The nuclear matrix: a critical appraisal

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Summary. It is becoming increasingly clear that the cell nucleus is a highly structured organelle. Because of its tight compartmentalization, it is generally believed that a framework must exist, responsible for maintaining such a spatial organization.

Over the last twenty years many investigations have been devoted to identifying the nuclear framework. Structures isolated by different techniques have been obtained in vitro and are variously referred to as nuclear matrix, nucleoskeleton or nuclear scaffold. Many different functions, such as DNA replication and repair, mRNA transcription, processing and transport have been described to occur in close association with these structures. However, there is still much debate as to whether or not any of these preparations corresponds to a nuclear framework that exists in vivo. In this article we summarize the most commonly-used methods for obtaining preparations of nuclear frameworks and we also stress the possible artifacts that can be created in vitro during the isolation procedures.

Emphasis is placed also on the protein composition of the frameworks as well as on some possible signalling functions that have been recently described to occur in tight association with the nuclear matrix.

Key words: Nuclear matrix, Isolation techniques, Stabilization, Protein composition, Functions

Introduction

It has commonly been thought that the cell nucleus is relatively unstructured, especially when compared with the tight compartmentalization found within the cytoplasm. An obvious reason for such a view is that there are no membrane structures inside the nucleus. Nevertheless, apart from very prominent domains such as the nuclear envelope with the pore complexes (Panté and Aebi, 1995), the nucleolus with its sub-compartments (Hernández-Verdun, 1991) and heterochromatin, the nucleus also contains a heterogeneous group of inclusions, such as interchromatin granules (Clevenger and Epstein, 1984; Thiry, 1993, 1995; Ferreira et al., 1994), nuclear bodies (Brasch and Ochs, 1992) and coiled bodies (Thiry, 1994), that have been known since a long time.

Thanks to the outstanding advances made by molecular biology techniques, our knowledge about the genetic machinery and its regulation or how DNA is replicated is continuously growing. Now genes can be identified, purified, sequenced, changed at our will, reintroduced into cells and expressed there as proteins. However, an overall view about how functions are structurally integrated within the nucleus is still lacking (Manfredi Romanini and Fraschini, 1996).

During the last few years, morpho-functional studies employing antibodies against molecules involved in DNA replication and transcription, DNA repair, mRNA processing and transport, steroid hormone binding sites, have revealed that these phenomena are highly compartmentalized in the nucleus, because they take place in well-distinct domains, of which some correspond to the aforementioned inclusions (e.g. Lawerence et al., 1989; Nakayasu and Berezney, 1989; Mazzotti et al., 1990; Carter et al., 1991; Neri et al., 1992; Hassan and Cook, 1993; Wansink et al., 1993; Blencowe et al., 1994; Jackson et al., 1994; Zhang et al., 1994; Bisotto et al., 1995; van Steensel et al., 1995).

Thus, these morpho-functional investigations have demonstrated that a division in compartments is indeed present in the nucleus, though it is not clearly evident at first glance. A belief common to many investigators is that, if the nucleus is indeed strictly compartmentalized, a structure must exist, responsible for maintaining such a rigid subdivision.

Investigations carried out over the last twenty years have demonstrated that when the nucleus is stripped of most DNA, RNA and soluble proteins (mainly histones) a structure remains that has been variously referred to as the nuclear matrix, or scaffold or the nucleoskeleton (Berezney, 1984, 1991; Verheijen et al., 1988; de Jong et al., 1990; van Driel et al., 1991; Jack and Eggert, 1992;
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Cook, 1995). Such a structure, acting as a framework, would provide attachment sites to the structural domains (or DNA loops) in which the eukaryotic genome is subdivided during interphase (e.g. Gerdes et al., 1994). Indeed, it is bewildering to realize that in a typical diploid mammalian cell about 2 μm of double-stranded DNA are packed within a nucleus with a diameter of approximately 10 μm. To achieve this, the DNA double helix is first coiled into nucleosomes (11 nm diameter), then nucleosomes into solenoids (30 nm diameter) which are thought to be folded into loops of tens of kilobasepairs (Cook, 1995). It is clear that only a highly structured organelle may handle the topological problems arising for example during duplication of DNA.

However, there is always uncertainty as to whether or not the nuclear matrix, isolated in vitro, could really represent a framework of the nucleus in vivo. In fact, the only nuclear framework of which the existence is universally accepted is the nuclear lamina, a continuous thin fibrous layer that underlies the inner nuclear membrane and is mainly composed from three related proteins named lamin A, B and C (Moir and Goldman, 1993; Dessev, 1994).

A problem that has plagued this field of investigation is the use of different names to denote very similar structures. Indeed, this has caused a remarkable confusion even between investigators involved in this kind of research. Throughout this paper, we will mainly refer to structures representing putative nuclear frameworks as nuclear matrix, also because of historical reasons.

In this review, we shall focus on the various methods that have been employed to isolate the nuclear matrix and then we shall tackle an issue that is becoming increasingly important, that is the «stabilization» procedures often used to prepare the nuclear matrix. Lastly, we will briefly review the protein composition as well as the numerous functions that have been described to occur in association with the matrix.

The high-salt matrix

More than 20 years ago, Berezney and Coffey (1974) first introduced the term «nuclear matrix» to denote a mainly proteinaceous residual structure, retaining the overall size and shape of the nucleus, that is obtained by sequential treatments of isolated nuclei with nuclease and buffers of low and high ionic strength. This extraction protocol is by far the most widely used to obtain preparations of nuclear frameworks, conceivably because of its inherent simplicity, and many of the functional studies regarding the possible roles performed by the nuclear matrix have been performed with this type of preparation (see for example the classic papers by Berezney and coworkers about DNA replication: e.g. Smith and Berezney, 1982, 1983; Tubo and Berezney, 1987a). Transmission electron microscope investigations revealed that the nuclear matrix is composed from three well-distinct domains: a peripheral lamina containing residues of the pore complex; an ill-defined inner fibrogranular network; and residual nucleoli (Maraldi et al., 1986). Using the EDTA regressive staining and thin sectioning, a similarity was seen between the matrix fibrogranular network and a framework that can be evidenced by transmission electron microscopy in the nucleus of intact cells (Berezney, 1984). Such an observation strengthened the belief that a matrix would also exist in vivo. The original protocol devised by Berezney and Coffey (1974) called for the use of 2 M NaCl to extract histones and soluble or loosely-bound nuclear proteins, but subsequently it has been claimed that use of lower salt concentrations (for example 0.25 M (NH₄)₂SO₄ may lead to a better preservation of the morphology without significantly affecting the efficiency of protein extraction (e.g. Belgrader et al., 1991a). It is very important to note that many variations from the original protocol have been described, depending on the cell type being used. Indeed, the original matrix isolation technique was described for rat liver nuclei, that are extremely resistant to repeated centrifugations.

Moreover, during nuclear isolation, in rat liver there is a spontaneous formation of disulfide bonds, with a consequent «stabilization» of the framework (see later). The introduction of endless variations is not without consequences, for even apparently insignificant changes can lead to dramatic variations. For example, if an enrichment of matrix-bound newly replicated DNA needs to be seen, it is imperative that extraction with high salt precedes nuclease digestion (Pardoll et al., 1980; Djondjurov et al., 1986) and the same holds true for enrichment of actively transcribed genes (Kirov et al., 1984).

The detergent-extracted matrix

In 1984 Lacmml and associates (Mirkovitch et al., 1984) described the use of an ionic detergent, lithium diodosalsicylate (LIS) for extracting histones and other nuclear proteins. The authors were concerned about possible artifacts created by the use of 2 M NaCl during their search to identify and characterize DNA sequences that remain bound to the nuclear matrix. However, when such an extraction agent is used, it is mandatory to «stabilize» isolated nuclei by exposing them by a brief time to heat in vitro (37 °C or 42 °C) or to millimolar concentrations (typically 0.5mM) Cu²⁺ (see later for problems created by this stabilization). If stabilization is indeed performed, the final structures (referred to as nuclear scaffolds) will contain all three classical matrix domains, otherwise only the peripheral lamina will be present, and the final structures are usually referred to as nuclear shells (Ludérus et al., 1992). It should be recalled that the term nuclear scaffold was originally employed to indicate matrix preparations obtained by extracting nuclei with polyanions such as dextran sulphate or heparin (Adolph, 1980).

The LIS extraction technique allowed the identification of matrix-associated regions (MARs), also called scaffold-associated regions (SARs), that is, AT-rich stretches of 300-1000 bp, highly conserved during the evolution and believed to anchor DNA loops to a nuclear framework (Mirkovitch et al., 1984; Izaurralde et al., 1988, 1989). It is worthwhile recalling that MARs sequences bind to heterologous nuclear matrices (Izuarralde et al., 1988, 1989). MARs are common at the boundaries of transcription units and are often found near enhancer-like regulatory sequences (see Wang et al., 1995) but have been implicated also in DNA replication and chromosome segregation, at least in yeasts (Amati and Gasser, 1988). However, subsequent studies have shown that MAR binding sites are present also in matrices prepared by 2M NaCl extraction (Izuarralde et al., 1988) but further evidence suggests that the major part of the binding sites are generated in vitro during stabilization of nuclei by heat (Jackson et al., 1990a,b). Moreover, in nuclei not exposed to nonionic detergent, an ectopic SAR sequence does not bind to the nuclear scaffold, whereas it does in permeabilized nuclei (Eggert and Jack, 1991). The classic biochemical studies by Laemmli and coworkers hinted at the fact that proteins binding MAR sequences may bind SARs in vitro have been identified (see later).

The agarose-embedded matrix

Since chromatin tends to aggregate under isotonic conditions, it is necessary to isolate nuclei using unphysiological (highly hypotonic) salt concentrations. The aggregation can also be prevented by using Mg++, but this has an adverse effect on chromatin structure and activates nucleases (Cook, 1988). For this reason, Cook and coworkers have developed a procedure in which HeLa cells, growing embedded in agarose microbeads (50-150 μm in diameter), are permeabilized with a mild nonionic detergent (0.1% Triton X-100) in a «physiological» buffer (e.g. Jackson et al., 1988). Then DNA is removed by restriction enzymes and subsequently electroeluted. However, it should be emphasized that, so far, nobody really knows the exact ionic composition of the nuclear interior, thus the term «physiological» must be interpreted with caution. Moreover, even if this technique has been claimed to be very mild, it should be noticed that evidence has demonstrated that when cultured cells are permeabilized with 0.1% Triton X-100, there is a redistribution of cytoplasmic proteins to the cell nucleus (Meland and Sluder, 1992).

Nevertheless, these kind of preparations (referred to as nucleoskeletons) offer several advantages over the others, because the gross structural morphology is preserved, there is DNA integrity (i.e. DNA is supercoiled after removing histones) and, most importantly, these structures synthesize DNA and RNA at a rate found in vivo (e.g. Jackson et al., 1992). It should also be noted that these nucleoskeletons do not require heat «stabilization» in vitro (see later, and Jackson et al., 1990a). In any case, this isolation protocol has not been frequently used, perhaps because the method is quite complex and the preparations are not well-suited to biochemical studies. Nevertheless, such a method has permitted to size in 86 kb the average length of DNA loops in HeLa cells (Jackson et al., 1990a,b). Moreover, electron microscope immunolabelling has shown in this type of framework the existence of «replication or transcription factories» (Hughes et al., 1995), that is, large structures containing, beside newly-synthesized DNA or RNA, several factors involved in DNA duplication or transcription, such as DNA polymerase α, proliferating cell nuclear antigen (or PCNA, i.e. the 36 kDa accessory protein to DNA polymerase δ), RP-A, RNA polymerase II, etc (Hozak et al., 1993; Hughes et al., 1995). It is of interest that at least some of these factories correspond to a sub-set of nuclear bodies observed in the past in thin sections (Brasch and Ochs, 1992; Hozak et al., 1994). Cook and associates have elaborated a model of matrix-associated DNA transcription and replication in which it is envisaged that both of these events take place as template slides through fixed sites (i.e. the factories) located around a nucleoskeleton (but see also Pandoll et al., 1980; Vogelstein et al., 1980; Tubo et al., 1987; Tubo and Bereczney, 1987b). Overall, many of the results obtained with this type of preparation are in agreement with data gained from nuclear frameworks obtained in a more conventional way. Lastly, it should be recalled that Hozak et al. (1995) have demonstrated, by immunolabelling at both the light and electron microscope level, the presence in the interior network, of nucleoskeletal structures, of nodes containing lamin A. Such an observation, which follows a number of previous reports dealing with the intranuclear presence of laminins (e.g. Moir et al., 1994), suggests that the inner framework could be somewhat related to the cytoskeleton, as nuclear lamins share much sequence homology with intermediate filaments (e.g. McKeon et al., 1986). Therefore, the authors envisioned the existence of a common cell skeleton, spanning from the plasma membrane to the nuclear interior (Hozak et al., 1995), in agreement with other suggestions (Pienta et al., 1989; Getzenberg et al., 1991a,b; Pienta and Coffey, 1992) and their own previous observation about lumps of chromatin attached to an intermediate-filament-like skeleton (Jackson and Cook, 1988).

The in situ matrix

Capco et al. (1982) and Staufenbiel and Deppert (1984) first described a method in which adherent cells are extracted in situ with nonionic detergents and salt solutions and digested with nucleases. These structures are referred to as in situ nuclear matrix or nuclear
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matrix-intermediate filament complexes. The obvious advantage of this type of isolation technique is that the time required for obtaining the final structures is much shorter than in conventional preparations, because there are no time-consuming centrifugations. Furthermore, there is probably a better preservation of the morphology because of the absence of centrifugations at high speed. The major disadvantage is that these preparations also retain cytoskeletal components, so that biochemical studies (for example electrophoretic analysis of polypeptides) must deal with this fact, even though Fey and Penman (1988) subsequently claimed the discovery of a method allowing the separation of the nuclear matrix from cytoskeletal components. Penman and coworkers have also emphasized the necessity to use 0.25M (NH₄)₂SO₄ as a milder extracting agent and they have stressed the role played by RNA in maintaining the structural integrity of the inner network by demonstrating that the RNase inhibitor, vanadyl ribonucleoside complex, is necessary for isolating intact filaments. Conversely, the use of RNase A completely destroys the inner matrix (Nickerson et al., 1989; He et al., 1990). If the nuclear matrix-intermediate filament complex (obtained after treatment with (NH₄)₂SO₄) is further extracted with 2M NaCl, a network of 9 and 13 nm "core filaments" becomes visible, which can be studied by whole mount and resinless thick section electron microscopy (He et al., 1990). The filaments can be completely disrupted by treatment with RNase A. By means of a monoclonal antibody, Nickerson et al. (1992) have identified a high molecular weight (240-kDa) protein (H1B2) that is a component of the core filament. Interestingly enough, the antigen is masked in the interphase nucleus while it is uncovered as chromatin condensation takes place during mitosis. It should be pointed out that protein composition of core filaments, though quite complex, is not very different from the polypeptide profile of the material extracted by 2 M NaCl (He et al., 1990).

The in situ matrix is well suited for morpho-functional studies having as a goal the identification of the distribution of nuclear matrix proteins and their relationship with phenomena like DNA replication and transcription, DNA repair, RNA splicing, mRNA transport etc. (see Introduction for the references).

This technique has demonstrated that some types of nuclear bodies can be considered part of the nuclear matrix, because at least some of their constituents resist treatment with salt and nucleases (Brasch and Ochs, 1992). This applies to coiled bodies, a structure first identified almost one hundred years ago by Ramón-y-Cajal (for a review see Bohmann et al., 1995a), and containing the small nuclear ribonucleoproteins (snRNPs) U1, U2, U4, U5 and U6, the autoantigen p80 collagen, as well as proteins deriving from the nucleolus like fibrillarin, Nopp 140, NAP 57 and ribosomal protein S6 (Bohmann et al., 1995a). Very recently Bohmann et al. (1995b) have shown that coiled bodies might interact with nucleoli in regulating ribosomal RNA transcription and maturation, while Frey and Matera (1995) have demonstrated that they also contain snRNP U7 and associate with specific DNA sequences. Another striking example is the doughnut-shaped nuclear body containing the 126-kDa matrix protein recognized by monoclonal antibody 5E10 (Stuurman et al., 1992a) as well as the PML protein, that is part of a fusion product with the retinoic acid receptor α (RARα), resulting from the t(15;17) chromosomal translocation associated with acute promyelocytic leukemia (De Thé et al., 1991). It is extremely interesting to note that the PML-RARα hybrid displays an altered nuclear localization when compared to that of its normal PML and RARα counterparts (Weis et al., 1994). In leukemic cells the major part of PML nuclear bodies are disorganized into several aberrant microstructures containing both PML and PML-RARα. Importantly, treatment of the neoplastic cells with retinoic acid restores both the normal morphology and the number of the bodies (Koken et al., 1994). Moreover, infection with adenovirus causes both a redistribution and morphological changes of the nuclear bodies (Puvion-Dutilleul et al., 1995). Several viral proteins (E4-ORF3 E1A and SV40 large T antigen) localize to or accumulate in close proximity to these bodies suggesting that they might be a preferential target for small DNA tumor virus oncoproteins (Carvalho et al., 1995). These data are in agreement with the results by Smith et al. (1985) and Pombo et al. (1994) concerning nuclear matrix and adenovirus DNA replication. This nuclear domain is the same as described by Ascoli and Maul (1991), identified by monoclonal antibodies and human auto antibodies. It is also indicated as ND10 and contains protein SP100, and autoantigen in primary biliary cirrhosis, as well as 55- and 52-kDa polypeptides (see Koriath et al., 1995). It should be recalled, however, that other nuclear domains have been identified that are not part of the nuclear matrix, because they do not resist treatment with DNase I. A well-known example is the region called PIKA (polymorphic interphase karyosomal association) described by Saunders et al. (1991) containing three to four structurally-related proteins (23-25-kDa in size) that are recognized by a human antisemur.

Stabilization of the matrix

In 1981 Kaufmann et al. first demonstrated that when the nuclear matrix is prepared from rat liver nuclei, formation of S-S bonds spontaneously occurs during isolation of nuclei. If formation of the bonds is inhibited by alkylating agents such as n-ethylmaleimide and/or iodoacetamide the inner matrix and residual nucleoli are barely recognizable while the peripheral lamina is still detectable (Kaufmann and Shaper, 1984). Treatment with RNase A and dithiothreitol results in even more empty structures showing only the peripheral lamina. For this reason, formation of disulfide bonds, acted by the cross-linking agent sodium tetrathionate (NaTT), has been deliberately used to stabilize the inner network.
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(Suurman et al., 1990; Nakayasu and Berezney, 1991). It is worth remembering that the stabilization created by NaTT had been erroneously interpreted as the result of protease inhibition (Berezney, 1979). Two important issues need to be emphasized about this form of stabilization. Firstly, available evidence suggests that proteins of the nuclear matrix are not cross-linked by disulfide bonds in vivo (Kaufmann and Shaper, 1991). Secondly, it seems that spontaneous formation of disulfide bonds occurs only when nuclei are isolated from normal rat liver or hepatoma cells (Kaufmann and Shaper, 1984, 1991) because treatment with iodoacetamide is ineffective when used in HeLa cells or mouse erythroleukemia cells (Van Eekelen et al., 1982; Belgrader et al., 1991a; Martelli et al., 1992a). In these cases, the inner matrix and nucleolar remnants are present even if nuclei were exposed to sulfhydryl blocking agents. Interestingly enough, the formation of intranuclear disulfides also takes place when the matrix is prepared in situ from hepatoma cells (Kaufmann and Shaper, 1991).

Laemmli and coworkers described the stabilizing effects of denaturing cations such as Cu++ or Ca++, especially when nuclei were exposed to these agents at a temperature of 37 °C or above (Lebkowski and Laemmli, 1982; Lewis et al., 1984). Mirkovitch et al. (1984) used a 37 °C incubation of isolated nuclei from Drosophila cells to stabilize the inner matrix before LIS extraction and to preserve interactions between the scaffold and SARS. In 1985, Evans and Hancock unequivocally demonstrated the stabilizing effect of a 37 °C incubation of isolated nuclei, leading to the insolubilization of a specific subset of nuclear proteins. It is very important to note that up to that time a 37 °C incubation has been inadvertently used by many investigators, for example during nuclease digestion. Therefore, an unnoticed stabilization of the matrix inner network had been obtained.

The stabilizing effect of heat is a universal phenomenon because it has been observed in nuclei isolated from a variety of cell lines and even from yeasts (Boyle and Baluda, 1987; Humphrey and Pigiet, 1987; McConnell et al., 1987; Berrios and Fisher, 1988; Martelli et al., 1990, 1991). So far, the biochemical mechanism(s) underlying this stabilization have escaped clarification. However, it should be stressed that an analogous stabilization occurs when nuclei are immediately prepared from cells exposed in vivo to temperatures that produce a heat shock response (Littlewood et al., 1987; Martelli et al., 1991; Wachsberger and Coss, 1993). It is commonly thought that during heat shock nuclear proteins become denatured with a subsequent exposure of hidden stretches of hydrophobic amino acids (Becker and Craig, 1994; Hartl et al., 1994). This in turn leads to aggregation and precipitation ensuing in insolubilization of nuclear proteins. It is very interesting to note that during heat shock a redistribution of nuclear proteins, of which some belong to the nuclear matrix, takes place (McConnell et al., 1987; de Graaf et al., 1992; Neri et al., 1995a). Our recent results also indicate that an in vitro incubation at 37 °C of isolated nuclei causes changes in the distribution of nuclear matrix proteins and, most interestingly, that changes seen after in vitro incubation closely resemble those visible after heat shock in vivo (Neri et al., 1994, 1995a). Moreover, changes are also seen when nuclei are incubated in a buffer without Mg++ (Neri et al., manuscript in preparation) which instead contains the polyamines, spermine and spermidine as well as KCl (Izuarralde et al., 1988). An example of how a thermal exposure can influence the distribution of nuclear matrix polypeptides is presented in Fig. 1. While the 240-kDa antigen, referred to as NuMA (nucleus-mitotic apparatus) was insensitive to a 20 min incubation of isolated nuclei at 37 °C in a buffer containing spermine/spermidine/KCl, a ribonucleoprotein (RNP) component showed dramatic changes in comparison with intact cells. In the same picture, it can be observed that when isolated nuclei were kept at 0 °C for 20 min no variations in the immunofluorescent pattern due to a 105-kDa splicing component were seen, while they were very evident after a 37 °C incubation (data not shown). Although Mg++ ions are usually held responsible for creating artifacts during isolation of nuclei (Cook, 1988), our data clearly show that other molecules can have a similar effect. It should also be emphasized that at least two nuclear matrix proteins contain hydrophobic domains (see later). Thus, the 37 °C stabilization of isolated nuclei could be due to protein aggregation and/or precipitation, likewise the stabilization caused by heat shock in vivo. However, there must be differences between the two phenomena, because the protein composition of the matrix prepared from nuclei heat-stabilized in vitro is different, when analyzed by two-dimensional gel separations, from the matrix isolated from nuclei deriving from cells subjected to heat shock in vivo (Martelli et al., 1995). The protein composition of the matrix after a heat shock in vivo closely resembles that seen after stabilization of isolated nuclei with NaTT (Martelli et al., 1995). For example, nucleolar proteins B23/nucleolin and C23/nucleolin are very abundant in the nuclear matrix derived from in vivo heated cells or chemically cross-linked nuclei, whereas they are very scarce (B23) or totally absent (C23) from the matrix after exposure of nuclei to 37 °C in vitro (Martelli et al., 1995).

Nevertheless, our data also suggest that formation of S-S bonds during in vitro heat stabilization of isolated nuclei is unlikely to occur in agreement with our previous suggestions (see Martelli et al., 1994a). Furthermore, two-dimensional gel analysis has revealed that the protein composition of the in vitro heat-stabilized matrix is not very different from that of structures isolated without this form of treatment (Martelli et al., 1995), thus suggesting that the increased recovery of protein, measurable in heat-exposed structures, is predominantly due to an additional recovery of the same types of polypeptides.

Functional studies have evidenced that in vitro heat
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stabilization can lead to the formation of artifacts and in these cases the available data do not fit well with the model envisaging the matrix as the active site for DNA replication and transcription (Fisher et al., 1989; Martelli et al., 1992b, 1994b). Moreover, the data from Jackson et al. (1990a,b) have clearly established that the «thermal stabilization» creates five new (artefactual) DNA loop attachment sites in HeLa cell nuclei for every one that pre-existed. Therefore, in our opinion, such an in vitro stabilization should be avoided. Our unpublished data have shown that in isolated nuclei, Cu ++ also changes the localization of matrix polypeptides (Neri et al., manuscript in preparation).

As far as stabilization with NaTT is concerned, it should be stressed that it does not change the distribution of some nuclear matrix proteins (Neri et al., 1995b). Stabilization with this chemical has also been used for preparing the in situ matrix (e.g. Wansink et al., 1993). However, since NaTT does not act only by inducing formation of S-S bonds, but also through other, as yet unidentified mechanism(s) (Stuurman et al., 1992b), we feel that caution is necessary when employing this

Fig. 1. Immunofluorescent staining of intact K562 human erythroleukemia cells (a, c, e) and isolated nuclei (b, d, f) reacted with antibody to NuMA protein (a,b), RNP constituent (c,d) and 105-kDa splicing component (e,f). In b and d nuclei were incubated for 20 min at 37 °C in a buffer containing spermine/spermidine/KCl, whereas in f they were kept in the same buffer for 20 min at 0 °C. Samples were analyzed by a confocal laser scanning microscope. Bar: 1 μm.
chemical, also because enzymic activities cannot be recovered after this treatment (Martelli and Cocco, 1994).

Protein composition of the nuclear matrix

Even though the first available reports indicated that the protein composition of the nuclear matrix was quite simple when compared with that of nuclei (e.g. Berezney and Coffey, 1977), the use of two-dimensional gel electrophoresis has evidenced that the polypeptide profile of the matrix is exceedingly complicated. This has been mostly demonstrated in the «nuclear matrix-intermediate complex» preparations (e.g. Fey and Penman, 1988; Dworetzky et al., 1990). These investigations have shown differences depending on the cell type and related to neoplastic transformation (Getzenberg et al., 1991b; Zini et al., 1994). Other polypeptides sharing such a property are SATB1 (Dickinson et al., 1992), particularly expressed in thymocytes, and SAF-A, a 120-kDa protein, identical to hnRNP-U (Fackelmayer et al., 1994), that has also been recognized and studied by other groups (e.g. Tsutsui et al., 1993; von Kries et al., 1994) and the 240-kDa protein referred to as NuMA (Luderus et al., 1994). Very recently, a nine amino acid sequence motif has been identified in SATB1, conferring to the protein unique MAR binding activity (Wang et al., 1995). Using nuclear matrices prepared by a high-salt extraction, Zong and Scheurmann (1995) have described a novel 33-kDa DNA binding protein (MAR-BP1) that specifically binds to MARs associated with the immunoglobulin heavy chain (IgH) enhancer. Results have also shown that in cells where the IgH locus is transcriptionally inactive, the nuclear factor-κ negative regulator binds to IgH enhancer and interferes with MAR-BP1/enhancer interactions, thus preventing attachment to the nuclear matrix. Also, protein C23/nucleolin binds MARs and it seems that polypeptides sharing such a property can be divided into two groups: those that bind to double-stranded DNA (possibly through recognition of a peculiar tertiary structure such as a narrow minor groove) like MAR-BP1 as well as SATB1; and those preferring single-stranded DNA, such as C23 and lamin B3 (Luderus et al., 1992; Zong and Scheurmann, 1995). Our unpublished results (Neri et al., manuscript in preparation) have shown that both SATB1 and SAF-A are localized in the nuclear interior and antibodies raised against them reveal a fibrogranular network. In this context it should also be recalled that laminas A and C are DNA-binding proteins, and bind MAR sequences (Hakes and Berezney, 1991a; Luderus et al., 1992, 1994). Very recently, Yagisawa et al. (1996) have identified a 114-kDa MAR-binding protein that is expressed only in human breast carcinomas and not in either normal or benign breast disease tissues or epithelial carcinomas. Undoubtedly, the number of known polypeptides showing MAR-binding capability will continue to grow in the future.

Then, there is a plethora of other proteins identified as minor components of the nuclear matrix: autoantigens like fibrillarin (Ochs and Smetana, 1991), fibronecctin (Zerlauth et al., 1988), keratin-like proteins (Alige et al., 1990), oncogene products (Evan and Hancock, 1985), tumor-suppressor genes (such as the retinoblastoma gene product, Durfee et al., 1994; Mancini et al., 1994), splicing factors (Smith et al., 1989), heterogeneous nuclear ribonucleoprotein (He et al., 1991), transcription factors like NMP-1 and NMP-2 (Guo et al.,
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1995; Merriman et al., 1995), trans-acting factors like NF1 (Sun et al., 1994), DNA binding proteins such as RFP (Isomura et al., 1992), primer recognition proteins (Vishwanatha et al., 1992), or enzymes like DNA polymerase α (Smith and Berezney, 1982, 1983), DNA primase (Tubo and Berezney, 1987c), RNA polymerase I (Dickinson et al., 1990) or II (Lewis et al., 1984), poly-ADP-ribosome polymerase (Kauffman et al., 1991), histone deacetylase (Hendzel et al., 1991), RNase H (Karwan et al., 1990), phospholipases (Tamiya-Koizumi et al., 1989; Bertagnolo et al., 1995), and protein kinases such as kinase C or casein kinase 2 (Capitani et al., 1987; Tawfic and Ahmed, 1994), etc. The biological significance of several of these molecules is still to be ascertained.

It could be hypothesized that, while the most represented matrix proteins are components of a common network (or a few frameworks) spanning the entire nuclear interior, the minor constituents are localized to specific matrix sub-domains, each of which conceivably performs a different task (Stuurman et al., 1992c).

Lastly, it should be noted that, by definition, the nuclear matrix is mostly composed from nonhistone proteins (Berezney, 1984). Usually, extraction with high ionic strength solutions (NaCl or (NH₄)₂SO₄) is effective in removing almost all core histones (about 95%) from the matrix preparations (histone H1 is completely removed at a lower ionic strength). According to Laemmli and coworkers LIS treatment also results in scaffolds that are devoid of core histones (Mirkovitch et al., 1984; Izzuaralde et al., 1988). Surprisingly, they have also reported that LIS is less efficient in removing histone H1, that could be extracted and purified from scaffold preparations and it has been shown that this protein specifically associates with SARS (Izzuaralde et al., 1989; Kas et al., 1989). Other investigators, including us, have come to opposite conclusions, because LIS extracted H1 but not most core histones (Belgrader et al., 1991a; Neri et al., manuscript in preparation).

Functions associated with the matrix

The functions associated with the nuclear matrix are endless and the reader is referred to other excellent reviews (e.g. Berezney, 1991) for a more comprehensive treatment of the topic.

Rather, we would like to stress that some of the functions that have been studied in more depth (for example DNA replication) have been found in frameworks prepared by different techniques, that is the high-salt nuclear matrix, the in situ matrix and the agarose-embedded matrix (e.g. Tubo and Berezney, 1987a; Nakayasu and Berezney, 1989; Hozak et al., 1993). Thus, the evidence that these functions take place attached to some sort of nuclear frameworks seems compelling.

For other functions, however, like the attachment of specific DNA sequences, the results are strictly dependent on the technique used for preparing the framework and a definitive conclusion cannot be drawn yet (Razin and Vassetzkzy, 1992; Razin and Gromova, 1995).

A function that has been recently linked to the nuclear matrix of which awareness is continuously increasing, concerns the autonomous inositide cell cycle operating within the nucleus (see Manzoli et al., 1989; Cocco et al., 1994). Following the original report by Capitani et al. (1987) regarding protein kinase C (PKC) bound to the nuclear matrix, Payrastre et al. (1992) have shown the association with the nuclear matrix of several key enzymes of the inositide cycle such as phosphoinositide kinases, diacylglycerol kinase and phospholipase C (PLC). Ultrastructural studies to DNAs obtained by means of colloidal gold-conjugated secondary antibodies have confirmed these findings based on enzymatic assays (Zini et al., 1995a,b; Maraldi et al., 1995; Mazzotti et al., 1995). In particular, the α isoform of PKC has been identified bound to the matrix after stimulation of quiescent Swiss 3T3 mouse fibroblasts with insulin-like growth factor I, and PLC β1 has been recognized as the major PLC isoform present in the nucleus, in agreement with previous data based on western blot experiments carried out on highly-purified nuclear preparations (Martelli et al., 1992d). Such a system might play a fundamental role in conveying signals from the cell periphery to active sites of DNA replication and transcription within the nucleus.

Concluding remarks

An intriguing new concept for the nuclear matrix has been recently formulated by Razin and Gromova (1995) in order to reconcile many of the contradictory data available in the literature. These authors envision the nuclear matrix as a system of channels connecting the nuclear interior with nuclear pores. The chromosomal domains are assembled around this channel system to which active DNA sequences (both in terms of replication and transcription) are attached, thus making the transport of different molecules possible (DNA precursors, enzymes and regulatory factors) from cytoplasm to the nucleus and, at the same time, migration in the opposite direction of mRNA molecules. Indeed, a recent observation from Panzeter and Ringer (1993) corroborates such a model by showing that DNA precursors are channeled directly to DNA replication sites present on the matrix. Such a model could also explain why antibodies to nuclear matrix proteins never decorate a uniformly-sized inner network but rather a fibrogranular framework showing branches and dilations (Stuurman et al., 1992c). Indeed, the channels would not have the same size but they would branch and expand, forming caverns around which replication and transcription factories are assembled. Certainly, this new attractive model for the nuclear matrix deserves further investigations.
An overview of nuclear matrix

A point that must be taken into consideration is that nuclear frameworks can be isolated not only from higher eukaryotes but also from plants (e.g. Beven et al., 1991), yeasts (e.g. Cárdenas et al., 1990), the slime mold *Physarum polycephalum* (e.g. Waiz and Loidl, 1988; Lang et al., 1993) or even Dinoflagellates (Minguet et al., 1994). These results hint at the universality of nuclear frameworks, showing that they should conceivably be an early acquisition of the eukaryotic cell nucleus. It is surprising that both in *Physarum polycephalum* and mammalian renal epithelia cell line LLC-PK, different extraction protocols do not yield final structures differing in a substantial way (either biochemically or immunologically) when the *in situ* procedure (Waiz and Loidl, 1988) or the agarose-embedded technique (Eberharter et al., 1993) are employed. It could be argued that these are the most gentle procedures so far devised for preparing frameworks but when the agarose-embedded technique was used in mouse cells, an inner framework could be seen in skin fibroblasts but not in the plasmacytoma MPC-11 cells, even if they had been treated with phorbol esters to induce vimentin synthesis and intermediate filament expression (Wang and Traub, 1991). Usually, cells of haemolympohopoietic lineage are thought to contain very little or no inner matrix (see Martelli et al., 1992c). However, given both the high rate at which these cell lines replicate and the fundamental role that the nuclear matrix is believed to play in DNA duplication, the absence of an internal framework appears puzzling and very difficult to explain. It is also bewildering that mouse erythroleukemia cells lack high levels of matrix-associated DNA polymerase α activity, even though heat stabilization has been performed (Martelli et al., 1993). Perhaps, in these cells an inner network constituted from ribonucleoproteins could represent the nuclear matrix (see Yancheva et al., 1986). Whatever the case, it is unquestionable that nuclei obtained from different cells exhibit a different sensitivity to variations in the extraction procedures. However, so far any understanding about the reason(s) responsible for such a different behaviour is completely lacking.

It might be that many of the problems scientists investigating nuclear frameworks have been facing stem from the fact that they have been looking for a structure using progressive stripping of nuclei to remove as many components as possible, using suitably «mild» conditions. The goal is to end up with a simplified residual structure that would provide, *in vitro*, insight into the organization of the nucleus *in vivo*. However, since nuclei usually do not resist even these «mild» treatments very well, some form of stabilization is necessary, but unavoidably these procedures lead to the formation of artifacts and also to retention of more material than the framework proper molecules.

It is out of the question, indeed, that every method so far devised for isolating nuclear frameworks has its own shortcomings. This has generated very pessimistic views about the possibility of isolating the real (if any) nuclear matrix (see for example Jack and Eggert, 1992).

Conceivably, nuclear frameworks exist, but it might be that they are «functional» rather than «structural» entities. Functional frameworks would also be easier to duplicate during S-phase of the cell cycle, a very critical issue that so far has not been investigated at all.

Therefore, only new approaches, now widely used, allowed by a combination of ultrastructural, biochemical, immunological and genetic techniques might rapidly and substantially increase our knowledge about the structural and functional organization of the nucleus. Furthermore, they should permit a definitive solution to the controversy still surrounding the existence of a nuclear matrix *in vivo*.

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