Journal of Cellular Biochemistry Supplements 30/31:238–242 (1998)

The New Paradigm: Integrating Genomic Function and Nuclear Architecture

Ronald Berezney* and Xiangyun Wei

Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260

Abstract A new view of the cell nucleus is emerging based on the functional dynamics of nuclear architecture. The striking structural preservation of a variety of genomic processes on the nuclear matrix provides an important approach for correlating nuclear form and function. In situ labeling coupled with three-dimensional microscopy and computer imaging techniques shows that DNA replication and transcription sites are organized into higher-order units, or ''zones,'' in the cell nucleus. The dynamic interplay and ''re-zoning'' of replication and transcription regions during the cell cycle may form the structural basis for the elaborate global coordination of replicational and transcriptional programs in the mammalian cell. J. Cell. Biochem. Suppls. 30/31:238–242, 1998. © 1998 Wiley-Liss, Inc.

Key words: nuclear matrix; DNA replication sites; transcription sites; confocal microscopy; nuclear domains; higher-level nuclear organization

IN SITU NUCLEAR MATRIX: HIGHER-ORDER ARRANGEMENT OF CHROMATIN AND RIBONUCLEOPROTEIN DOMAINS

When considering the organization and function of the cell nucleus, it is important to recognize the multiplicity of events that occur within this fundamental structure of the eukaryotic cell. The nucleus is the repository for the genetic information as well as its major site of both expression and regulation. Understanding how the DNA is arranged into a chromatin structure in the cell nucleus is therefore of paramount importance for understanding both the packaging of the genetic information and its expression. Current knowledge suggests that there is a hierarchy of organization of DNA from the DNA double helix to nucleosomes to folded chromatin fibers. While many details of chromatin folding remain to be elucidated, the arrangement of chromatin into repeating 50- to 200-kb loops or domains has been extensively documented [Cook and Brazell, 1975; Paulson and Laemmli, 1977; Dijkwel et al., 1979; Vogelstein et al., 1980; Basler et al., 1981; Berezney and Buchholtz, 1981a,b; Pienta and Coffey, 1984; Nelson et al., 1986; Laemmli et al., 1992]. The precise karyotypic banding properties of mitotic chromosomes further implies even higher levels of chromatin organization, some of which at least, are characteristic of the species. Recent application of the fluorescent in situ hybridization (FISH) technique have confirmed earlier observations that the chromatin in the interphase nucleus is not randomly arranged but remains organized into chromosome-specific territories [van Driel et al., 1995].

Despite this progress, a prevalent textbook view depicts the cell nucleus as a spherical structure with a surrounding double-membraned nuclear envelope, distinct nucleoli, and chromatin fibers spread throughout in spaghetti-like fashion. The regions between the chromatin are generally left "empty" or labeled as "nucleoplasm." Current knowledge necessitates two major alterations in this model. First, the chromatin is more precisely arranged into chromosome-specific territories. Second, there is elaborate nonchromatin structure in the nucleus as well. Unfortunately, the nonchromatin structures in the cell nucleus are difficult to visualize by standard thin-sectioning microscopy and staining procedures. An important breakthrough occurred with the development of the EDTA regressive staining method [reviewed in Berezney, 1984; Berezney et al., 1995].

Contract grant sponsor: National Institutes of Health; Contract grant number: GM 23922.

*Correspondence to: Ronald Berezney, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260. E-mail: berezney@acsu.buffalo.edu

Received 27 October 1998; Accepted 29 October 1998

In this way, the nonchromatin granular and fibrous structures stand out against a background of lightly stained or "bleached" chromatin regions. Taken together, these nonchromatin regions comprise what is termed the *in situ nuclear matrix* (Berezney, 1984). Resinless section electron microscopy has resulted in improved visualization of the in situ nuclear matrix [Nickerson et al., 1995]

Previous studies have demonstrated that the in situ nuclear matrix regions in the nucleus are concentrated in ribonucleoproteins and RNA splicing components as well as containing the diffuse or active euchromatin [reviewed in Berezney, 1984, 1995; Nickerson et al., 1995]. Numerous electron autoradiographic studies also indicated that the functional regions of chromatin are contained within or border the in situ nuclear matrix regions [reviewed in Berezney et al., 1984]. The nuclear matrix is therefore envisioned as the regions in the nucleus involved in the organization and expression of chromatin and ribonucleoprotein [Berezney, 1984, 1991; Berezney et al., 1995].

THE FUNCTIONAL NUCLEUS: ORGANIZATION OF FUNCTIONAL DOMAINS IN THE CELL NUCLEUS

Recent developments in microscopy, imaging, and labeling have lead to a new view of the cell nucleus based on genomic function. It is now known that genomic functions and factors that mediated these functions are compartmentalized in the cell nucleus in discrete domains, including DNA replication and transcription sites, RNA transcript tracks and splicing factors [Berezney et al., 1995; Nickerson et al., 1995; van Driel et al., 1995]. A schematic of this "functional nucleus" is shown in Figure 1. In this model, the classic nuclear structures readily visible with light and/or electron microscopy are not depicted in order to emphasize the "invisible world" of genomic function that is shown for the first time. Moreover, the development of chromosome-specific DNA "paints" has demonstrated that the individual chromosome territories are maintained in the functional genome of the interphase nucleus [reviewed in van Driel et al., 1995].

These findings support the view that the cell nucleus is a well-ordered structure in terms of both genomic organization (chromosome territories) and genomic function (DNA replication, transcription, RNA transcript tracks, and splicing factors). They also provide the foundation for a new and exciting field in gene expression and regulation in which the role of nuclear architecture in the organization and function of genes and gene products is under investigation [Baskin, 1995]. In this regard, Nakayasu and Berezney [1989] reported that DNA replication sites are strikingly maintained on the nuclear matrix after extraction of cells grown on coverslips. Moreover, the isolated nuclear matrix was capable of synthesizing DNA on nuclear matrix-bound sites that were indistinguishable from those visualized in intact cells [Nakayasu and Berezney, 1989]. Reports from other investigators have confirmed these findings and extended them to studies of transcription sites, RNA transcript tracks, and splicing factor-rich sites [reviewed in Berezney et al., 1995; Jack-

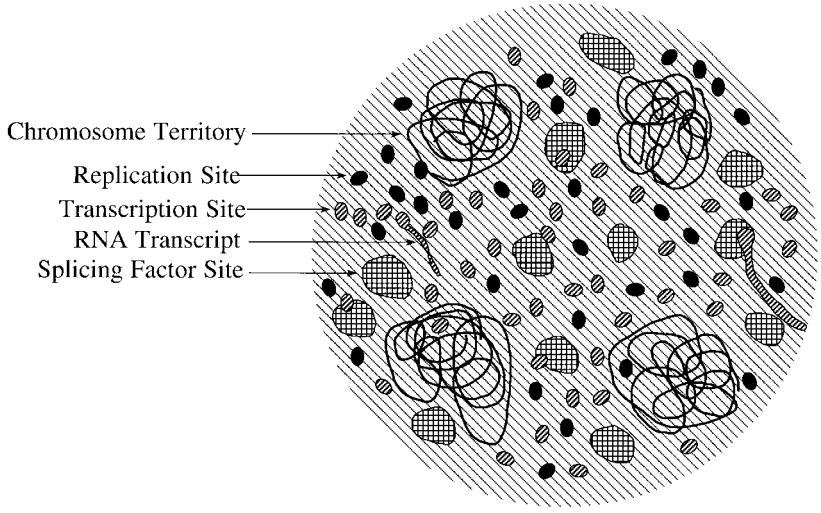

Fig. 1. Schematic illustration of the domains of genomic function in the mammalian cell nucleus. The known structures of the nucleus are not depicted in order to stress the presence of discrete functional domains.

son and Cook, 1995; Nickerson et al., 1995; van Driel et al., 1995].

Figure 2 summarizes the relationships between function domains and nuclear matrix association. Importantly, the spatial organization of the functional domains is strikingly similar to that in intact cells. This is consistent with the proposed role of the nuclear matrix in the higher-order arrangement of the genome and its expression [Berezney, 1984, 1991; Nelson et al., 1986; Berezney et al., 1995; Nickerson et al., 1995]. Recently, our group has extended this relationship to the chromatin. We have found that chromosome territories are anchored to the nuclear matrix and that disruption of the nuclear matrix internal structure results in a corresponding disruption of discrete chromosome territories (H. Ma and R. Berezney, unpublished results).

The remarkable maintenance of genomic organization and function on the nuclear matrix provides a potentially important approach for further investigation of the role of nuclear architecture in these processes. In this regard, the isolation of the nuclear matrix [Berezney et al., 1974, 1977] and the plethora of functional properties found in association with these isolated structures [reviewed in Berezney, 1991, 1995] take on added significance as an in vitro system for identifying specific components involved in higher-order genomic organization and function.

NUCLEAR ZONING OF REPLICATION AND TRANSCRIPTION

While the findings of discrete nuclear domains of replication, transcription, and RNA splicing have provided a foundation for studying the relationships of nuclear form and function, many questions remain unanswered and are providing the basis for current and future experimentation. One fundamental question is: Are the sites of DNA replication or transcription "randomly" arranged in the nucleus, or are there higher levels of organization above the individual sites? We have recently directly addressed this question by simultaneously labeling replication and transcription sites in permeabilized mammalian cells and applying three-dimensional microscopy and computer imaging techniques. Our results demonstrate for the first time that replication and transcription sites are clustered into separate higher-order domains or "zones" in the cell nucleus [Wei et al., 1998]. Figure 3A illustrates schematically the arrangement of individual replication sites into higher-order zones (green shaded areas). Since replication sites contain an average of 1 mbp DNA [Jackson and Pombo, 1998], and each

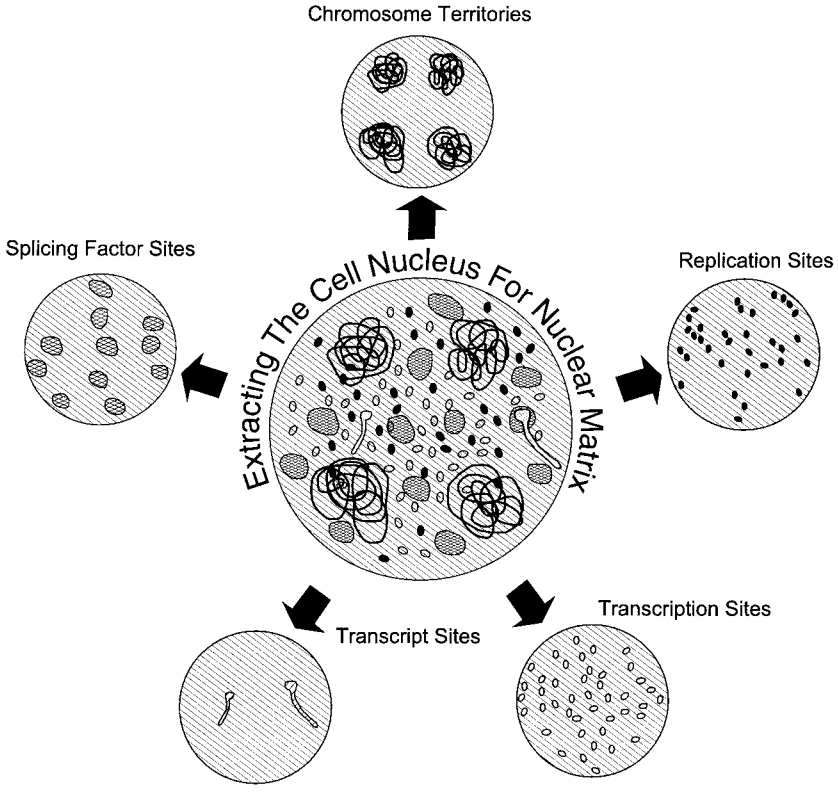

Fig. 2. Maintenance of genomic functional domains after extraction for nuclear matrix. When cells grown on coverslips are extracted for nuclear matrix, the structural organization of the various genomic functional domains are strikingly preserved. These include DNA replication sites, transcription sites, transcript tracks or sites and splicing factor sites (see text for references). Recently we have observed that chromosome territories are also maintained.

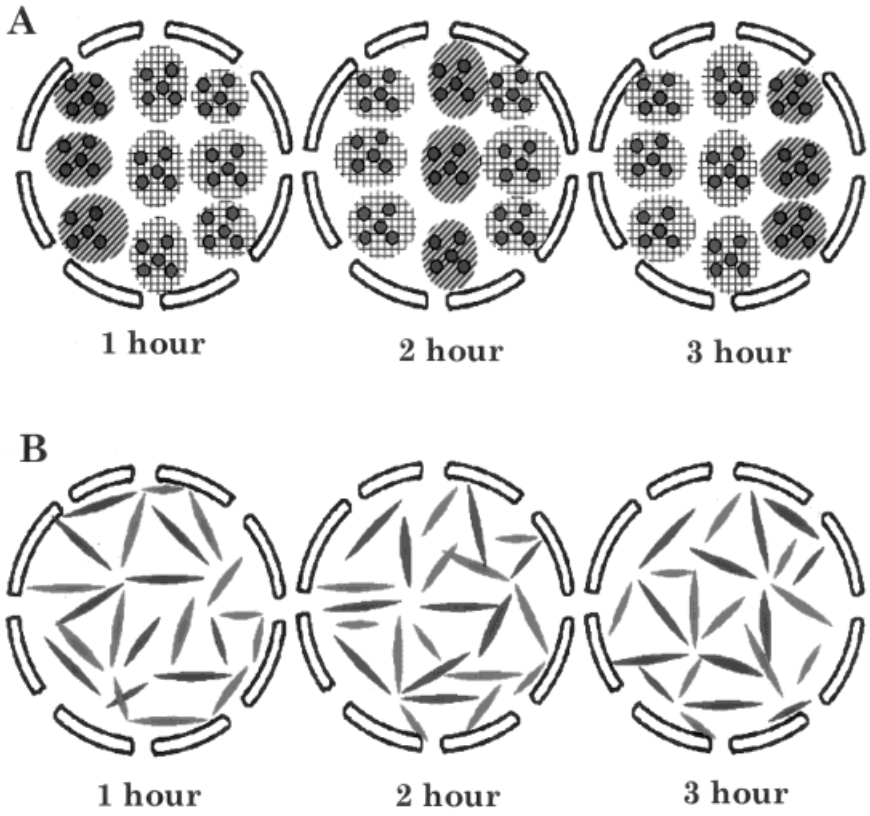

Fig. 3. Spatio-temporal dynamics of replication and transcription zones in the mammalian cell nucleus. **A:** Replication and transcription sites are shown to occupy distinct and spatially separate regions of the nucleus termed replication and transcription zones (*green and red shaded regions*, respectively). Each zone contains massive amounts of DNA (e.g., >10 mbp) and is composed of numerous replication (*green solid circles*) or transcription sites (*red solid circles*), which in turn contain multiple replicons or genes. Different times in early S-phase (1–3 h) are depicted to illustrate the progressive temporal program of DNA replication. Massive shifts in "zoning" are postulated to occur as replication zones finish replicating, and are re-zoned for transcription. Similarly, other zones commissioned for transcription are decommissioned and re-zoned for replication. **B:** The same dynamics of zoning and re-zoning of replication and transcription is illustrated as described in **A**. In this case, however, the individual zones (stick-like structures) are shown to form a discontinuous network-like pattern in three dimensions. The networks for replication (*green*) and transcription (*red*) are spatially distinct and dynamically change in accordance with the temporal program of replication and transcription in the cell. **Color plate on page 326.**

replication zone likely contains a dozen or more individual replication sites, the chromatin contained within these replication zones must be huge (>10 mbp) and may represent a fundamental level for the coordination of replication timing among multiple genes and gene families [Hatton et al., 1988; Dhar et al., 1988; Selig et al., 1992; Kitsberg et al., 1993]. Similarly, individual transcription sites are also believed to contain multiple genes and the higher-order arrangement into separate transcription zones (red shaded areas in Fig. 3A) may be the structural expression of transcriptional programming in the cell.

This analysis was performed in early S-phase, when discrete sites of both replication and transcription are readily observed [Wei et al., 1998]. Since actively transcribed genes are preferentially replicated in early S-phase, these finding have important implications for our understanding of the coordination of replication and transcription of genes. What is suggested is that huge regions of chromatin are "recruited" for replication or transcription at a specific moment in S-phase. At another moment in time (e.g., 1 h later in S-phase), another group of replication zones would be active. This implies a dynamic process of re-zoning [Cook, 1998] in which regions in the nucleus previously commissioned for replication are re-zoned into transcription zones, while other regions involved in transcription are decommissioned for replication zones (Fig. 3A).

Three-dimensional analysis further shows that the individual replication and transcription zones form network-like patterns that extend throughout the nucleus [Wei et al., 1998].

While the networks of replication and transcription zones appear similar, they are completely separated in three dimensions (Fig. 3B). Moreover, each network is composed of neighboring zones that appear to be structurally discontinuous [Wei et al., 1998](Fig. 3B). Coupled with the re-zoning model depicted in Figure 3A, we propose the presence of dynamic three-dimensional networks of transcription and replication in the cell nucleus that reflect the corresponding replicational and transcriptional programming of the cell and may be an important property for the global coordination of genomic function.

In summary, replication and transcription are arranged in successively higher levels of organization in the cell nucleus: from individual sites that contain multiple genes, to zones that contain numerous sites, to three-dimensional networks that are the structural basis for the exquisite coordination of replication and transcription in the mammalian cell. Recently, we have found that these higher levels of organization are remarkably maintained after extraction for the nuclear matrix (X. Wei, S. Somanathan, and R. Berezney, unpublished observations). Further studies in this direction should provide important clues for dissecting the components involved in the interrelationships of nuclear architecture with the coordination of genomic function.

ACKNOWLEDGMENTS

We are extremely grateful to Jim Stamos, Suryanarayan Somanathan, and Allan Siegel, who did the illustrations and printing.

REFERENCES

Baskin I (1995): Mapping the cell's nucleus. Science 268: 1564–1565.

Basler J, Hastie ND, Pietras D, Matsui S-I, Sandberg A, Berezney R (1981): Hybridization of nuclear matrix attached deoxyribonucleic acid fragments. Biochemistry 20:6921–6929.

Berezney R (1984): Organization and functions of the nuclear matrix. In Hnilica LS (ed): "Chromosomal Nonhistone Proteins." Vol 4. Boca Raton, FL: CRC Press, pp 119–180.

Berezney R (1991): The nuclear matrix: A heuristic model for investigating genomic organization and function in the cell nucleus. J. Cell Biochem 47:109–123.

Berezney R, Buchholtz LA (1981a): Dynamic association of replicating DNA fragments with the nuclear matrix of regenerating liver. Exp Cell Res 132:1–13.

Berezney R, Buchholtz LA (1981b): Isolation and characterization of rat liver nuclear matrices containing high molecular weight deoxyribonucleic acid. Biochemistry 20:4955–5002

Berezney R, Coffey DS (1974): Identification of a nuclear protein matrix. Biochem Biophys Res Commun 60:1410–1417.

Berezney R, Coffey DS (1977): Nuclear matrix: Isolation and characterization of a framework structure from rat liver nuclei. J Cell Biol 73:616–637.

Berezney R, Mortillaro MJ, Ma H, Wei X, Samarabandu J (1995): The nuclear matrix: A structural milieu for genomic function. Int Rev Cytol 162A:1–65.

Cook P (1998): Duplicating a tangled genome. Science 281: 1466–1467.

Cook PR, Brazell IA (1975): Supercoils in human DNA. J Cell Sci 19:261–279.

Dhar V, Mager D, Iqbal A, Schildkraut CL (1988): The coordinate replication of the human beta-globin gene domain reflects its transcriptional activity and nuclease hypersensitivity. Mol Cell Biol 8:4958–4965.

Dijkwel PA, Mullenders LHF, Wanka F (1979): Analysis of the attachment of replicating DNA to a nuclear matrix in mammalian interphase nuclei. Nucleic Acids Res 22:219–230.

Hatton KS, Dhar V, Gahn TA, Brown, EH, Mager D, Schildkraut CL (1988): Temporal order of replication of multigene families reflects chromosomal location and transcriptional activity. Cancer Cells 6:335–340.

Jackson DA, Cook PR (1995): The structural basis of nuclear function. Int Rev Cytol 162A:125–149.

Jackson DA, Pombo A (1998): Replicon clusters are stable units of chromosome structure: Evidence that nuclear organization contributes to the efficient activation and propagation of S phase in human cells. J Cell Biol 140: 1285–1295.

Kitsberg D, Selig S, Brandeis M, Simon I, Keshet I, Driscoll DJ, Nicholls RD Cedar H (1993): Allele-specific replication timing of imprinted gene regions. Nature 364:459–463.

Laemmli UK, Kas E, Poljak L, Adachi Y (1992): Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. Curr Opin Genet Dev 2:275–285.

Nelson WG, Pienta KJ, Barrack ER, Coffey DS (1986): The role of the nuclear matrix in the organization and function of DNA. Annu Rev Biophys Chem 15:457–475.

Nickerson JA, Blencowe BJ, Penman S (1995): The architectural organization of nuclear metabolism. Int Rev Cytol 162A:67–123.

Paulson JR, Laemmli UK (1977): The structure of histone-depleted metaphase chromosomes. Cell 12:817–828.

Pienta KJ, Coffey DS (1984): A structural analysis of the role of the nuclear matrix and DNA loops in the organization of the nucleus and chromosome. J Cell Sci Suppl 1:123–135.

Selig S, Okumura K, Ward DC, Cedar H (1992): Delineation of DNA replication time zones by fluorescence in situ hybridization. EMBO J 11:1217–1225.

van Driel R, Wansink DG, van Steensel B, Grande MA, Schul W, de Jong L (1995): Nuclear domains and the nuclear matrix. Int Rev Cytol 162A:151–189.

Vogelstein B, Pardoll DM, Coffey DS (1980): Supercoiled loops and eucaryotic DNA replication. Cell 22:79–85.

Wei X, Samarabandu J, Devdhar RS, Siegel AJ, Acharya R, Berezney R (1998): Segregation of transcription and replication sites into higher order domains. Science 281: 1502–1505.