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The Nuclear Matrix: A Heuristic Model for Investigating Genomic Organization and Function in the Cell Nucleus

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Abstract Despite significant advances in deciphering the molecular events underlying genomic function, our understanding of these intergrated processes inside the functioning cell nucleus has, until recently, met with only very limited success. A major conundrum has been the "layers of complexity" characteristic of all cell structure and function. To understand how the cell nucleus functions, we must also understand how the cell nucleus is put together and functions as a whole. The value of this neo-holistic approach is demonstrated by the enormous progress made in recent years in identifying a wide variety of nuclear functions associated with the nuclear matrix. In this article we summarize basic properties of in situ nuclear structure, isolated nuclear matrix systems, nuclear matrix-associated functions, and DNA replication in particular. Emphasis is placed on identifying current problems and directions of research in this field and illustrating the intrinsic heuristic value of this global approach to genomic organization and function.

Key words: nuclear structure and function, nuclear matrins, cDNA cloning and sequencing, DNA binding proteins, zinc finger motifs, hydropathy plot, DNA replication, replicon clusters, clustersome model, replication granules

THE IN SITU NUCLEAR MATRIX AND GENOMIC FUNCTION

The cell nucleus is the repository for the genetic information of all eucaryotic cells including that of man. Despite considerable progress in defining basic molecular properties of the primary genomic functions of DNA replication, transcription, and RNA splicing and processing, our knowledge of how these processes are organized and regulated within the confines of the cell nucleus is extremely limited. Similarly, although the genetic code behind the DNA (the nucleotide sequence) has long been broken, our understanding of the organization of this DNA into chromatin and higher order structures in the cell nucleus is still in its infancy.

Clearly the old model of the interphase nucleus as a bag of chromatin immersed in a homogeneous nucleoplasm is undergoing radical change. Indeed, classical ultrastructural studies have demonstrated that the cell nucleus is an extremely elaborate structure where regions containing chromatin are in close association with an elaborate nonchromatin infrastructure in the nuclear interior [Fawcett, 1966; Monneron and Bernhard, 1969; Derenzini et al., 1977, 1978; Berezney, 1984]. Electron microscopic autoradiography and cytochemical studies have revealed that the functions of DNA replication and transcription are localized over those sites of interaction between the active chromatin and the nonchromatin structure [Fakan and Bernhard, 1971; Fakan & Hancock, 1974; Berezney, 1984]. Moreover, RNA splicing and other post-transcriptional regulatory processes presumably occur within this structural milieu of the cell nucleus [Fakan and Bernhard, 1971; Berezney, 1984].

This led to the proposal that the regions in the nucleus where functions occur comprise an elaborate three-dimensional structure termed the in situ nuclear matrix [Berezney, 1984]. A schematic model of the cell nucleus is presented in Figure 1. In this model the in situ matrix is presented as the major structural region in the nucleus where nuclear function occurs.

The presence of a nonchromatin matrix as a universal feature of the eucaryotic cell nucleus is now well established. What remains to be resolved is the significance of this structure. Presumably it is related to the nuclear functions of replication, transcription, and RNA processing and transport to the cytoplasm. In support of this conclusion a direct relationship between the relative amount of nonchromatin nuclear matrix in the nucleus and the transcriptional

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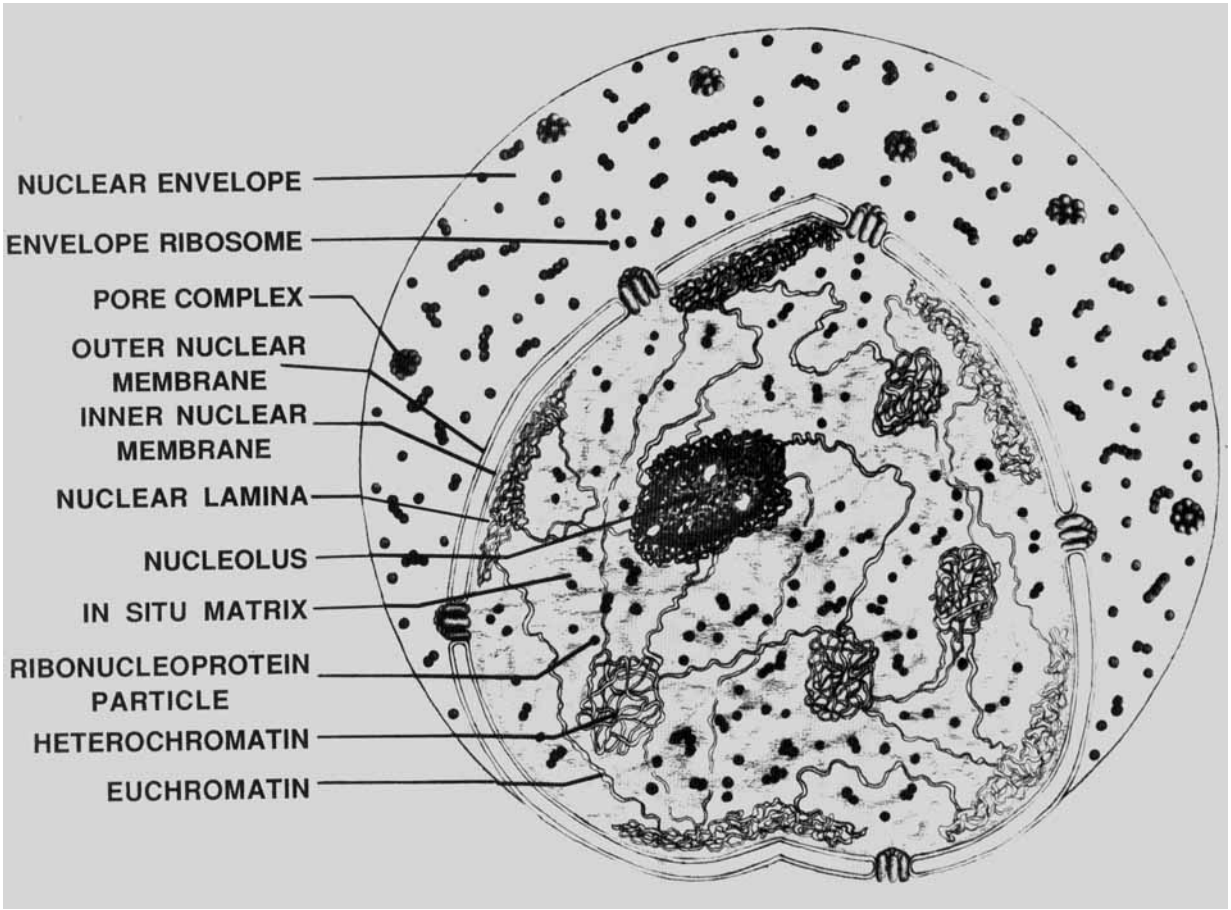

Fig. 1. Schematic model of a typical cell nucleus. The nucleus is surrounded by a double-membraned nuclear envelope containing nuclear pore complexes. Ribosome-like structures are found on the surface of the outer nuclear membrane as individual particles and "polysome-like" arrays. The chromatin in the nuclear interior is interpreted as a continuous system of condensed (heterochromatin) and diffuse (euchromatin) regions. The nonchromatin region of the nuclear interior is simplified to contain the nucleolus, RNP (ribonucleoprotein) particles, and an in situ matrix forming a diffuse network which associates with the chromatin and nucleoli in the interior and the nuclear pore complexes at the periphery. The peripherally localized matrix may correspond to the nuclear lamina often observed in close association with the inner nuclear membrane. (Drawn by L.A. Buchholtz and reproduced from Berezney [1979] with permission of Academic Press.)

activity of the cell has been observed [LaFond et al., 1983; Setterfield et al., 1983]. Indeed, it has been proposed that the *raison d'être* of the in situ matrix is to organize and orchestrate the dynamics of genomic function and regulation [Berezney, 1984].

Since a variety of functional processes presumably occur in the matrix region of the nucleus, the in situ matrix is likely to be composed of a variety of components distinct for the different functional domains [Berezney, 1984]. The definition of the individual components (e.g., proteins), how they are assembled into the in situ structures, and how different components might be integrated into higher order structure are important questions that need to be addressed in future research. At present we can only conclude that complex nonchromatin structures exist in this region of the nucleus with little understanding of how the structure is actually put together.

This raises a commonly addressed question in this field. Is the nuclear matrix an independent skeletal structure in the nucleus which determines three-dimensional organization and functioning of chromatin and ribonucleoproteins? Or is the matrix simply the "phenotypic expression" of nuclear functions with no independent existence devoid of function? In my mind this is a loaded question akin to "What comes first, the chicken or the egg?" It is a universal theme of all biology that structure and function although often studied as separate entities are actually two sides of the same coin: the fundamental biological processes of the living state. It is our own ignorance of the underlying

mechanisms that lead to this dichotomization of what is inevitably a unified process.

In other words, rather than the cell making the nuclear matrix and the nuclear matrix driving nuclear functions, it is likely that the matrix and nuclear functions both make and drive each other in a sort of unified symbiosis at the supramolecular level.

THE ISOLATED NUCLEAR MATRIX

If the in situ nuclear matrix is indeed a real structure of the cell nucleus, then it might be possible to isolate these structures. In 1974, Berezney and Coffey, following up on previous studies of residual nuclear structures, reported the first characterization of nuclear matrix isolated from rat liver tissue. Subsequent studies by this group [Berezney and Coffey, 1977] and many others [Berezney, 1984; Berezney, 1979; Shaper et al., 1979] have led to the characterization of nuclear matrices from a wide variety of eucaryotic cells throughout the phylogenetic scale from unicellular organisms to man.

Most procedures for nuclear matrix isolation are based on the original protocols reported by Berezney and Coffey [1974, 1977]. Basically, morphologically intact nuclei are isolated and subjected to a series of treatments involving nuclease digestion, salt extractions, and detergent (Triton X-100). A key point is that morphologically recognizable nuclear structures are maintained throughout the extraction protocol including the final nuclear matrix fraction, despite the removal of most of the chromatin and protein and disruption of the nuclear membranes with detergent.

A major modification for nuclear matrix isolation was introduced by Laemmli and his associates [Mirkovitch et al., 1984] who used the chaotrophic agent and detergent LIS (lithium 3,5-diiodosalicylate) instead of salt solutions for extraction. This preparation has been termed the *nuclear scaffold* to distinguish it from its salt extracted counterpart and has been widely used for the study of specific DNA sequences associated with the residual nuclear structure [Gasser and Laemmli, 1987]. Other preparations of these types of structures have been termed *nucleoskeletons, nuclear ghosts,* and *nuclear cages,* although the term *nuclear matrix* is clearly the most widely used. Cook and his associates use the term *nucleiod* for nuclear matrices which have intact, supercoiled DNA associated with them (McCready et al., 1979). The term *DNA-rich nuclear matrices* has also been used to describe these types of preparations [Berezney and Buchholtz, 1981a].

Isolated nuclear matrices maintain many of the major architectural features of the intact nucleus despite the removal of 75–90% of the total nuclear protein and virtually all of the chromatin. The isolated matrices also contain large amounts of tightly bound RNA, lesser amounts of DNA (dependent on the degree of nuclease digestion), and only trace amounts of lipids if nonionic detergent extraction (e.g., Triton X-100) is performed. The major macromolecular component is protein and is composed of a multitude of different proteins with an enrichment of the higher molecular weight nonhistone proteins in the nucleus and a depletion of lower molecular weight proteins, especially the histones [Berezney, 1979, 1984]. Three of the major proteins are lamins A, B, and C which migrate between 60–70 kDa on SDS-polyacrylamide gel electrophoresis and compose the major components of the surrounding residual nuclear envelope or *nuclear lamina.*

Laemmli and co-workers first demonstrated that isolated chromosomes extracted with nuclease and high salt maintain a residual protein chromosomal structure termed the *chromosome scaffold* [Adolph et al., 1977]. Further studies showed that the chromosomal DNA loops are attached to the scaffold structure [Paulson and Laemmli, 1977]. Since the DNA loops are attached to the nuclear matrix in interphase cells, it has been widely suggested that at least certain components of the interphase nuclear matrix (i.e., the DNA attachment sites) are maintained in mitotic cells as the chromosome scaffold. Despite this belief, our knowledge of the precise relationships between the proteins composing the interphase matrix versus the chromosome scaffold is very limited.

Three main structural regions typically compose the isolated nuclear matrix, including a surrounding residual nuclear envelope or nuclear lamina (containing morphologically recognizable nuclear pore complexes), residual components of nucleoli, and an extensive fibrogranular internal matrix (Fig. 2). The latter structure is believed to represent residual components of the in situ nuclear matrix structure observed in whole cells. Using EDTA regressive staining, a similarity is seen between the fibrogranular internal matrix of the isolated nuclear matrix and

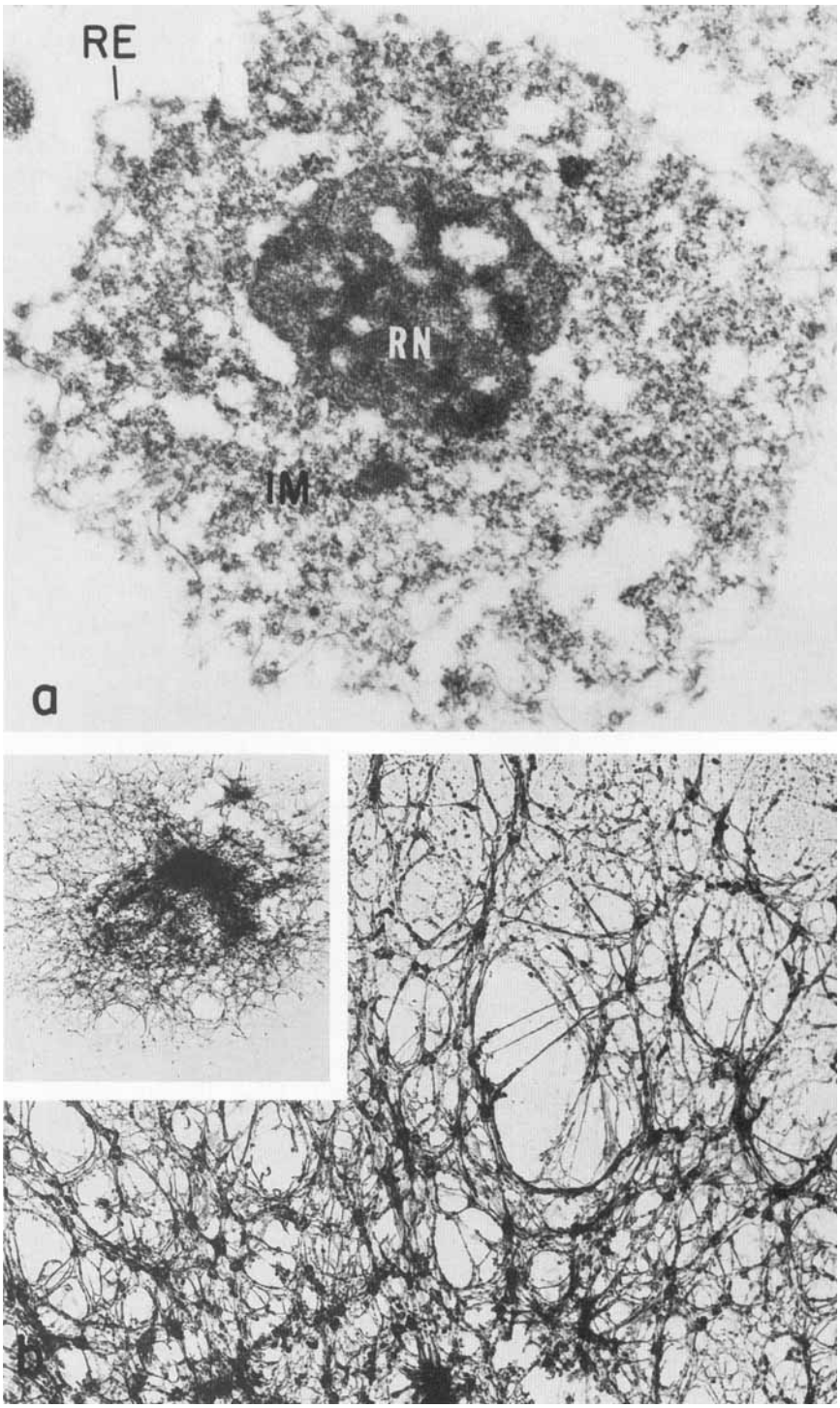

Fig. 2. Study of nuclear matrix structure by thin sectioning and whole mount electron microscopy. **a:** Thin sectioning of an isolated rat liver nuclear matrix reveals the basic tripartite structure of the nuclear matrix. IM, internal matrix; RE, residual nuclear envelope or nuclear lamina; RN, residual nucleoli. **b:** Whole mount electron microscopy of an isolated rat liver nuclear matrix spread on an aqueous surface reveals an overall fibrous network structure (inset). At higher magnification details of the elaborate fibrogranular network are seen. The specimen was critically point dried and rotary shadowed with platinum-palladium. The delicate matrix lacework is considerably disrupted in the absence of critically point drying. (Reproduced from Berezney, R. [1981] with permission of Springer-Verlag.)

the in situ nuclear matrix visualized in whole cells [Berezney, 1984].

While standard thin sectioning electron microscopy and EDTA regressive staining enable the visualization of a fibrogranular structure in whole cells and isolated nuclear structures, the structural information obtained by these procedures is limited. Moreover the nucleus is a large three-dimensional structure. With these points in mind Penman and co-workers [Capco et al., 1982; Nickerson et al., 1990] have studied in detail nuclear matrix structures three-dimensionally by whole mount and resinless thick section electron microscopy. A complex three-dimensional network of filaments with associated granular structures is observed similar to that of the cytoskeleton. This anastomising network of filaments is shown in Figure 2b which is a whole mount image of an isolated rat liver nuclear matrix. The structures visualized with these techniques demonstrate a much greater degree of structure order in the nuclear matrix compared to standard thin sectioning electron microscopy.

Penman and others have also pioneered the development of procedures to extract nuclear matrices directly from cells grown on cover slips [Fey et al., 1986; Nickerson et al., 1990]. These so-called in situ nuclear matrix preparations are particularly valuable for the electron microscopic studies of three-dimensional structure discussed above as well as for immunolocalization studies in the nucleus and nuclear matrix. Numerous studies have documented that such in situ matrix preparations offer the advantage of better maintenance of nuclear morphology with very similar properties of the isolated matrices.

In earlier studies of nuclear extraction some investigators found that procedures related to those used for nuclear matrix isolation (nuclease, high salt, and detergent) could also lead to so-called "empty" nuclear matrices which contained the surrounding nuclear lamina with nuclear pore complexes but were devoid of internal matrix structure. After some initial confusion it became apparent that the internal nuclear matrix is much more sensitive to extraction than the surrounding residual nuclear envelope [Kaufman et al., 1981]. This has led to more optimized preparations for both nuclear matrix with well-preserved internal matrix structure and nuclear lamina free of internal matrix components [Smith et al., 1984; Belgrader et al., 1991b]. If isolated nuclei are digested with RNase A and extracted for nuclear matrix with salt in the presence of sulfhydryl reducing agents such as dithiothreitol, the internal matrix is destabilized and empty matrices consisting exclusively of nuclear lamina are obtained. Preparation of nuclear matrix in the absence of RNase and dithiothreitol leads to typical tripartite matrices with elaborate internal matrix structure.

NUCLEAR MATRIX PROTEINS

Nuclear matrix proteins are the nonhistone proteins which comprise the nuclear matrix subfraction following nuclease, salt, and detergent extraction of isolated cell nuclei (see preceding section on nuclear matrix isolation). While virtually all known nuclear functions are associated with this proteinaceous nucleoskeletal structure (see following section on functional properties of nuclear matrices), our knowledge of the proteins which compose this intriguing nucleoskeletal structure is very limited. There is no doubt, however, that a detailed molecular analysis of the individual nuclear matrix proteins is of paramount importance for deciphering the structural organization and molecular properties of nuclear matrix structure and the associated functions.

Previous studies of nuclear matrix proteins using one-dimensional SDS-PAGE (polyacrylamide gel electrohoresis), while useful for providing an initial indication of the overall polypeptide profile of the nuclear matrix, are extremely limited due to the enormous complexity of the protein composition. This was not fully realized until two-dimensional gels were run. Thus attempts to identify similarities and differences among the polypeptide profiles obtained from nuclear matrices of different species, cellular origins, cell cycle stages, or physiological states should only be regarded as preliminary results which must be extended to two dimensional analysis.

Another difficulty is that nuclear matrices prepared from tissue culture cells are invariably contaminated with large amounts of cytoskeletal proteins, particularly the intermediate filament proteins [Capco et al., 1982; Belgrader et al., 1991b; Staufenbiel and Deppert, 1983; Verheijen et al., 1986]. In contrast, nuclei isolated from tissues such as rat liver are relatively devoid of such proteins [Staufenbiel and Deppert, 1983]. Thus polypeptide profiles of nuclear matrices obtained from highly purified rat liver nuclei are likely to largely reflect the true nuclear proteins in this preparation, while those from tissue culture cells are likely to contain

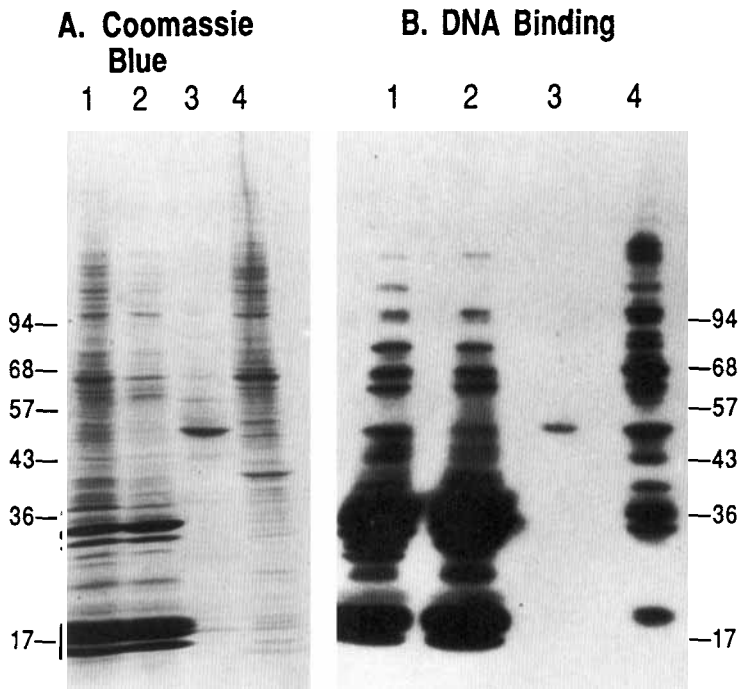

Fig. 3. Identification of DNA binding proteins in the nuclear matrix by one-dimensional Southwestern blots. Total rat liver nuclear proteins (**lanes 1**), nuclear matrix proteins (**lanes 4**), and the proteins from the salt (**lanes 2**) and Triton X-100 (**lanes 3**) extraction steps of the nuclear matrix isolation procedure were separated on 5–18% SDS polyacrlamide gradient gels and either Coomassie blue stained (**A**) or electrophoretically transformed to nitrocellulose paper and probed with labeled genomic DNA (Southwestern blot). Note the enrichment in higher molecular weight DNA binding proteins in the nuclear matrix. The positions of molecular weight markers are shown in kilodaltons. (Reproduced from Hakes and Berezney [1991a] with permission of The American Society For Biochemistry & Molecular Biology.)

cytoskeletal proteins as major components with many of the true nuclear proteins appearing as only minor components. Naturally, any in situ nuclear matrix preparations would have this limitation. Recently Fey and Penman [1988] have circumvented this problem by an extraction procedure which separates intermediate filament proteins from the true nuclear matrix components.

Two-dimensional analyses of nuclear matrix proteins performed by several different groups all stress the high degree of complexity of these polypeptide profiles. Using ^{35}S-methionine labeling for detection, Fey and Penman [1988] have detected over 200 proteins in the nuclear matrix. Stuurman et al. [1990] have also found enormous complexity in the two-dimensional profiles with the sensitive silver procedure. Despite this complexity, these studies are already providing valuable information. For example, the total nuclear matrix proteins can be separated into two major classes: those which are found in a variety of cell lines (common matrix proteins) and those which are both cell type and differentiation state dependent [Fey and Penman, 1988; Stuurman et al., 1990; Dworetzky et al., 1990].

Studies in our laboratory are concentrating on the major proteins of the nuclear matrix which are common at least among mammalian cells. Using a two-dimensional PAGE system we have detected in rat liver nuclear matrix about one dozen major Coomassie blue stained proteins along with over fifty more minor spots. As a initial step we have screened nuclear matrix proteins for DNA binding activity on Southwestern blots. Using one-dimensional SDS-PAGE we have shown that the nuclear matrix is enriched in the higher molecular weight DNA binding proteins found in total rat liver nuclear protein [Fig. 3; Hakes and Berezney, 1991a]. Approximately one dozen major DNA binding proteins with apparent molecular weights exceeding 40,000 were detected on the 1-D blots. Further studies indicated that these proteins preferentially bound DNA when competed with excess RNA [Hakes and Berezney, 1991a].

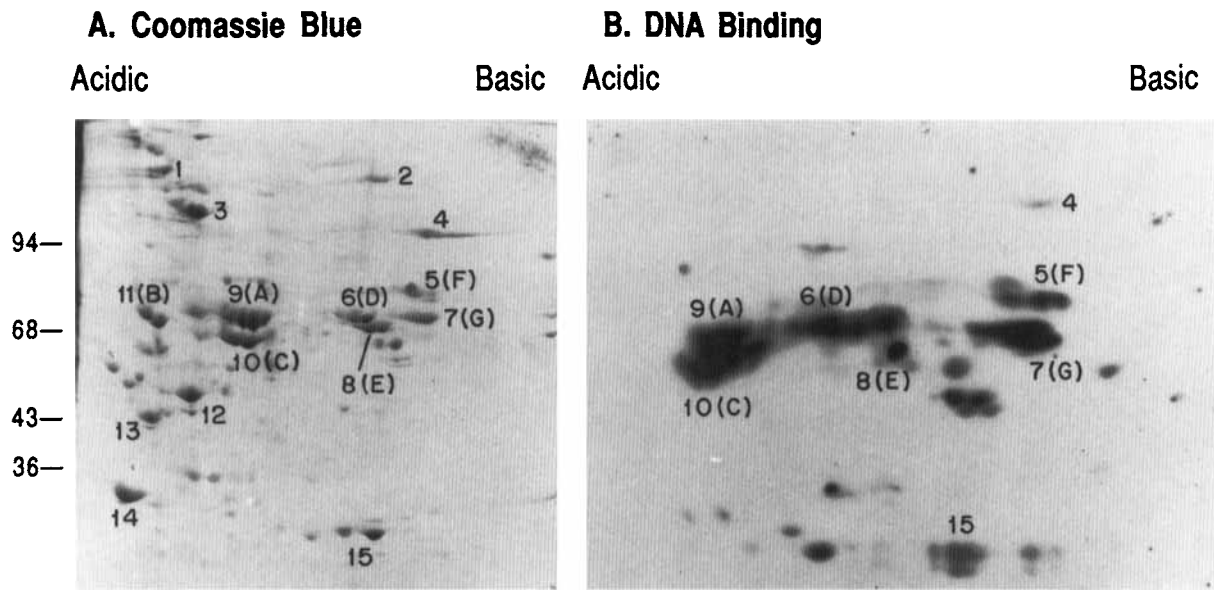

Fig. 4. Identification of DNA binding proteins in the nuclear matrix by two-dimensional Southwestern blots. Total nuclear matrix proteins were separated on two-dimensional gels, stained with Coomassie blue (**A**) or transferred to nitrocellulose paper for DNA binding with labeled genomic DNA (**B**). The major DNA binding proteins identified on the two-dimensional Southwesterns were lamins A, C and matrins D, E, F, G and an unidentified protein migrating at about 48 kDa. (Reproduced from Hakes and Berezney [1991a] with permission of The American Society for Biochemistry & Molecular Biology.)

Two-dimensional Southwestern blots were then performed to identify the specific DNA binding proteins (Fig. 4). Approximately twelve distinct spots were detected including lamins A and C but not B, several internal nuclear matrix proteins (termed *nuclear matrins*), matrins D, E, F, G, 4 (but not 3), and an unidentified protein of about 48 kDa. The identity of these major nuclear lamins and matrins as DNA binding proteins was then confirmed by Southwestern analysis following purification of the individual proteins from the 2-D gels.

As a step toward the further characterization of the nuclear matrins and their putative role as DNA binding proteins, we have been screening λ-gt11 cDNA expression libraries with our polyclonal antibodies to these matrins. A 2.7 kb rat liver DNA clone which contains the entire 544 amino acid coding sequence for matrin F/G was identified and sequenced [Hakes and Berezney, 1991b]. The predicted amino acid sequence from the coding region of the matrin F/G cDNA showed that this protein contains approximately 50% hydrophobic amino acid residues. A hydropathy plot of this sequence, based on the axiom of Kyte and Doolittle [1982], revealed that the predicted protein sequence for matrin F/G has several large hydrophobic domains which are puncuated by short hydrophilic domains (Fig. 5). A secondary structure prediction based on the Chou-Fasman algorithm indicates that the protein has the potential to form approximately 45% beta sheet, 25% alpha helix, and 20% reverse turns (Fig. 6).

Since matrins F and G were both identified as DNA binding proteins, we searched the predicted protein sequence for known DNA binding motifs. We discovered two overlapping putative zinc finger domains which have similarity to the cysteine-cysteine type zinc finger motif [Fig. 6; Hakes and Berezney, 1991b]. This putative zinc finger domain is located in a region of the protein which has some interesting structural predictions. First of all this region does not have a large hydrohobic character, suggesting a potential external location. Secondly, it is predicted to be composed nearly exclusively of repeating reverse turns secondary conformation. Both of these properties are consistent with one or more zinc fingers extending from this region.

Within one zinc finger and flanking the other, there is a palindromic sequence of seven amino acids (Ser-Ser-Thr-Asn-Thr-Ser-Ser; see Fig. 6). Computer searches have failed to identify this sequence in any other known protein. This sequence contains a potential glycosylation site flanked on each side by potential phosphorylation sites. Since nuclear matrix proteins have been shown to be both phosphorylated [Allen et al., 1977] and glycosylated [Hart et al., 1989], it

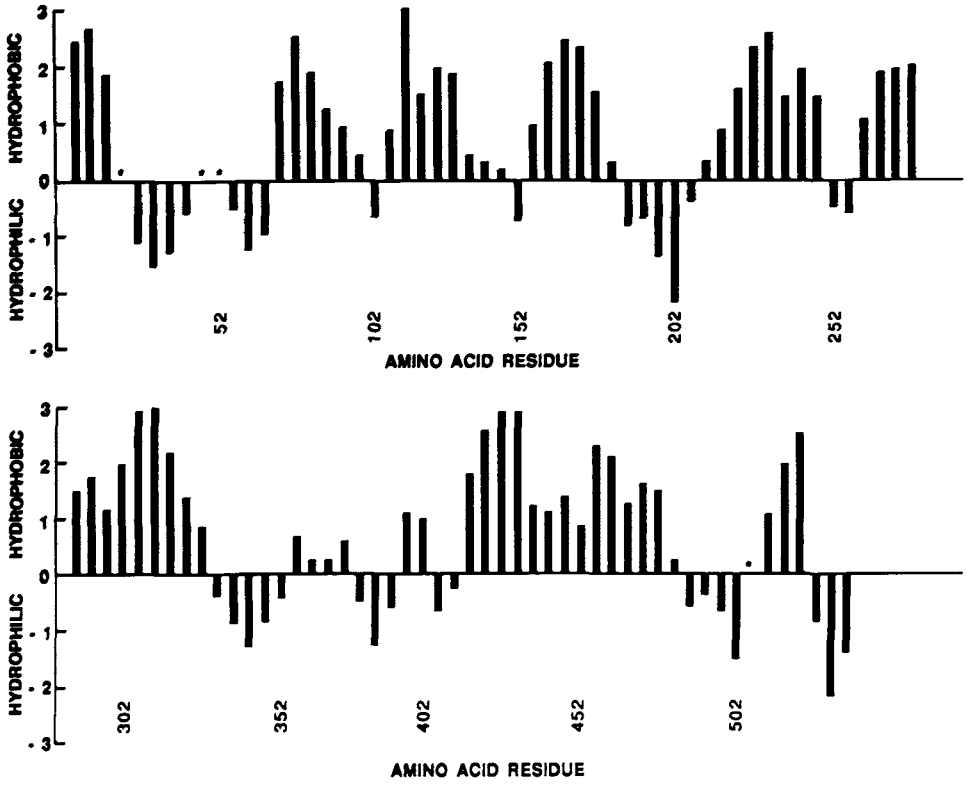

Fig. 5. Hydropathy plot of matrin F/G. The relative hydrophobicity/hydrophilicity is ploted along the amino acid sequence according to the algorithm of Kyte and Doolittle (1982). (Reproduced from Hakes and Berezney [1991b] with permission.)

is interesting to speculate that this sequence may serve as a regulatory site within the zinc finger domain. In this regard we have confirmed that Thr-380 in the palindromic sequence is a predicted casein kinase II phosphorylation site as is Ser-350 in another region of the zinc finger domain [Hakes and Berezney, 1991b].

It is likely that the next few years will see the elucidation of many of the nuclear matrix proteins using this molecular cloning approach. This will provide fundamental information about a family of proteins which are of obvious significance for nuclear organization and likely function but that have, until recently, defied analysis. For example, we have cloned and sequenced the cDNA for another nuclear matrix protein, matrin 3 [Belgrader et al., 1991a]. Matrin 3, in contrast to matrin F/G, is a very hydrophilic protein and shows no sequence homology to matrin F/G. These studies along with polyclonal and monoclonal antibody approaches will also set the stage for a more vigorous pursuit of defining the function of nuclear matrix proteins via DNA transfection, transgenic mice, and microinjection experiments.

NUCLEAR MATRIX FUNCTIONS

The nuclear matrix was first identified in whole cells as that region of the nucleus where the actively functioning chromatin is located along with the nonchromatin fibrogranular matrix structures [Berezney, 1984]. It is, therefore, not surprising that isolated nuclear matrices, which show a structural correspondence to the in situ defined structures, have a vast array of functional properties associated with them. Table I summarizes many of these major properties along with sample references for those readers interested in reading more about any particular topic. I apologize to the authors of the literally hundreds of other pertinent studies which cannot be directly referenced in this brief overview.

It is important to stress that while it is no surprise to see this multitude of functional properties ranging from DNA loop attachment sites, to DNA replication, to transcriptional associations, to RNA transcripts, to RNA splicing, to viral associations and their associated functions, to a vast number of regulatory proteins involved

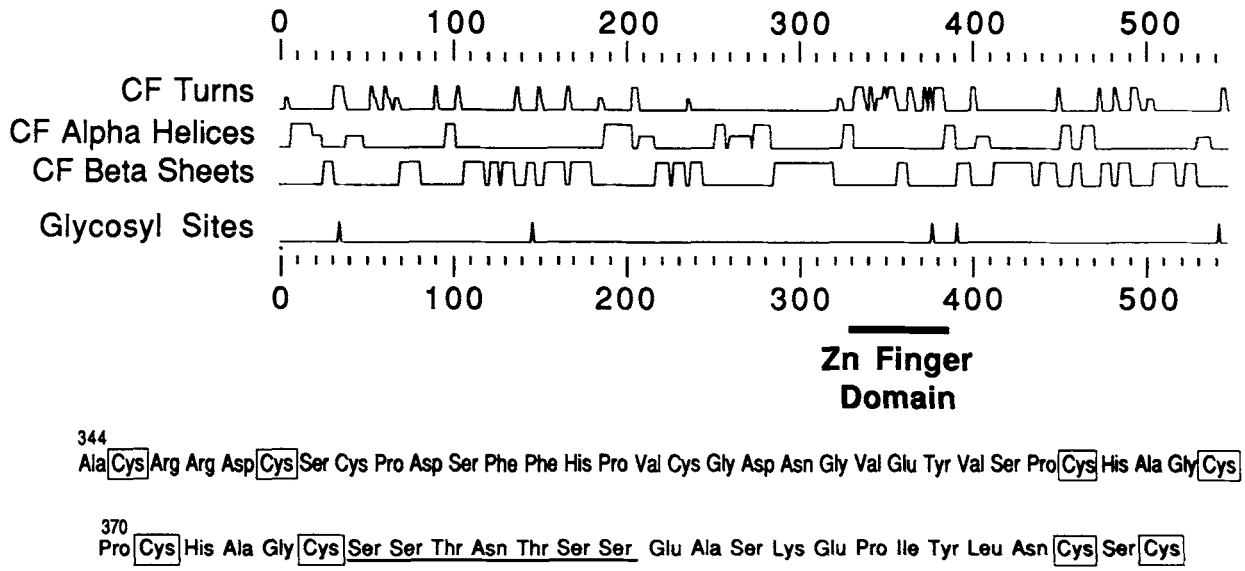

Fig. 6. Secondary structure prediction for matrin F/G and identification of a putative zinc finger domain. The predicted amino acid sequence for matrin F/G was run through a computer program which predicts secondary structure based on the algorithm of Chou-Fasman. The predicted regions of α-helices, β-sheets, and reverse turns are indicated. The region containing the putative zinc finger domains is indicated at the bottom of the plot. The cysteine residues which might occupy coordinate positions on the zinc fingers are boxed. A 7-mer palindromic sequence containing possible phosphorylation sites flanking a central predicted glycosylation site is underlined. (Reproduced from Hakes and Berezney [1991b] with permission.)

in the functioning and regulation of these properties (e.g., steroid hormone receptor receptors, oncogene proteins, heat shock proteins, calmodulin binding proteins, protein kinases), the true significance of these associations remains to be determined. Initial results, however, suggest that the isolated nuclear matrix is a potentially powerful in vitro approach for studying the molecular biology of higher order nuclear structure and function.

Since only limited studies have been performed on many of these properties, more studies are needed to better define the nature of the associations and the actual role(s) of the nuclear matrix structure in these processes. This is true even for those properties that have been studied in more detail, such as DNA loop attachment sequences (so-called "MAR" or "SAR" sequences [Gasser & Laemmli, 1987]), active gene sequences, RNA transcripts, viral associations, steroid hormone binding, and DNA replication. What is needed for each functional property is a detailed description of the associated function, what molecular constituents are involved, and the relationship of the in vitro function to in situ associations. Naturally, this last evaluation is most difficult and may require continued studies of the in vitro associations until enough is known to plan appropriate experiments at the level of whole cells. Hopefully molecular biology and "reverse genetic" approaches now possible with the development of recombinant DNA and molecular cloning research will provide considerable insight into these problems.

The last section of this article concentrates on one of the best-studied functions associated with the nuclear matrix: DNA replication. In particular recent experiments will be described that are designed to bridge the gap between in vitro matrix systems and replication in situ.

THE NUCLEAR MATRIX AND DNA REPLICATION

It is known that each enormous molecule of eucaryotic chromosomal DNA is divided into 100s to 1,000s of independent subunits of replication termed *replicons* [Hand, 1978]. Replication proceeds bidirectionally within each replicon subunit. Individual replicons are further organized into families or clusters of tandemly repeated subunits which replicate as a unit at particular times in S phase [Hand, 1975, 1978; Lau and Arrighi, 1981; Fig. 7A] Up to 100 or more replicons may be organized into each replicon cluster with an estimated average size of

TABLE I. Functional Properties Associated With Isolated Nuclear Matrix

Functional property	Reference
DNA loop attachment site sequences	Gasser and Laemmli, 1987
DNA binding proteins	Hakes and Berezney, 1991a,b
DNA topoisomerase II	Fernandes and Catapano, 1991
Replicating DNA	van der Velden and Wanka, 1987
Replication origins	Dijkwel et al., 1986
DNA polymerase alpha and primase	Tubo and Berezney, 1987c
Other replicative factors	Tubo and Berezney, 1987a
Active gene sequences	Zehnbauer and Vogelstein, 1985
RNA polymerase II	Abulafia et al., 1984
Transcriptional regulatory proteins	Feldman and Nevins, 1983
HN-RNA and RNP	Verheijen et al., 1988
Pre-ribosomal RNA and RNP	Ciejek et al., 1982
SN-RNA and RNP	Harris and Smith, 1988
RNA splicing	Zeitlin et al., 1987
Steroid hormone receptor binding	Rennie et al., 1983
Viral DNA and replication	Smith et al., 1985
Viral pre-messenger RNA	Mariman et al., 1982
Viral proteins	Covey et al., 1984
Carcinogen binding	Gupta et al., 1985
Oncogene proteins	Eisenman et al., 1985
Heat shock proteins	Reiter and Penman, 1983
Calmodulin binding proteins	Bachs and Carafoli, 1987
HMG-14 and HMG-17 binding	Reeves and Chang, 1983
ADP-ribosylation	Cardenas-Corona et al., 1987
Protein phosphorylation	Allen et al., 1977
Protein kinase C	Capitani et al., 1987
Reversible size changes	Wunderlich and Herlan, 1977

approximately 25 [Painter and Young, 1976; Hand, 1978]. The numerous reports that specific DNA sequences are duplicated at precise times within the S phase of eucaryotic cells [Goldman et al., 1984; Hatton et al., 1988] further support the conclusion that replicon cluster synthesis is temporally and spatially regulated along the chromosomal DNA molecule.

While the existence of replicon subunits, their bidirectional replication, and organization into functional replicon clusters are well documented, the mechanistic and molecular basis for these fundamental properties remains a long standing but unsolved mystery. Even less understood is what controls the exquisite spatial and temporal patterns of replicon cluster synthesis during S phase. It is nothing short of remarkable that the approximately 50,000–100,000 individual replicons that comprise the typical mammalian genome are programmed to replicate once and only once in a precisely choreographed process. This all implies a great deal of structural order underlying DNA replication in the cell nucleus. Somehow the molecular details of replication are integrated within the complex three-dimensional organization of the cell nucleus. As summarized below, the key player in this process may be the nuclear matrix.

Numerous studies of in vivo replicated DNA associated with isolated nuclear matrix have led to a radically new view of DNA replication inside the cell nucleus [Berezney and Coffey, 1975; Dijkwel et al., 1979; McCready et al., 1979; Pardoll et al., 1980; Berezney and Buchholtz, 1981b]. It is envisioned that replicating DNA loops corresponding to individual replicon subunits are bound to the nuclear matrix. Bidirectional replication then occurs by the reeling of DNA at the two ends of the loops through matrix-bound replisomes (Fig. 7B). Topographical organization of the replicating DNA loops and the associated replisomes into functional clusters or "clustersomes" may then provide the basis for replicon clustering.

Consistent with this clustersome model, DNA polymerase alpha, primase, and other replicative components have been found associated with isolated nuclear matrix. The in vitro synthesis of Okazaki-sized DNA fragments [Smith and Berezney, 1982], density shift experiments which indicate that the matrix-bound synthesis continues replication along in vivo–initiated DNA strands [Tubo et al., 1985], the striking replicative and pre-replicative association of DNA polymerase alpha, primase, and other replicative components with the nuclear matrix [Smith & Berezney, 1983; Tubo & Berezney, 1987a], and the ATP stimulated processive synthesis by the matrix bound polymerase [Tubo et al., 1987] all point towards a replicative related role of these matrix-bound activities.

The clustersome model further predicts that the replicational machinery (replisomes) for a large number of individual replicons are correspondingly clustered at nuclear matrix-bound sites (Fig. 7B). As a step toward testing this

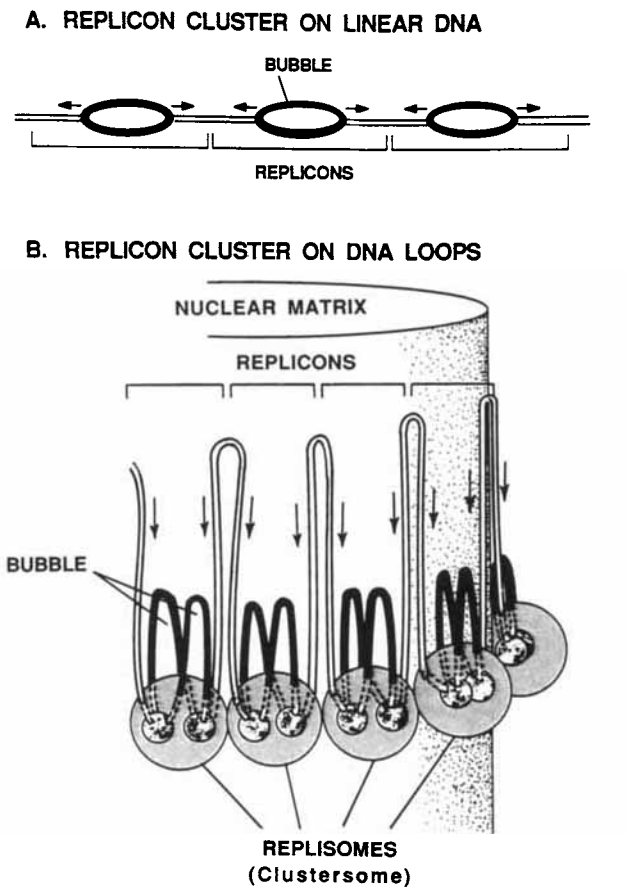

Fig. 7. The clustersome model of nuclear matrix-associated DNA replication. **A:** Replicon cluster on linear DNA. Bidirectional replication along three tandomly arranged replicons in a hypothetical replicon cluster is illustrated along a linear DNA molecule. The arrows show the directions of the growing replicational bubbles. Unduplicated DNA is shown in *white;* duplicated DNA (replicational bubbles) in *black.* **B:** Replicon cluster on nuclear matrix-attached DNA loops. The DNA of the same replicon cluster shown in A is now arranged in a series of loops attached to the nuclear matrix at fixed replicational sites. Each of these sites, known as *replisomes,* also contains the apparatus for copying the DNA. This occurs when there is reeling of DNA across the matrix-bound replisomes as shown by the arrows. Unduplicated DNA loops are shown in white; duplicated DNA loops (replicational bubbles) in black. Groups of replisomes cluster together to form a higher order assembly for replicon cluster synthesis termed the *clustersome.* (Reproduced from Tubo and Berezney [1987c] with permission of The American Society for Biochemistry & Molecular Biology.)

aspect of the model, we developed methods to extract the matrix-bound replicational complexes [Tubo and Berezney, 1987b]. Most of the matrix-bound DNA polymerase alpha and primase activities were released in the form of discrete megacomplexes sedimenting on sucrose gradients at approximately 100S and 150S. In contrast, complexes extracted from nuclei during nuclear matrix preparation sedimented at about 8–10S which is typical of DNA polymerase-primase complexes purified from cells. The rapid conversion of the megacomplexes into the more typically sized 10S complexes following release from the matrix structure suggested that the megacomplexes were composed of clusters of 10S complexes and might, thus, represent the in vitro equivalent of the predicted clustersome [Tubo and Berezney, 1987b].

A model for the arrangement of these putative nuclear matrix-bound clustersomes is shown in Figure 8A. While our biochemical results supported this model, the possibilities of rearrangements or aggregations during nuclear and/or nuclear matrix isolation could not be completely ruled out. What was needed was a method to directly visualize the sites of DNA replication in the nuclei of whole cells.

With this in mind, we developed a permeabilized mammalian cell system to study the incorporation of biotin-11-dUTP into newly replicated DNA. The sites of biotinylated, newly synthesized DNA were then directly visualized

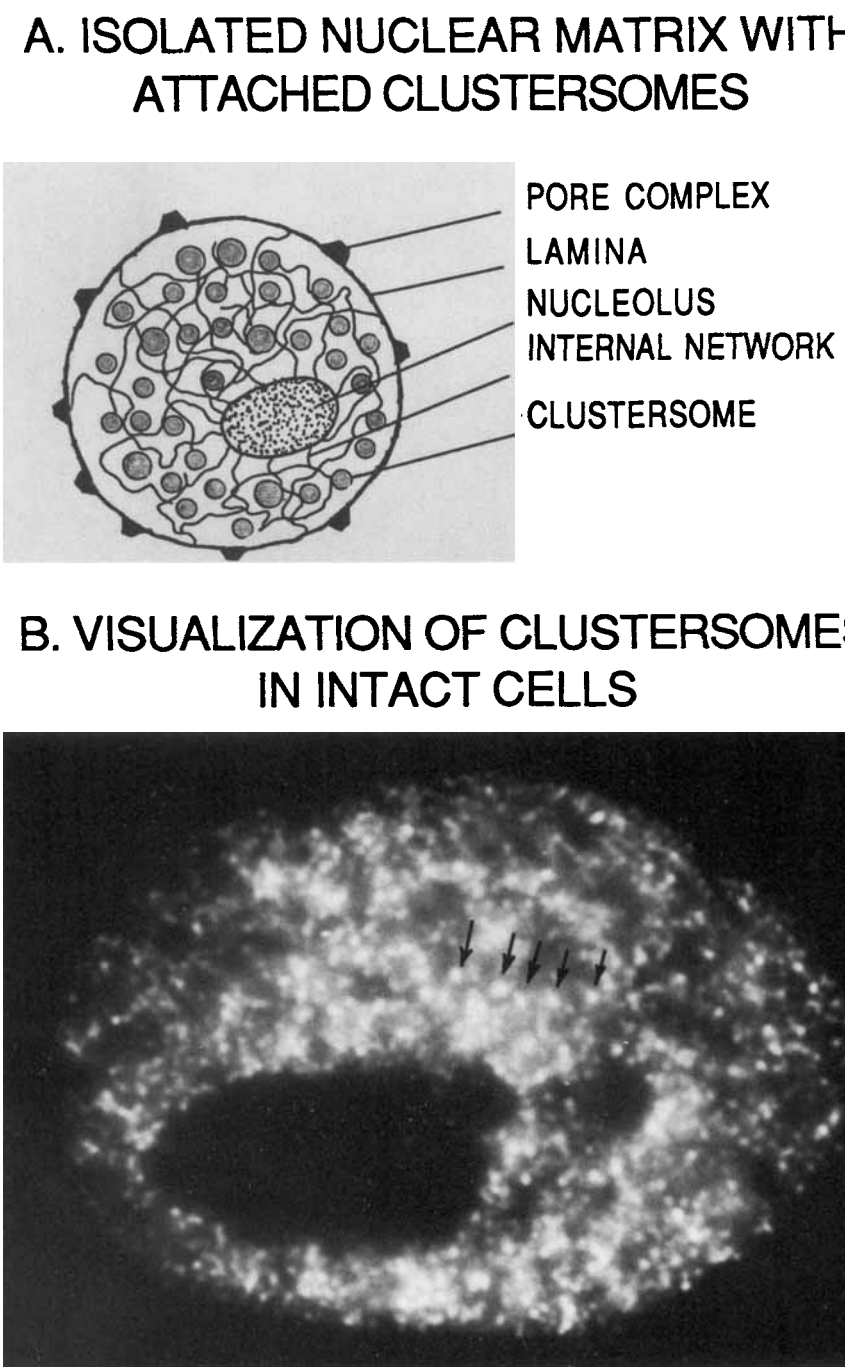

Fig. 8. Schmatic diagram of clustersomes attached to the nuclear matrix and direct visualization of replication sites with fluorescence microscopy. **A:** Schmatic diagram of an isolated nuclear matrix with associated clustersomes. The isolated nuclear matrix retains many of the basic architectural landmarks of the intact cell nucleus. In nuclear matrices from cells active in DNA replication, the replication sites are organized into large assemblies termed *clustersomes*. In **B** these clustersomes were directly visualized in whole cells by a fluorescence microscopic technique. The individual replication granules or clustersomes (arrows) are distinguished by their intense fluorescence (white granules). Hundreds of clustersomes are detected in each nucleus active in DNA replication.

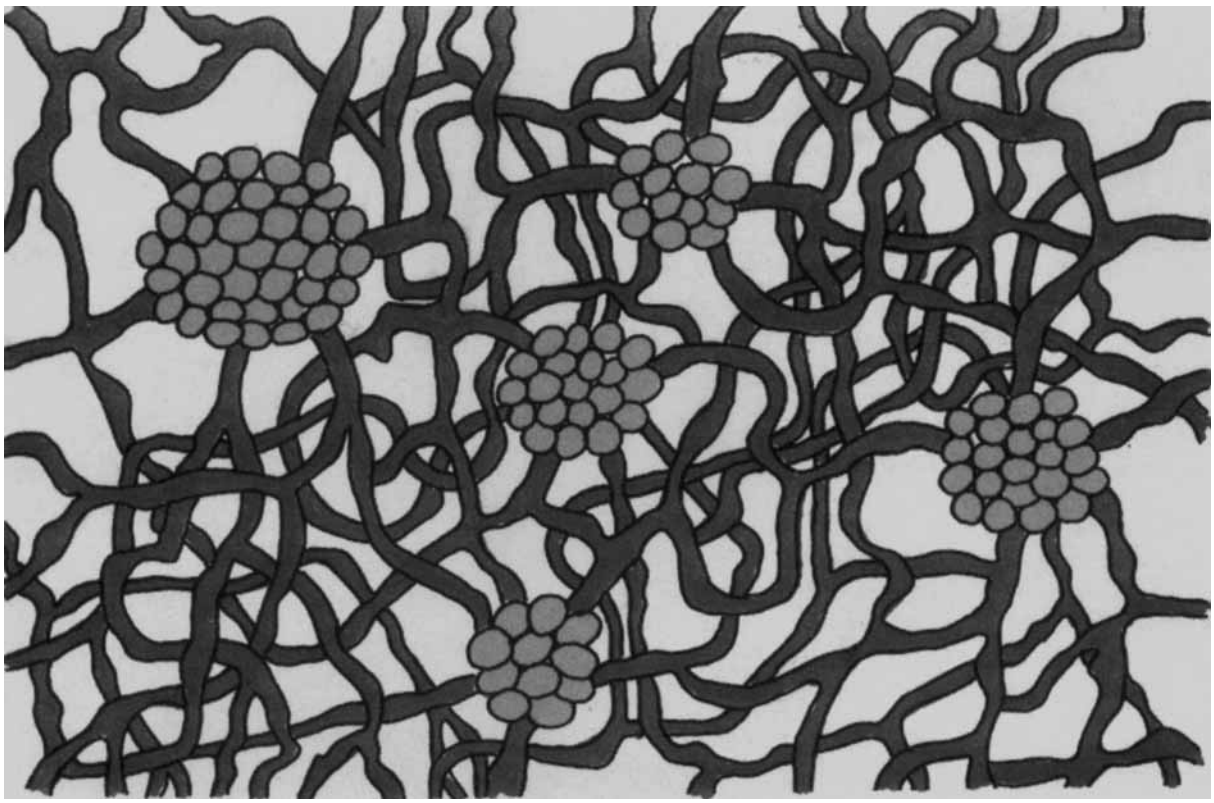

Fig. 9. Cartoon of clustersomes anchored to the three-dimensional matrix protein network. Our combined structural and biochemical studies lead us to propose that the clustersome is the basic functional unit of replication in the cell nucleus. Each clustersome is composed of a "cluster" of DNA loops (replicons) and a corresponding cluster of individual replisomes that mediate the duplication of each DNA loop. The clustersomes are in turn anchored to the three-dimensional matrix protein network. The individual replisome subunits that compose each clustersome are shown in light gray and the matrix scaffolding structure in dark gray. For simplicity the DNA loops for each replisome are not shown. It is believed that up to several hundred of these clustersomal functional units of replication are active at any time during the S phase.

by fluorescence microscopy following reaction with Texas red–streptavidin [Nakayasu and Berezney, 1989]. As demonstrated in Figure 8B, discrete granular sites of replication were observed. The number of replication granules per nucleus (150 to 300) and their size (0.4–0.8 microns in diameter) are consistent with each replication granule being the site of synthesis of a replicon cluster. At any given time in S phase one would anticipate that thousands of replicons would be active and arranged in up to several hundred clusters.

In addition, the characteristic size and shape of the individual replication granules remained the same while the fluorescence intensity progressively increased in pulse periods ranging from 2 to 60 minutes [Nakayasu and Berezney, 1989]. The size of the individual replication granules, therefore, is not determined by the amount of DNA which is replicated but is rather an inherent organizational property of each replication site. These results strongly support the previously proposed clustersome model.

To what extent are the in situ nuclear patterns of DNA replication maintained following nuclear matrix isolation? To address this question biotin-dUTP was first incorporated into permeabilized cultured cells (e.g., 3T3 fibroblasts or PtK_1 cells) followed by in situ extraction for nuclear matrix [Nakayasu and Berezney, 1989]. Alternatively, nuclear matrix structures were prepared followed by in vitro incorporation of biotin-dUTP via the nuclear matrix-bound DNA synthesis system. Replication granules were observed on the nuclear matrix which were virtually identical in size and number to those in cells. Identically appearing granules were also detected following DNA synthesis on the short fragments of DNA (circa 1–5 kb) attached to the in situ prepared nuclear matrices. These results demonstrate that components of the replicational machinery maintain

sites on the nuclear matrix which closely correspond to the presumed replicon cluster sites (clustersomes) in intact cells.

In conclusion, our combined structural and biochemical studies, both in vitro and in vivo, lead us to propose that the clustersome is the basic functional unit of replication in the cell nucleus. The clustersomes are assembled along the nuclear matrix where both their structural organization and function can be regulated.

As visualized in the schematic model of Figure 9, the clustersomes are part of a larger nuclear matrix network in the cell nucleus. Deciphering the components that compose both the clustersomes and the nuclear matrix network is a necessary step if we are to unlock the secrets of replicon clustering and their regulation in the eucaryotic cell. It is conceivable, for example, that the three-dimensional network of nuclear matrix-attached clustersomes may provide the structural basis for the functional networking and molecular cross-talking that is likely underlying the spatial and temporal regulation of replicon clusters.

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