer FO: Scaffold attachment factor A (SAF-A) is concentrated in inactive X chromosome territories through its RGG domain. *Chromosoma* (2003) 112: 173-182.
 40. Andersen A and Panning B: Epigenetic gene regulation by noncoding RNAs. *Curr Opin Cell Biol* (2003) 15: 281-289.
 41. Dickinson LA, Joh T, Kohwi Y and Kohwi-Shigematsu T: A tissue-specific MAR/SAR DNA-binding protein with unusual binding site recognition. *Cell* (1992) 70: 631-645.
 42. Dickinson LA, Dickinson CD and Kohwi-Shigematsu T: An atypical homeodomain in SATBI promotes specific recognition of the key structural element in a matrix attachment region. *J Biol Chem* (1997) 272: 11463-11470.
 43. Alvarez JD, Yasui DH, Niida H, Joh T, Loh DY and Kohwi-Shigematsu T: The MAR-binding protein SATBI orchestrates temporal and spatial expression of multiple genes during T-cell development. *Genes Dev* (2000) 14: 521-535.
 44. Yasui D, Miyano M, Cai S, Varga-Weisz P and Kohwi-Shigematsu T: SATBI targets chromatin remodelling to regulate genes over long distances. *Nature* (2002) 419: 641-645.
 45. Cai S, Han H and Kohwi-Shigematsu T: Tissue-specific nuclear architecture and gene expression regulated by SATBI. *Nat Genet* (2003) 34: 42-51.
 46. Tsutsui K, Tsutsui KM, Sano K, Kikuchi A and Tokunaga A: Involvement of DNA topoisomerase II β in neuronal differentiation. *J Biol Chem* (2001) 276: 5769-5778.
 47. Dekker J, Rippe K, Dekker M and Kleckner N: Capturing chromosome conformation. *Science* (2002) 295: 1306-1311.
 48. Kellum R and Schedl P: A group of scs elements function as domain boundaries in an enhancer-blocking assay. *Mol Cell Biol* (1992) 12: 2424-2431.
 49. Zhao K, Hart C and Laemmli UK: Visualization of chromosomal domains with boundary element-associated factor BEAF-32. *Cell* (1995) 81: 879-889.
 50. Gaszner M, Vazquez J and Schedl P: The Zw5 protein, a component of the scs chromatin domain boundary, is able to block enhancer-promoter interaction. *Genes Dev* (1999) 13: 2098-2107.
 51. Blanton J, Gaszner M and Schedl P: Protein: Protein interactions and the pairing of boundary elements in vivo. *Genes Dev* (2003) 17: 664-675.