INTRODUCTION

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Classical ultrastructural studies on the cell nucleus have demonstrated an association of euchromatin and heterochromatin with an extensive non-chromatinous structure in the interior of the nucleus (Agutter, 1991 ; Hoffman, 1993). It was shown 50 years ago that a fraction of nuclear proteins resist extraction even with buffers of very high ionic strength (Mayer and Gulick, 1942). This subfraction was found to be made of a nucleoprotein fibrillary network. The term 'Nuclear Matrix' was assigned to this structure by Berezney and Coffey (1974). DNA associated with the nuclear matrix contained A-T rich sequences and therefore termed as matrix associated regions (MAR) or scaffold associated regions (SAR). The scaffold forms the base chromatin loops during DNA replication in organisms ranging from yeast to man. SARs were shown to be usually located at the borders of different genes, close to 5' or 3'-end cis-regulatory sequences or DNase I hypersensitive sites suggesting that SARs might participate in the transcriptional regulation through regulatory domains (Avramova and Paneva, 1992). The nuclear matrix is now considered to be a valid structure with a few defined functions and probably several yet to be identified functions. Some of the nuclear matrix functions supported by experimental proof are :

(a) organization of chromatin in the interphase nucleus of eukaryotes into loops of 30-100 kbp and each of these loops represents either a replicational or transcriptional single unit.

(b) the localization of replicational machinery (replisomes) in the nuclear matrix (Tubo *et* a/., 1985) which is considered to be the site of DNA replication.

(c) the nuclear matrix is enriched with actively transcribed genes (Zehnbauer and Vogelstein, 1985) and is associated with the processing of RNA.

(d) the nuclear matrix acts as a site of interaction for viral proteins (Covey *et al.*, 1984) and viral DNA (Rennie *et al.*, 1983).

(e) the nuclear matrix contains binding sites for some hormones, carcinogens (Gupta *et al.*, 1985), tumor promoters (Eisenman *et al.*, 1985), drugs and other substances (Kaufmann *et al.*, 1986 ; Bareack and Coffey, 1982).

Methodological options for nuclear matrix preparation

The *in situ* nuclear matrix was first isolated and characterized by Berezney and Coffey (1974) in liver. Subsequently many others (Faken and Hancock, 1974 ; Cook and Brazell, 1975 ; Paulson and Laemmli, 1977 ; Grasser and Laemmli, 1986b) have isolated and characterized nuclear matrices from a variety of organisms. Several of these isolation methods involved treatment of isolated nuclei with nucleases, non-ionic detergent and high salt buffers. This sequential extraction of nuclei finally yielded a nuclear matrix fraction usually containing the granular and fibrous internal matrix, which forms a web throughout the interior of the nucleus, the residual elements of the nuclear envelope (also termed as the pore complex lamina) and residual nucleoli. A major modification of the foregoing method was suggested by Mirkovitch *et al.* (1984) who used lithium 3, 5-diiodosalicylate (LIS) a chaotropic agent and a detergent instead of buffers with high ionic strength. The resultant preparation, termed as "nuclear scaffold" has been widely used to study the DNA sequences specifically associated with the matrix. The nuclear scaffold also

has been described as the nuclear skeleton, nuclear ghost or nuclear cage. The nuclear matrices are predominantly proteinaceous. Depending on the type of isolation methods employed, several studies showed minor variations in the biochemical constitution of the nuclear matrix, for example, if ribonuclease was omitted, the isolated nuclear matrix contained RNA as the second most abundant component. Matrix preparations examined by electron microscopy showed several common structural entities such as fibrillary and granular internal nuclear matrix, pore complex lamina and residual nucleoli.

Some studies suggested that structural integrity of the nuclear matrix involves metallo-protein interactions during matrix isolation, based on the inclusion of Ca^{++} or Cu^{++} (Lebkowski and Laemmli, 1982) or Mg^{++} (Bouvier *et al.,* 1985a). Matrix preparations using Mg^{++} were found to be enriched with residual RNP complexes which formed a salt-resistant intra-nuclear network. Digestion with RNase in the presence of low ionic strength EDTA was shown to alter the morphology of network, suggesting a biochemical role for Mg^{++} (Bouvier *et al.*, 1985b). The internal fibrous network of the nuclear matrix is more labile than that of the nuclear lamina. Therefore, most of the nuclear matrix protocols involve a stabilization step to avoid dissociation of the fibrous network. Various methods entail stabilization by fixation with acroline (de Graaf *et al.,* 1991), brief incubation at 37 or 42^0C, treatment with Cu^{++} (Razin *et al.*, 1985) and oxidation with sodium tetrathionate (NaTT) (Kaufmann and Shaper, 1984). The mechanisms involved in stabilization are not known excepting for the method of oxidation with NaTT. NaTT oxidizes sulfhydryl groups to disulfide bridges, which results in the stabilization of nuclear matrix (Kaufmann *et al.*, 1986). Basing on this data, it was subsequently shown that a reduction of disulfides is important in disassembly of the nucleus at prophase (Sturrman *et al.,* 1992).

Nuclear matrix and DNA replication

Wanka *et al.*, (1982) suggested that the nuclear matrix might be involved in unwinding the DNA double helix in a specific manner so that the daughter molecules can be separated easily from the parent template during DNA replication. But their study could not conclusively demonstrate the matrix binding region on the DNA molecule or specific attachment sites for DNA on the nuclear matrix. Tubo and Berezney (1985) provided evidence, using density shift experiments, for the replisome loop model in eukaryotic DNA replication. On the basis of the relation between loop sites and replication sites, the bases of the DNA loop behave as replication origins. Studies of Dijkwel *et al.* (1986) showed the position of replication origin relative to the nuclear matrix by autoradiographic analysis of nuclear matrix halo structures. Using synchronized BHK cells, a label at the beginning of S-phase remained matrix associated and later on migrated into the DNA halo, suggesting that replication origins remain matrix bound after the initiation of DNA synthesis. Tubo *et al.* (1985) provided enzymatic evidence that approximately 10 % of the total cellular DNA primase activity was associated with isolated nuclear matrix, suggesting that the nuclear matrix is an important entity in the eukaryotic replication of DNA in the cell nucleus. Further studies by Dave *et al.* (1989) showed terminal deoxyribonucleotidyl transferase(s), a class of DNA polymerase, is involved in the postembryonic DNA synthesis of Immunoglobulin gene recombination events in thymus nuclei and is associated with the nuclear matrix for its expression. This enzyme catalyses the addition of deoxyribonucleotides to the 3'-(OH) terminus of DNA without template direction. All these findings conclusively demonstrate the involvement of the nuclear matrix in DNA replication and DNA synthesis.

Enzymes of DNA, RNA metabolism and the nuclear matrix

Several enzymes involved in DNA and RNA metabolism have been shown to be associated with the nuclear matrix. These include DNA α- and β-polymerases (Foster and Collins, 1985), topoisomerases I and II (Berrios *et* a/., 1985), RNA polymerase II (Lewis *et al.,* 1984), poly (A) polymerase (Schroder *et al.,* 1984), DNA methylase (Burdon *et al.,* 1985) and DNA primase (Tubo and Berezney, 1987a). Though the function of these enzymes is known, it is not understood how and why these enzymes are associated with the nuclear matrix.

Topological states of DNA and the nuclear matrix

The nuclear matrix has also been implicated in the conversion of different topological states of DNA. DNA Topoisomerase II, mediates the interconversion of DNA through transient double strand breaks and rejoining. Topoisomerase II can relax positive and negative supercoiled DNA, catanate and decatanate DNA rings and unknot the knotted DNA (Wang, 1985). DNA Topoisomerase II has been shown to bind in a cooperative manner to SAR. This suggests a role for the nuclear matrix in confirming specific topology to DNA through DNA topoisomerase II. Studies by Tsutsui *et al.* (1988) suggested that the nuclear scaffold exhibits at least two classes of DNA binding sites of which one is specific to supercoiled DNA and does not bind relaxed or linear forms, while the other lacked this specificity. Cockerill and Garrard (1986a) suggested direct anchorage of topoisomerase II to the chromosomal loop domains. They mapped the binding sites in the mouse immunoglobulin-k gene and found common sequences in the binding site and in the corresponding region of other genes (Cockerill and Garrdard, 1986b ; Udvardy *et at,* 1985). The DNA binding sites on the nuclear scaffold exhibited a recognition mechanism which was

not based on nucleotide sequence but rather was conformation directed, in the sense that tortional stress generated in the looped domain served as a recognition signal. Similarly in another study (Hinzpeter and Depperet, 1987), it was shown that concatemerized oligonucleotides posses an unwinding nucleation site with strong affinity for the nuclear scaffold, and with augmented SV40 promoter activity. Mutated concatemerized oligonucleotides resisted unwinding, showed weak affinity and a lack of enhancement of promoter activity, suggesting that relaxation of the superhelical structure of DNA by topoisomerase II is important for SAR functions (Bode *et al.*, 1992).

Nuclear matrix and gene expression

Buttyan and Olsson (1986) using androgen dependent genes, demonstrated that actively transcribed genes are protected from nuclease digestion by their association with the nuclear matrix. They further used nuclear matrix protection assays to analyse the tissue specific expression in a highly related gene family and to predict transcriptional activity of this gene family in a specific tissue. In support of foregoing, Keppel (1986) provided evidence that transcribable human ribosomal RNA (rRNA) genes are attached to the matrix and that the tandom repeats of ribosomal DNA (rDNA) are not randomly associated with the matrix but are probably attached at transcriptional complexes. Studies on the visualization of mRNAs of fibronectin and neurotensin using fluoresence hybridization with cDNA and intron specific probes indicate that there is a highly ordered structural organization in association with the nuclear matrix (Xing *et al.*, Carter *et al.*, 1993). Also, it has been demonstrated that a receptor binding factor (RBF-1) for the avian oviduct progesterone receptor (PR) has high affinity binding sites on avian genomic DNA, which

are localized in the nuclear matrix. The direct action of progesterone results in the rapid expression of nuclear matrix protooncogenes *c-myc* and *c-jun* (Schuchard *et al.*, 1991).

Nuclear matrix association with heterogeneous nuclear RNA (hnRNA)

Herman *et al,* (1978) in a two-step extraction of chromatin were able to remove 99 % of the chromatin. The remainder of the RNA is associated with the nuclear matrix. This fraction of residual RNA was found to be chiefly heterogeneous nuclear RNA (hnRNA). They suggested that the integrity of the nuclear matrix is dependent on this RNA. There are pros and cons for this interpretation. For example, Miller e/ *al.* (1978a) showed that RNase treatment of the nuclear matrix does not alter its morphology, while electron microscopic studies by Fey *et al.* (1986a) showed a drastic alteration in the morphology of the nuclear matrix when RNase was included in the preparation of the nuclear matrix. The data reported by Smith *et al.* (1986) agrees with other studies showing that internal matrix structures are distorted when nuclear matrix associated RNA is degraded or metal ions are chelated. Their data further suggested that internal nuclear matrix assemblies are present *in situ* and their absence in biochemical preparations might be an artifact. Knowledge of the composition and organization of hnRNA in the granular and fibrous internal nuclear matrix structure is required to understand how RNA is associated with the nuclear matrix. Gallinaro *et al.* (1983) showed that pre-mRNA in the nuclear matrix and in the salt resistant complexes derived from hnRNP share a common constitutive unit, suggesting that hnRNP and mRNA are structurally similar.

Small nuclear ribonuclear particles (snRNP) and the nuclear matrix

Like hnRNA, small nuclear ribonuclear particles (snRNPs) were found associated with nuclear matrices prepared from chicken oviduct (Ciezek *et al.*, 1982). The snRNPs (also called U RNAs) play a role in the processing of pre-mRNA. Of these, U1 to U6 RNAs are major U RNAs and U7 to U10 are minor U RNAs in eukaryotes. Zieve and Penman (1976) showed that U2, U3, U4, U6 RNAs were associated with the nuclear matrix. Though Ul RNA is also associated with the nuclear matrix, it was lost during chomatin extraction. The function of snRNPs is not known ; but one hypothesis is that they can act like a backbone structure thereby facilitating packaging (Berezney, 1984), post-transcriptional modification (Herman *et al.*, 1978) and transport of RNA into the cytoplasm (Gallinaro *et al.,* 1983). A specific interaction between snRNPs and nuclear matrix structures has been indicated by a limited number of studies (Padgett *et al.,* 1986 ; Bringmann and Luhrmann, 1986). However, these fail to establish the functional significance of such an interaction.

RNA transport and the nuclear matrix

Different RNA species are transported at different rates from the nucleus, and in general, smaller RNAs are transported rapidly into the cytoplasm than larger mRNAs. Most of the mRNAs are polyadenylated while some are non-polyadenylated such as histone mRNA. Studies by Mariman *et al.*, (1982) showed that adenovirus PIX mRNA (about 9s polyadenylated and unspliced) reaches the cytoplasm within 4 minutes after the start of synthesis while late adenovirus mRNA of same the size reaches the cytoplasm only after 16 minutes. The latter mRNA was shown to be matrix bound. The rate of transcription of mRNA depends

on the rate of maturation. Reasons for the different rates of transport of matrix bound mRNAs remain to be elucidated.

Virus specific proteins and the nuclear matrix

Studies have shown that the nuclear matrix is an important site for viral interaction. Viral DNA and viral specific proteins have been found to be enriched in the nuclear matrix. Studies by Hinzpeter and Deppert (1987) showed that the interaction of viral antigens with chromatin and the nuclear matrix is mediated by protein-protein interactions rather than by protein–DNA interactions.

The viral genomes such as the bovine papilloma virus type 1 (BPV–1) has a stretch of sequences which are located immediately adjacent to the origin of DNA replication. These sequences (672 bp DNA fragment) have been reported to interact with the nuclear matrix which is ultimately responsible for the replication of virus in the nuclei of tumor and transformed cells (Adorn and Richard-Fay, 1991).

Phosphorylation of the nuclear matrix

Post–translational modification of proteins is one of the regulatory mechanisms in cellular responses. Phosphorylation reactions involving protein kinases and phosphatases are often involved. The interplay between these two enzyme activities, results in the phosphorylation and dephosphorylation of specific substrates which ultimately brings about signal transduction events. Ca^{++}/ Calmodulin dependent protein kinases mediate several neuronal events in the cytosol, at synaptosomes and in the nucleus. Most nuclear Ca^{++}/ Calmodulin protein kinases have been shown to be associated with the nuclear matrix. Such an association leads

to compartmentalization, which provides a mechanism for the regulation of the enzyme access to substrates critically involved in nuclear functions. Halikowska and Leiw (1987) characterized a highly phosphorylated nuclear protein (68 kDa ; pI 6.5-8.2) associated with mononucleosomal particles and also with the nuclear matrix. This protein showed a high degree of phosphorylation in regenerating liver after partial hepatectomy. Extensive phosphorylation of this protein and its association with the nuclear matrix suggests that it plays a key role in nuclear organization and function. Further, Zhelev *et al.* (1990) showed a marked increase of 125 kDa (pI 6.5) protein in mitotic mammalian cells. The protein was named "mitotin" and was shown to be associated with the nuclear matrix. Accumulation of mitotin in premitotic and mitotic cells was related to the phosphorylation of this protein and the metabolic stability of its phosphorylated forms. These events suggest a role for phosphorylation and for the nuclear matrix in the complex events of mitosis. In another study, differences in the nuclear matrix phosphoproteins in wild type and nitrogen mustard-resistant rat breast carcinoma cell lines have been reported (Moy and Tew, 1986). Though the antigens in the two tumor cell lines were similar, one of them, due to its hypophosphorylation, failed to bind cyclic-AMP (c-AMP) and thus differed from the other. Phosphorylation of matrix proteins leading to structural alterations in the nuclear matrix suggested that the nuclear matrix can change its configuration depending on the functional requirements of the cell.

Cytoskeletal proteins and the nuclear matrix

During transcription in eukaryotes, RNA polymerase II has to be activated by binding to promoter elements such as TATA box (Breathnach and Chambon, 1981). Eukaryotic cells contain multiple factors which

enable the binding of RNA polymerase II to this promoter element. One of these multiple factors has been purified, characterized and was found to be similar to nuclear actin (Egly *et al.*, 1984). Moreover, antibodies to nuclear actin when injected into oocytes, stopped transcription by RNA polymerase II, suggesting a possible role for nuclear actin in RNA metabolism. Nakayasu and Ueda (1984) have shown an interaction between pre mRNA and actin filaments in the nuclear matrix of mouse leukemia L5178Y cells. Other studies also detected actin as a major protein in nuclear matrices (Nakayasu and Ueda, 1986). These observations suggest that actin might be involved in a well defined function in RNA synthesis. However, the forgoing possibility requires further study.

Nuclear matrins are major nuclear matrix proteins

The protein composition of the nuclear matrix was recently analysed in detail (Nakayasu and Berezney, 1991). Approximately 12 major proteins were identified, of which nuclear lamins A, B and C and the nucleolar protein B-23, and residual hnRNPs constitute already identified proteins. The rest of the proteins termed nuclear matrins consist of matrin 3 (125 kDa slightly acidic), matrin 4 (105 kDa, basic), matrin D-G (60-75 kDa, basic) and matrins 12 and 13 (42-48 kDa, acidic). Peptide mapping studies showed no homology of these matrins to nuclear lamins. Matrin-3 had an extensive acidic domain with a nuclear targeting signal sequence, and is a highly conserved protein (Belgrader *et al.*, 1991a). Interestingly, matrins D-G comprise two pairs of related proteins (matrins D/E and F/G). The F/G matrin was found to be a DNA binding protein containing two putative zinc finger motifs (Hakes and Berezney, 1991a). A palindromic seven amino acid sequence (Ser-Ser-Thr-Asn-Thr-Ser-Ser) was discovered within one of the zinc finger DNA binding regions.

This sequence appears to be a potential site for phosphorylation and glycosylation and therefore might be involved in a regulatory role within the DNA binding domain.

In addition to F/G, **lamina** A and C (but not B), matrins D and E were reported to be specific DNA binding proteins having preference for single strand DNA (Hakes and Berezney, **1991b),** probably each with a separate sequence specificity. In DNA binding assays they showed preference for nuclear matrix DNA over total genomic DNA. These studies directly demonstrated that internal nuclear matrix proteins (D,E,F,G and 4), in addition to the **lamina** A and C, can bind to DNA, suggesting that loop attachment sites are internal as well as peripheral in the nucleus during DNA replication.

In another study, a DNA binding protein (SATB1) (human cDNA clone) from thymus having selective binding to MARs has been reported (Dickinson *et al.*, 1992). SATB1 showed an unusual binding site recognition in the sense that it binds to a ATC rich sequence, wherein one strand consists of mixed A's, T's and C's excluding G's. In mutants deficient **in** the ATC rich sequence, there was a significant reduction in binding, even when the direct contact sequence remained intact. This suggested that **SATB1** binding to MAR's is through the recognition of the ATC sequences by sugar-phosphate back bone structure of DNA. Luderus *et al.*, (1992), employed a heterogenous binding system using matrix preparations from rat liver and the MARs from the histone cluster of *Drosophila*, MAR binding nuclear proteins were identified. They measured MAR binding to lamins from calf thymus and *Drosophila.* They found a 67 kDa matrix protein, which was identified as **lamin B1** and also another minor protein lamin B2. They further suggested lamin B interacts with chromosome by

directly binding to MAR sequences leading to a decondensation of the chromatin, the repolymerization of lamina or both. This type of mechanism probably safeguards chromatin organization and argues for a key role of lamin B in certain cells that lack lamin A and C.