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Review

The controversial nuclear matrix: a balanced point of view

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Summary. The nuclear matrix is defined as the residual framework after the removal of the nuclear envelope, chromatin, and soluble components by sequential extractions. According to several investigators the nuclear matrix provides the structural basis for intranuclear order. However, the existence itself and the nature of this structure is still uncertain. Although the techniques used for the visualization of the nuclear matrix have improved over the years, it is still unclear to what extent the isolated nuclear matrix corresponds to an in vivo existing structure. Therefore, considerable scepticism continues to surround the nuclear matrix fraction as an accurate representation of the situation in living cells. Here, we summarize the experimental evidence in favor of, or against, the presence of a diffuse nucleoskeleton as a facilitating organizational nonchromatin structure of the nucleus.

Key words: Nucleus, Protein, Skeleton, Structure, Function

Introduction

There is no doubt that the cell nucleus is one of the best known but least understood and most complicated of cellular organelles. Its discovery and description date back to the very beginning of the 19th century (Qumsiyeh, 1999). The first successful isolation of cytologically intact nuclei was achieved, by an extraordinarily harsh technique, by Friedrich Miescher in 1871 (Pederson, 1998; Dundr and Misteli, 2001), and such an accomplishment testifies the remarkable resistance of the nucleus to in vitro manipulations. Furthermore, Miescher's results suggested that there was a resistant nuclear structure, whether based on chromatin or on something else. Ever since, the nucleus attracted much attention. From the 1940s to the early 1960s, the nucleus was the subject of many biochemical studies that highlighted the existence of a class of nuclear proteins resisting extraction with high concentrations (up to 2 M NaCl) of salt. Morphological investigations, carried out by means of light and electron microscopy, revealed that high salt-extracted nuclei considerably retained their overall shape and exhibited nucleolar remnants and clearly demarcated heterochromatin (reviewed in Dundr and Misteli, 2001). Then, in 1974, Berezney and Coffey published a paper reporting virtually the same type of preparation of high salt-extracted nuclei. However, these authors for the first time introduced the term "nuclear matrix" to indicate the residual framework which resists removal of the nuclear envelope, chromatin and other soluble components of the nucleus, by a series of sequential treatments with non-ionic detergents, nucleases and buffers of both low and high ionic strength (Berezney and Coffey, 1974). A publication which followed-up (Berezney and Coffey, 1975) conceivably had a very strong impact because it was proposed that the nuclear matrix was a critical, facilitating element in nuclear functions and played a key role in DNA replication. Indeed, in none of the countless previous studies that produced a nuclear fraction not materially different in biochemical composition or morphological appearance from the nuclear matrix, there was a speculation on possible functions. Therefore, the concept of a nuclear matrix quickly became quite popular among cell biologists and led to the appearance of thousands of papers on the subject. It may appear ironic that many of these publications gave a strong relevance to the structural aspects of the issue. In fact, from the mid 1970s onwards, once the process of gene expression became experimentally tractable in vitro, for many investigators the emphasis in nuclear studies shifted to

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biochemical and molecular approaches, so that the study of nuclear structure became a secondary concern. Especially molecular biologists viewed the nucleus as a largely disordered bag of chromatin immersed in a homogeneous nucleoplasm, in which all “structures”, except for the nucleoli and the double-layered nuclear envelope, were no more than transient complexes that form and dissolve as a result of DNA transcription and replication, and RNA processing activities. In contrast, the nuclear matrix was promoted as the fundamental organizing principle of almost all nuclear functions (Berezney, 1991; Jackson and Cook, 1995; Berezney et al., 1996). Over the past 10 years, a new outburst of interest in understanding the detailed structure of the nucleus took place in parallel with the development and application of immunocytochemical techniques coupled to the widespread use of the confocal laser scanning microscope. These new tools allowed the establishment of the existence of several morphologically identifiable intranuclear structures (Lamond and Earnshaw, 1998; Matera, 1999). Major efforts have been devoted to correlate these structural landmarks of the nucleus with nuclear functions such as DNA replication and gene expression (Ma et al., 1998; Sleeman and Lamond, 1999a,b; Wei et al., 1999).

Lately, these studies have been enormously facilitated by the availability of genetically encoded fluorescent tags (of which a typical example is represented by green fluorescent protein, GFP) that now routinely allow the visualization and quantitative analysis of chromatin, mRNA and proteins within the nucleus of living cells. Even though there is no doubt that these sophisticated techniques have their own drawbacks and can lead to erroneous conclusions, they nonetheless revealed that the nucleus contains distinct subcompartments or domains and that it is an extremely dynamic organelle (Shopland and Lawrence, 2000). The nuclear domains are obviously not bound by membranes but they must be considered "subcompartments" for a number of reasons. Firstly, they contain defined subsets of components, mainly proteins (Lamond and Earnshaw, 1998). Secondly, their identification is possible by light and electron microscopy in fixed cells and at least some of them have recently been visualized in living cells by GFP technology (e.g. Broers et al., 1999; Kruhlak et al., 2000; Phair and Misteli, 2000). Thirdly, some of these domains can be isolated by biochemical fractionation in an enriched form (e.g Mintz et al., 1999). Progress in elucidating higher order structure of the nucleus is not limited to strictly functional domains, but applies to the very genome itself. Indeed, it is now evident that the chromatin in the interphase nucleus is arranged in spatially separate, chromosome-specific territories (see Ma et al., 1999, and references therein). Because the nuclear domains and their constituents retain their position after removal of the nuclear envelope, soluble proteins and chromatin, it has been proposed that they are anchored to the nuclear matrix which, therefore, would organize the overall nuclear structure (Dundr and Misteli, 2001). However, whether the cell nucleus is organized by an underlying structure analogous to the cytoskeleton has been a contentious issue since the original isolation of a nuclease and salt-resistant nuclear matrix. Recently, some critical reviews about the nuclear matrix have appeared in the literature (Hancock, 2000; Pederson, 2000). In stark contrast, another recent article reviewed all the experimental observations in favor of the existence of such a framework without taking into consideration those against it (Nickerson, 2001). Here, we shall try to provide the reader with a more "balanced" view of the current knowledge about the nuclear matrix. Thus, we shall review the evidence in favor of, as well as that against, its existence in living cells. However, for a better comprehension of the issue it is first necessary to briefly review the methods that, over the years, have been developed to prepare the nuclear matrix.

The methods for preparing a nuclear matrix and the issue of nuclear matrix “stabilization”

One of the main problems in the nuclear matrix field is the existence of several distinct protocols to prepare this fraction. The original method proposed by Berezney and Coffey (1974) called for the sequential treatment of envelope-stripped, isolated nuclei with nonspecific nucleases (either endogenous or exogenous DNase and RNase) and buffers of low and high (2 M NaCl) ionic strength. Subsequently, it has been claimed that use of lower salt concentrations [for example 0.25 M $(NH_4)_2SO_4$] may lead to a better preservation of the morphology without significantly affecting the efficiency of protein extraction (e.g. Belgrader et al., 1991). The original method has been successfully applied to tissues (rat liver, for example) or tissue culture cells. The matrix is mainly composed of nonhistone proteins and RNA and forms an elaborate anastomosing three-dimensional internal fibrogranular network through the nucleus, intersecting with the peripheral lamina. Nucleolar remnants are also present (e.g. Belgrader et al., 1991; Berezney, 1991). At present, the peripheral lamina is the only "rigid" structure of the nucleus whose existence is universally accepted. In unfractionated cells, the internal fibrogranular network of the nuclear matrix would correspond to the ribonucleoprotein-containing network of perichromatin fibrils and interchromatin granule clusters selectively stained by the EDTA-regressive staining method and present in the spaces between chromatin (Nickerson et al., 1997). Perichromatin fibrils are typically 3 to 5 nm in diameter, even though in some cases they can reach approximately 20 nm. Interchromatin granule clusters consist of 20 to 25-nm diameter particles interconnected by anastomized fibrils (3 to 8 nm in diameter) that, however, are distinct from perichromatin fibrils (Pederson, 1998). Perichromatin fibrils and interchromatin granule clusters have been functionally related to sites of pre-RNA transcription and processing (Spector, 1993).

Laemmli and coworkers (Mirkovitch et al., 1984) pioneered the use of an ionic detergent, lithium diiodosalicylate (LIS), for extracting histones and other nuclear proteins after digestion of isolated nuclei with restriction enzymes. The structure they obtained was named the nuclear "scaffold", but it should be recalled that this term was originally employed to indicate preparations obtained by extracting nuclei with polyanions such as dextran sulphate or heparin (Adolph, 1980). The LIS extraction technique allowed the identification of scaffold-associated regions (SARs), also called matrix-associated regions (MARs), that are AT-

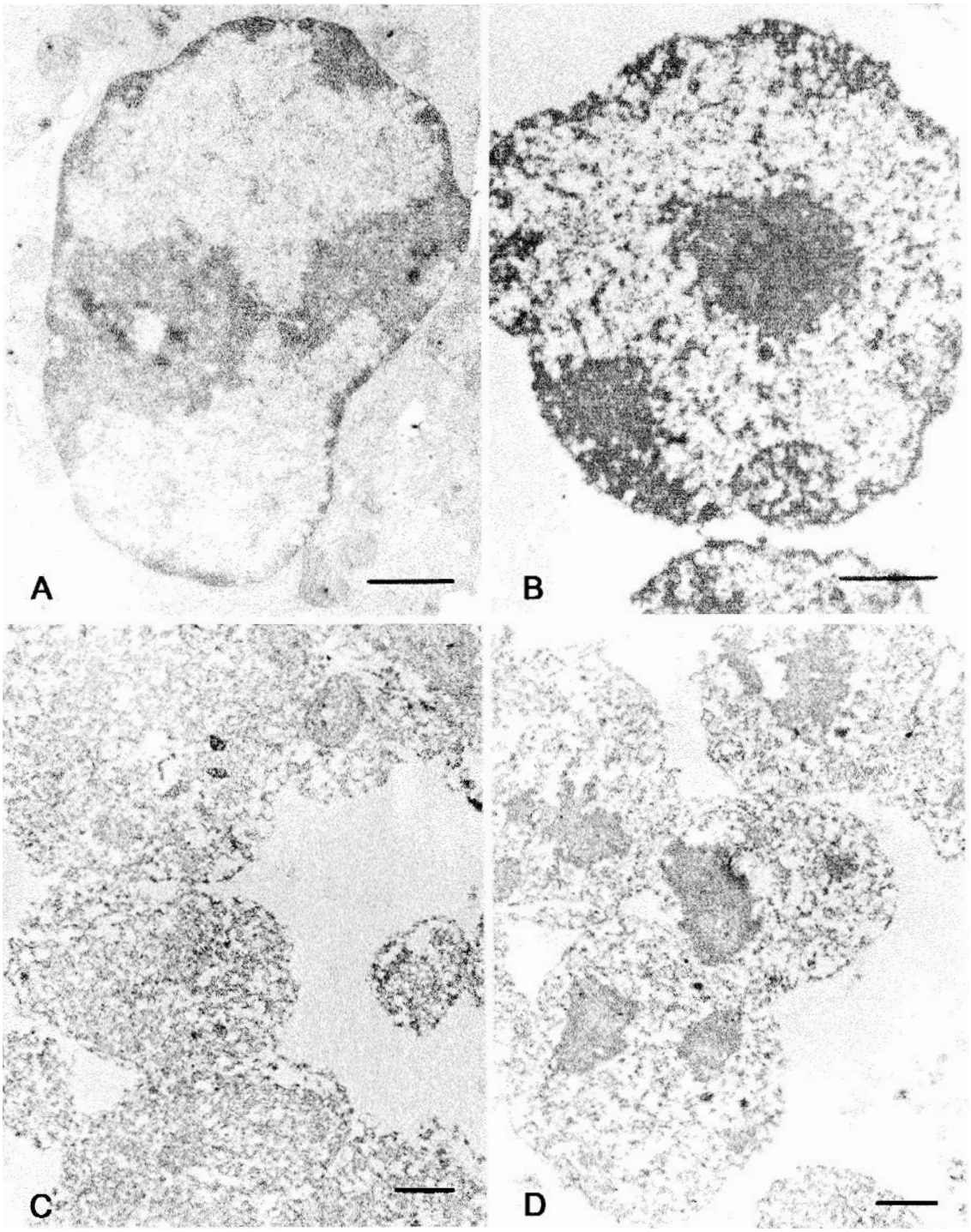

Fig. 1. Transmission electron microscope (TEM) analysis of an intact murine erythroleukemia (MEL) cell **(A)**, of a freshly isolated MEL cell nucleus **(B)**, and of MEL cell nuclear matrices prepared after NaTT stabilization performed at either 0° C **(C)** or 37° C **(D)**. Note how, when compared with an intact cell **(A)**, substantial nuclear chromatin clumping is visible **(B)** due to the use of hypotonic solutions for isolating nuclei. The nuclear matrices show a diffuse fibrillar internal network. However, nucleolar remnants are visible only in samples stabilized with NaTT at 37 °C **(D)**. Scale bar: 1 μm.

rich stretches of 300-1000 bp, highly conserved during the evolution, believed to anchor DNA loops to a nuclear framework (Mirkovitch et al., 1984; Izaurralde et al., 1988, 1989). Several S/MARs-binding proteins have subsequently been identified (Martelli et al., 1996). Overall, the LIS technique has not been widely used.

Penman and associates (e.g. Capco et al., 1982) described a method in which adherent cells are extracted in situ with nonionic detergents and salt solutions [0.25 M $(NH_4)_2SO_4$] followed by nuclease digestion. These structures are referred to as "in situ nuclear matrix" or "nuclear matrix-intermediate filaments complex", because they also retain cytoskeletal components. This technique was devised to avoid the use of hypotonic solutions to isolate nuclei. Indeed, the solutions employed for this scope usually contain 2-5 mM $Mg2^+$ ions that have an adverse effect on chromatin structure (see Fig. 1). Although it was originally proposed for cells growing as monolayers, Penman's method can also be employed for cells growing as suspension (e.g. Martelli et al., 1999a). It is worth recalling here that recently the same group replaced 0.25 M $(NH_4)_2SO_4$ with N-hydroxysulfosuccinimide (sulfo-NHS), a reagent which modifies protein amino groups without cross-linking (Wan et al., 1999). Use of sulfo-NHS allowed removal of chromatin under isotonic solution conditions, most likely because chromatin cut by nucleases is held in place by charge interactions involving nucleosomal amino groups. However, the preparations did not look very different from those in which chromatin was removed by a more conventional high salt extraction.

Finally, Cook and associates (Jackson et al., 1988) have developed quite a complex technique to obtain "nucleoskeletal preparations". The main goal of this technique is, once again, to avoid the use of unphysiological (i.e. highly hypotonic) salt concentrations to isolate nuclei. To this end, cells, growing embedded in agarose microbeads (50-150 μm diameter), are permeabilized with a mild nonionic detergent (0.1% Triton X-100) in a “physiological” buffer. Then, DNA is removed by restriction enzymes and subsequently electroeluted.

The existence of these distinct methods is further complicated by the fact that for one of each several variations have been introduced. The need for these variations comes from the fact that, more often than not, a given cell line behaves in a unique manner when sequentially extracted with the various solutions employed for nuclear matrix isolation. Additionally, modifications are also required according to the functions to be investigated. For example, if an enrichment of nuclear 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).

This leads us to another critical problem, which is the necessity of including "stabilization" steps in some of the aforementioned matrix isolation protocols. In the early 1980s it was demonstrated that spontaneous formation of disulfide bonds occurred during nuclear matrix preparation from rat liver. More importantly, if formation of disulfides was inhibited by alkylating agents, the inner matrix network and residual nucleoli were barely recognizable, whereas the peripheral lamina was still detectable (Kaufmann and Shaper, 1984). For this reason, formation of disulfide bonds, promoted by the cross-linking agent sodium tetrathionate (NaTT), has deliberately been used to "stabilize" the inner network and nucleolar remnants (Stuurman et al., 1990; Nakayasu and Berezney, 1991) (Fig. 1). Two important issues need to be emphasized about this form of stabilization. First, available evidence suggests that proteins of the nuclear matrix are not cross-linked by disulfide bonds *in vivo* (Kaufmann and Shaper, 1991). Second, it seems that spontaneous formation of disulfide bonds only occurs when nuclei are isolated from normal rat liver or hepatoma cells (Kaufmann and Shaper, 1984, 1991) because treatment with iodoacetamide was ineffective when used in HeLa or mouse erythroleukemia cells (Belgrader et al., 1991; Martelli et al., 1992). Laemmli and coworkers reported the stabilizing effects of divalent cations such as Cu^{2+} or Ca^{2+}, 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 to stabilize the inner matrix prior to LIS extraction for "preserving" interactions between the scaffold and SARs. 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 (Mc Connell et al., 1987; Berrios and Fisher, 1988; Martelli et al., 1990, 1991) (Fig. 2).

At the extreme end of these stabilization steps, we must consider the extensive cross-linking with 4% formaldeheyde employed by Penman's group (Nickerson et al., 1997) prior to DNase digestion to improve the preservation of the fine structure of their "in situ nuclear matrix" preparations, because with such a fixation it was possible to avoid salt extraction or electroelution of chromatin.

It is very hard to believe that such a technique does not create at all “new” macromolecular interactions among nuclear constituents, even though, in some selected cases, it has been employed as a non-perturbing method to maintain native interactions between macromolecules (Pederson, 1998).

Evidence in favor of, or against, the existence in vivo of a nuclear matrix

1. Visualization of a structure resembling the nuclear matrix

The nuclear matrix is considered by many authors to be the counterpart of the cytoskeleton. While the fibrillar structures formed by cytoskeletal proteins are clearly seen by immunocytochemical methods, the majority of

the proteins that have been isolated from the nuclear matrix and localized by immunofluorescence are not found in fibrillar structures but rather in punctate elements (Hancock, 2000). Nevertheless, the nucleus might contain internal filamentous structures composed of lamins or actin (Pederson, 2000). The main problem is that these fibers do not form a visible network which extends throughout the nuclear interior. There are other nuclear proteins with a potential for forming filamentous structures. These include NuMA (Zeng, 2000), INMP

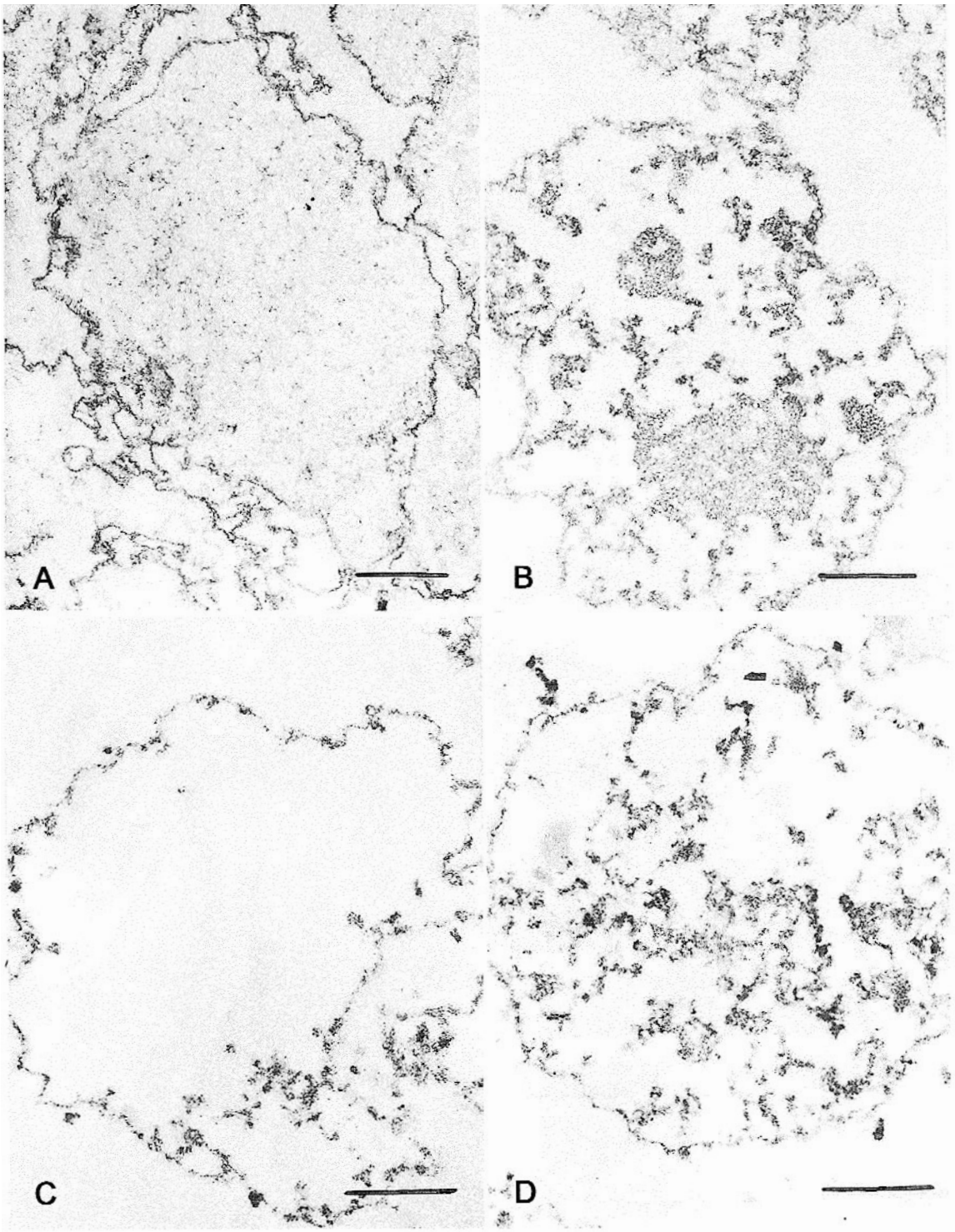

Fig. 2. TEM analysis of MEL cell nuclear matrices prepared with **(A, C)** or without **(B, D)** a 37 °C stabilization. In **(C)** and **(D)** a RNase digestion was included in the protocol. Note how 37°C is necessary for the detection of a fibrogranular internal network and nucleolar remnants (compare **A** with **B**). Treatment with RNase causes the internal network and nucleolar remnants to disappear if nuclei were not stabilized at 37 °C **(C)**, whereas, if they were, only the nucleolar remnants are no longer identifiable **(D)**. Scale bar: 1 μm.

(Menz et al., 1996), and several polypeptides exhibiting extensively reassembling from urea solution (Gerner et al., 1999), i.e. a distinctive feature of putative filament-forming components. In this connection, the protein referred to as NuMA (for nuclear mitotic apparatus) appears very interesting. It has the capability of forming networks of interconnected 5 nm filaments (Saredi et al., 1996) and its overexpression led to the appearance of three-dimensional, ordered lattices that filled the nucleus and were stable to detergent extraction (Gueth-Hallonet et al., 1998; Harborth et al., 1999). Moreover, forced expression of a mutated form of NuMA caused a drastic reorganization of the nucleus with relocation of DNA and nucleoli (Gueth-Hallonet et al., 1998). However, some highly differentiated cell types (neurons, fibroblasts, smooth and skeletal muscle cells) lack NuMA without detectable effects on nuclear organization (Taimen et al., 2000). Therefore, it is difficult to consider NuMA a universal and essential component of a nuclear matrix.

Our inability to visualize a true nuclear framework by immunofluorescence is considered by some investigators as the main evidence against the existence of a nuclear matrix (Hancock, 2000). On the other hand, the existence of a diffuse intranuclear network of irregular and intricately structured fibers, connected with the peripheral lamina, has been reported by electron microscopy of resinless preparations either in "nuclear matrix-intermediate filaments complex" (He et al., 1990; Nickerson et al., 1997; Wan et al., 1999) or in the nucleoskeleton (Jackson and Cook, 1988; Philimonenko et al., 2001). These fibers consist of 9- to 13-nm-thick "core filaments" covered with material mostly constituted of ribonucleoprotein (Nickerson et al., 1997). The core filaments are highly branched and morphologically resemble cytoplasmic intermediate filaments (He et al., 1990), but they are more abundant and heterogeneous in diameter than perichromatin fibrils are. Moreover, they show positive immunoreactivity with an antibody directed against an epitope shared by all intermediate filament proteins, and in addition, the diffuse nucleoskeleton is labeled by anti-lamin A antibodies at nodes of the branching core filaments (Hozak et al., 1995). Zeng and coworkers (1994) localized NuMA at the core filaments by immunoelectron microscopy, but there was no further confirmation. In any case, in immunofluorescence experiments, antibodies to NuMA never stain fibrillar structures but rather reveal a punctate pattern (e.g. Neri et al., 1997c; Martelli et al., 1999b). Associated with the filaments, numerous spherical structures having diameters of 20 to 25 nm can be seen and it is possible that at least some of them are interchromatin granules (Jackson and Cook, 1988; He et al., 1990). In any case, it would seem reasonable to expect that the extensive filament system revealed in some types of isolated nuclear matrix preparations is frequently caught, in sectioned nuclei, in longitudinal, oblique or cross sections. Nevertheless, the extremely numerous ultrastructural studies of sectioned nuclei never showed the expected transections of the presumably abundant filament system of the nuclear matrix.

The existence of an underlying nuclear protein network was also suggested recently by the exploitation of electron spectroscopic imaging (ESI) carried out on paraformaldeheyde-fixed cell lines (Hendzel et al., 1999). ESI couples a conventional electron microscope with an analytical imaging spectrometer. The separation of electrons that vary in energy after interacting with a specimen can then be employed to extract compositional information from the electron microscope using fixed, unfractionated cells (Bazett-Jones and Hendzel, 1999). By employing fixation methods identical to those used for indirect immunofluorescent analysis of nuclear organization, it was shown that interchromatin spaces and granules (that mainly correspond to the internal nuclear matrix network) are embedded and linked together by a protein-based architecture (Hendzel et al., 1999).

2. The methods employed for preparing the nuclear matrix may lead to creation of new macromolecular interactions and/or redistribution of nuclear proteins

Many of the protocols for isolation of the nuclear matrix generally employ nuclei prepared in a hypotonic medium and permeabilized by a nonionic detergent, followed by digestion with nucleases and extraction with high ionic strength solutions in this or in the inverse sequence. Available evidence demonstrates that each of these steps may affect the interactions of nuclear components by creating new links and lead to changes in the spatial distribution of nuclear polypeptides.

The in vitro formation of disulfide bonds in some types of nuclei has already been mentioned (see above). Recently, it has been shown that the creation of disulfide bonds in isolated preparations of heterogeneous nuclear ribonucleoproteins (hnRNPs) causes the appearance of regular helical filaments ranging in length from 100 nm to > 10 μm (Tan et al., 2000). These filaments are formed of proteins that initially exist as tetramers $(A2)_3B1$ and in conventional negatively-stained preparations the diameters of the thinnest filaments range from 7 to 10 nm, i.e. a size which is very close to the reported size of nuclear matrix fibers studied by others (He et al., 1990; Jackson and Cook, 1988). The filaments observed by Tan et al. (2000) frequently revealed spherical complexes of A2-B1 hnRNPs often distributed at regular intervals. Treatment with RNase rendered the filaments insoluble in 2 M NaCl solutions. These results might assume a particular significance in light of the fact that hnRNPs are the main constituents of the internal nuclear matrix network (Mattern et al., 1997). However, it should not be overlooked that these results, although suggestive, were obtained with essentially pure preparations of hnRNPs A2 and B1, so that we cannot be sure if they are also applicable to isolated nuclei. Moreover, treatment with RNase is not

always included in the protocols for preparing a nuclear matrix, so that resistance of some proteins to 2 M NaCl extraction cannot be explained exclusively on this base. As far as redistribution of nuclear components is concerned, our laboratory has repeatedly demonstrated that some nuclear matrix components (i.e. ribonucleoproteins) change their spatial distribution during the isolation steps leading to the final matrix fraction. Indeed, we have shown that stabilization with heat (Neri et al., 1994, 1997a) or Cu^{2+} (Neri et al., 1997b,c) caused a redistribution of nuclear matrix constituents. However, we have also identified a few matrix proteins (topoisomerase IIα, NuMA, and lamin B1) that are unaffected by these stabilization procedures (Neri et al., 1997c, 1999). Stabilization with NaTT did not usually alter the spatial distribution of nuclear matrix proteins, even of those sensitive to heat treatment (Neri et al., 1995). We, therefore, suggested that the nuclear matrix proteins not affected by the stabilization techniques may act as true structural components of internal matrix network(s).

3. The lesson from apoptosis

Apoptosis is a form of regulated cell death which results in the orderly removal of cells that are senescent,

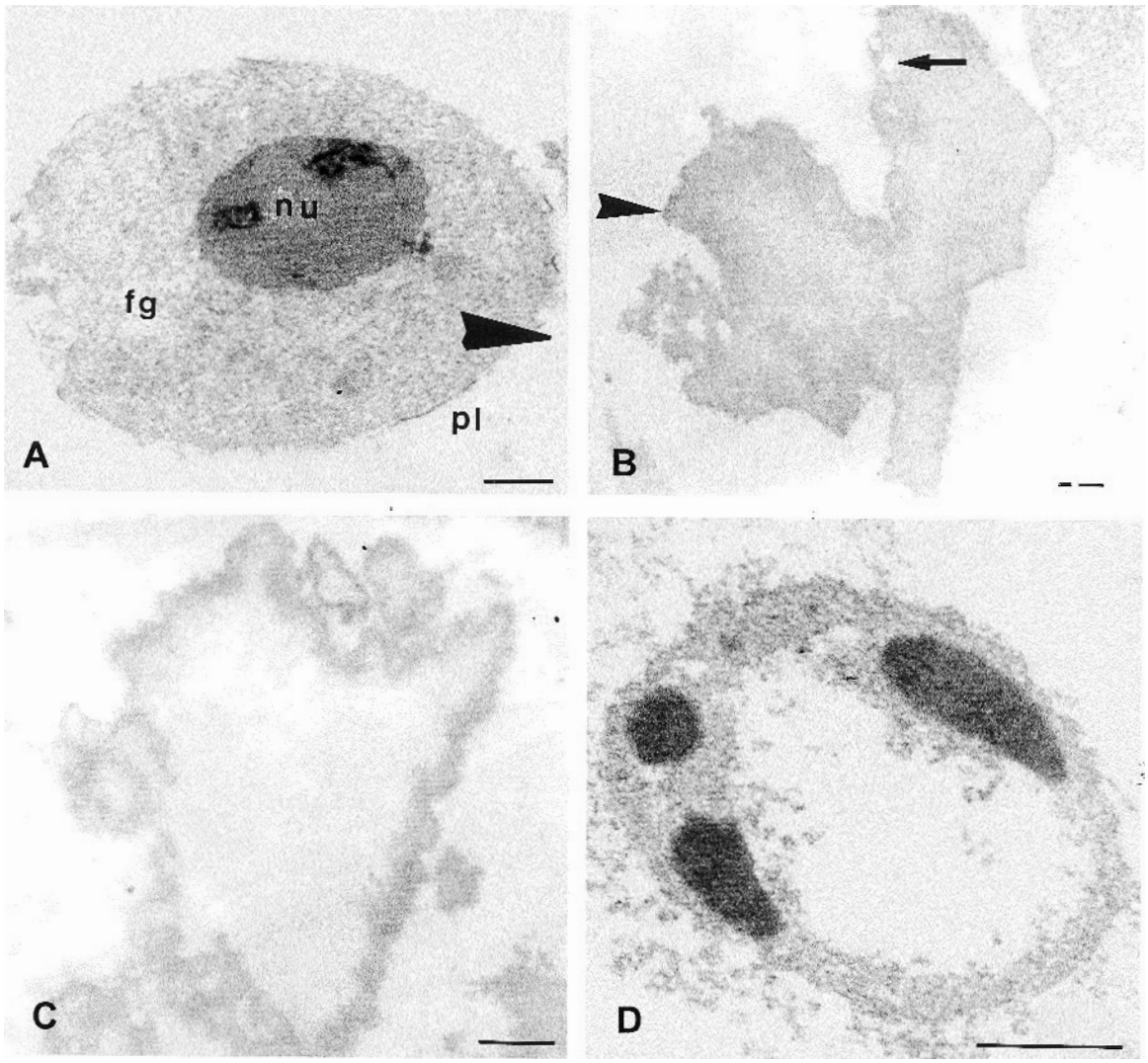

Fig. 3. TEM analysis of nuclear matrix-intermediate filaments complex isolated from normal **(A)** and apoptotic **(B-D)** HL-60 cells. The cells were treated with the topoisomerase I inhibitor, camptothecin, a powerful apoptosis inducer (Martelli et al., 1999a). **A.** A nuclear matrix-intermediate filaments complex prepared from non-apoptotic cells by DNase digestion and extraction with 0.25M $(NH_4)2SO_4$, pl, peripheral lamina; fg, fibrogranular network; nu: nucleolar remnant. **B.** After extraction with 0.25M $(NH4)2SO_4$, apoptotic nuclear matrix-intermediate filaments complex appear dense and homogeneous. Small transparent inner areas can be observed (arrow). The arrowhead indicates the peripheral lamina. **C.** When, in addition to 0.25 M $(NH_4)2SO_4$, a further extraction is carried out with 2 M NaCl, the nuclear matrix-intermediate filaments complex appears almost empty in the interior part. **D.** a nucleolar remnant with a large inner electron-transparent area, showing round and lenticular dense masses. Scale bars: 1 μm.

unneeded or defective and destined to die. Apoptosis is also complementary to mitosis, and these two phenomena determine maintenance, growth or involution of tissues. Therefore, apoptosis plays a major role during development, maturation of the immune system, and aging. Furthermore, while excessive apoptosis results in some neurodegenerative disorders due to cell loss, defects in apoptosis promote tumorigenesis by prolonging cell life span and hence cell accumulation (Hengartner, 2000). Apoptotic cell death was first described by Kerr and colleagues (1972) almost 30 years ago and was distinguished from necrosis exclusively on the basis of morphological criteria such as chromatin condensation and the formation of apoptotic bodies. Subsequently, it has become clear that apoptosis is also characterized by a variety of biochemical changes, which occur in several organelles, including the nucleus. The events taking place in the nucleus are striking, and some of them (e.g. DNA degradation and proteolysis of selected polypeptides) are routinely used as biochemical markers of apoptosis. The morphological changes typical of apoptosis are well known: they consist of a collapse of chromatin (which, in many cases adopts a striking crescent or "half-moon" shape) against the nuclear periphery, a progressive condensation of chromatin, a shrinkage of the entire nucleus into a single ball or, in other cases, chromatin budding outward into smaller balls resembling a cluster of grapes, with each grape being surrounded by a nuclear envelope (Earnshaw, 1995). The nuclear envelope remains morphologically intact even though the nuclear pores redistribute by sliding away from the surface of the condensed chromatin domains and accumulating between them (Earnshaw, 1995). The apoptotic nuclear changes are conceivably due to both DNA fragmentation and proteolysis of key nuclear polypeptides (Counis and Torriglia, 2000; Durrieu et al., 2000; Robertson et al., 2000). Regarding proteases, a family a cysteine-aspartate enzymes (referred to as caspases) are of critical importance in apoptosis (see Earnshaw et al., 1999, and references therein).

The first report dealing with the fate of nuclear matrix during apoptosis ruled out any involvement of this nuclear structure during glucocorticoid-induced cell death in thymocytes (Arends et al., 1990). This observation appeared highly surprising, also considering that several nuclear matrix proteins are cleaved during the apoptotic process (Casiano et al., 1996; Martelli et al., 1997). Clearly, the fact that a morphologically intact nuclear matrix could be isolated from apoptotic cells appeared as an indication that such a structure might be artifactually created in the test tube. However, there is now a clear indication that the nuclear matrix is markedly affected during apoptotic cell death. The evidence which supports this involvement comes from both morphological and biochemical investigations. Our laboratory has shown that marked ultrastructural changes are detectable in the nuclear matrix fraction prepared from apoptotic HL-60 cells (Figs. 3, 4). These changes are always detectable, independently of the methods employed to isolate the matrix (Martelli et al., 1999a,b). We, and others, also reported striking modifications in the spatial distribution of NuMA, as revealed by immunofluorescence analysis (Sodja et al., 1998; Martelli et al., 1999a), a further indication of the changes occurring at the nuclear matrix level (Fig. 5). As far as biochemical alterations are concerned, the current opinion holds that early proteolysis of key nuclear matrix proteins may open sites of nuclease hypersensitivity similar to those observed in transcriptionally active regions of chromatin (Krystosek, 1999), given that both protease and endonuclease activities are required for DNA fragmentation in apoptotic cells. Moreover, proteolysis probably facilitates the breakdown of the nuclear matrix itself and, ultimately, of the entire nucleus (Robertson et al., 2000). Consistently with this model of nuclear destruction, some M/SAR-binding proteins are cleaved during apoptosis. These include topoisomerase IIα (e.g. Casiano et al., 1996), NuMA (e.g. Gueth-Hallonet et al., 1997), SAF-A (Göhring et al., 1997; Kipp et al., 2000), lamin B1 (e.g. Dynlacht et al., 1999), lamins A and C (e.g.Takahashi et al., 1996), and SATB1 (Gotzmann et al., 2000). It is of great interest that, in the case of SAF-A (a protein which can bind both DNA and RNA), the cleavage occurs within the bipartite DNA-binding domain, resulting in loss of DNA-binding activity and a concomitant release of the protein from the nuclear matrix (Göhring et al., 1997). In contrast, cleavage of SAF-A did not affect the association of the protein with hnRNP complexes. This indicates that the function of SAF-A in RNA metabolism is not compromised in apoptosis whereas its cleavage may contribute to apoptotic nuclear breakdown. The behavior of SAF-A differs from that of SATB1, because in the latter case the cleavage left a C-terminal fragment still possessing both the proposed M/SAR-binding domain and the homeodomain (Gotzmann et al., 2000), so that the cleaved protein still associates with the nuclear matrix.

Therefore, the fact that the nuclear matrix is severely affected during apoptosis might be an evidence in favor of its existence in vivo. It is difficult to reconcile all the results that have demonstrated changes in the nuclear matrix of apoptotic cells with the findings originally reported by Arends et al. (1990). It should be emphasized that apoptosis is a highly asynchronous process. It is almost impossible to obtain a preparation of cells that are 100% apoptotic. Since, as we have demonstrated (Martelli et al., 1999a,b), the nuclear matrix of apoptotic cells is exceedingly fragile, it might be that the intact matrix structures isolated by Arends et al. (1990) derived from normal cells, while those from apoptotic cells did not survive the isolation procedure.

Concluding remarks

There are some reasons to believe that the cell nucleus contains a structural component that is capable

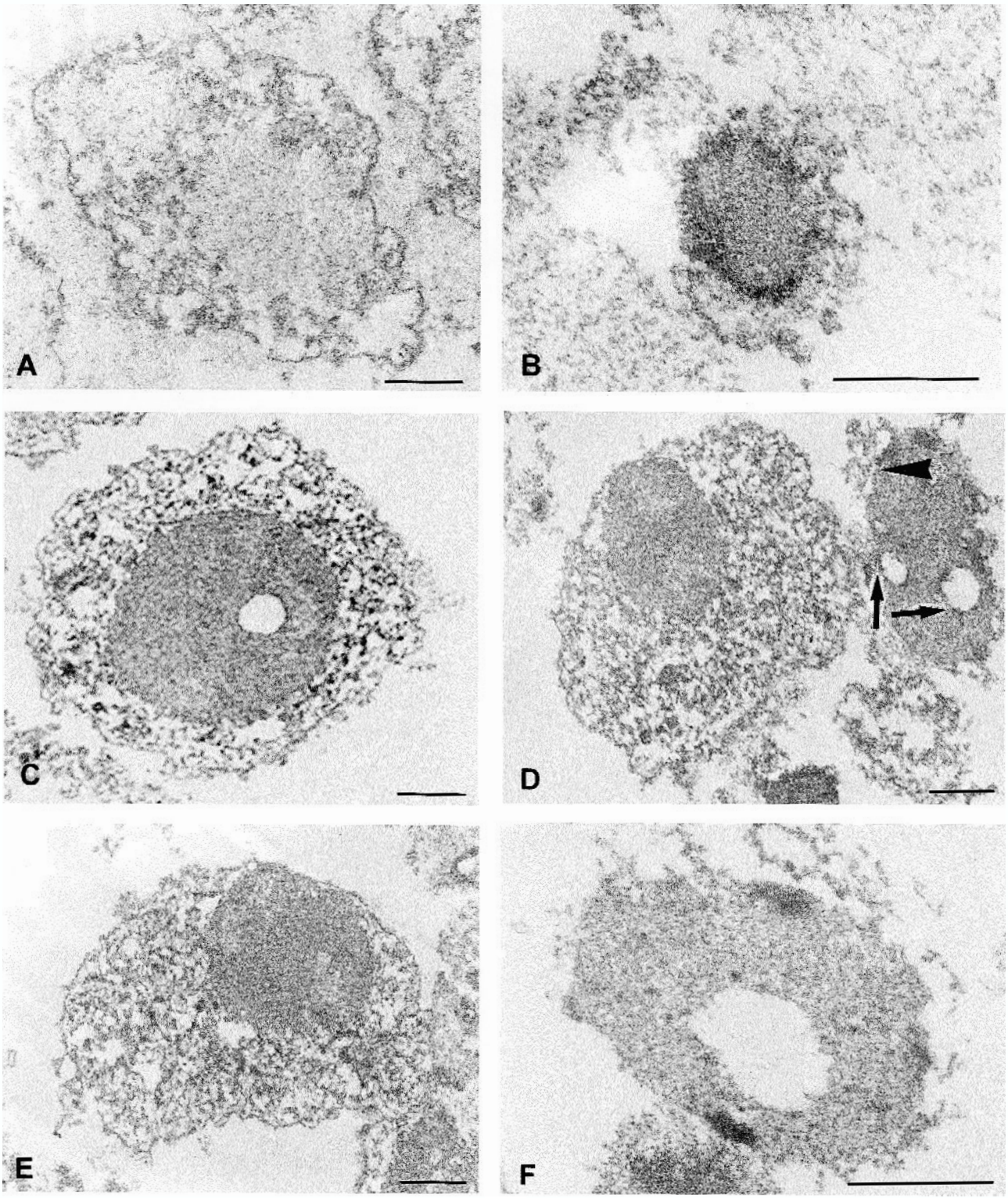

Fig. 4. TEM analysis of the nuclear matrix prepared from control **(A and E)** and apoptotic HL-60 cells **(B-D, F)**. The cells were treated with the topoisomerase I inhibitor, camptothecin. The nuclear matrix was prepared essentially according to Belgrader et al. (1991). In **A** and **B**, nuclei were stabilized at 37°C. In **C** and **D** they were exposed to NaTT at 0°C, whereas in E and F NaTT treatment was performed at 37°C. **A.** A normal nuclear matrix, showing the peripheral nuclear lamina, the inner fibrogranular network, and a nucleolar remnant. **B.** A nucleolar remnant from an apoptotic matrix displaying an electrondense peripheral area, surrounded by residues of the fibrogranular network. **C.** A nucleolar remnant with an inner cavity in a nuclear matrix isolated from an HL-60 cell during the early phase of apoptosis. **D.** A normal nuclear matrix in proximity to a nucleolar remnant from an apoptotic cell. Two inner cavities can be seen, with electrondense granules at the periphery (arrows); dense clusters of granules are also present at the periphery of the nucleolar remnant (arrowhead). **E.** A nuclear matrix from a normal HL-60 cell. **F.** A nucleolar remnant during apoptotic process. We can observe an empty central area and two lenticular clusters of granules at the nucleolar periphery. Scale bars: 1 μm.

of organizing and spatially sequestering nuclear domains. For example, both chromatin and some nonchromatin structures are constrained from substantial Brownian motion, even though transmission electron micrographs demonstrate that the density of chromatin and nonchromatin structures is not sufficient to constrain the diffusion of chromatin itself within the cell nucleus (Hendzel et al., 1999). However, other nuclear constituents, such as particles containing pre-mRNA and poly(A) RNA show in vivo mobilities that are consistent with free diffusion in the interchromatin spaces, thus implying that they are neither stably attached to, nor impeded by, a structural element (Hancock, 2000).

As reviewed above, the reality of the nuclear matrix remains uncertain. The original biochemical fractionation procedure of Berezney and Coffey (1974) and subsequent variations on this protocol (see above) have merit as a means of studying high-affinity interactions between specific nuclear components. As a cytological preparation for the investigation of structure-function relationship by transmission electron microscopy, however, this kind of procedure has serious shortcomings. Indeed, the identification and characterization of the nuclear matrix is absolutely dependent on elution of chromatin before or after fixation. Not only chromatin elution per se carries the potential to reorganize nuclear components but it also calls for the "stabilization" steps that are a further source of artifacts.

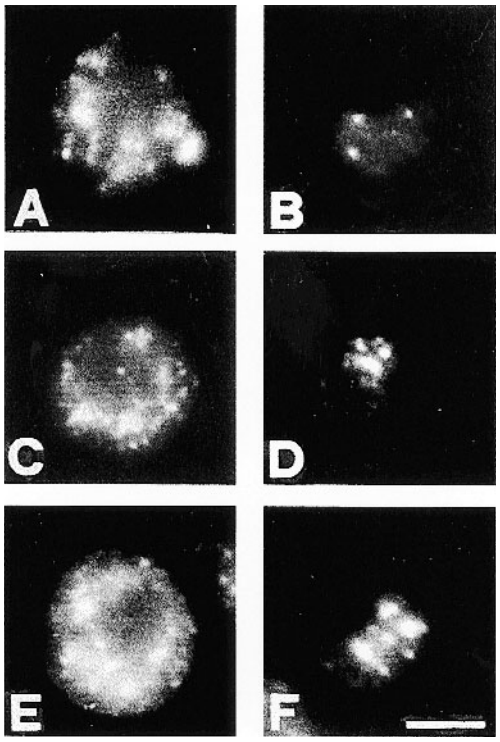

Fig. 5. Immunofluorescent staining for NuMA protein in isolated nuclear matrices. **A, C, E.** Matrices from control HL-60 cells **A.** Stabilization with 37 °C. **C.** Stabilization with NaTT at 0 °C. **E.** Stabilization with NaTT at 37 °C). **B, D, F:** Matrices from apoptotic cells. **B.** Stabilization with 37 °C. **D.** Stabilization with NaTT at 0 °C. **F.** Stabilization with NaTT at 37 °C incubation). Bar: 5 μm.

The main conceptual problem of the nuclear matrix is that it should be constituted of an extended filament system highly branched in an extensively arborized pattern. In vivo, this extensive network would correspond to the interchromatin spaces and granules. However, the interchromatin spaces of living cells appear as a sinusoidal, interconnected system bounded by chromatin contours (Pederson, 2000). Therefore, it is difficult to understand how such an in vivo topography of the interchromatin spaces could accomodate a crisscrossing network of mostly very straight filaments as observed in nuclear matrix preparations.

A reconciling hypothesis should take into account the fundamental concept of nuclear compartmentalization. If it is hard to imagine the existence of a multimicrometer-spanning, diffuse nuclear matrix, are there any clues of the presence of shorter-range structural elements of the nucleus? The nucleus contains some proteins such as nonmuscle actin, NuMA, nuclear pore complex-associated proteins Nup 153 and Tpr, and nuclear lamins that are capable of forming filaments (see Pederson, 2000, and references therein). These proteins, and others that remain to be identified, might form short arrays of filaments strategically placed around or even inside the various nuclear domains. Thus, the nucleus would have many "local" nuclear matrices involved in various aspects of the complex processes taking place in this organelle. The "local" matrices might also play a role in mediating chromosome territory organization. Indeed, Ma et al. (1999) have shown that disruption of the chromosome territories is related to the release from the nuclear matrix of a small subset of acidic proteins. These proteins are distinct from the major nuclear matrix proteins. This observation may be the first clue of the existence of several, "specialized" nuclear matrices.

Such a view is also easier to reconcile with the fact that the nucleus is so dynamic and with the results of immunofluorescent studies that have not revealed the existence of a long-range filament system in the nucleus. The name nuclear matrix was chosen to indicate a common structural element of the nucleus serving as a skeleton. The term skeleton implies a static structure, but as the cytoskeleton turns over, a nucleoskeleton must also be capable of dynamic reorganization. The fact is that, when we speak about a skeleton, we invariably think of the human body skeleton and of its long bones. However, our skeleton is not exclusively composed of long structures. The name nuclear matrix also implies that there is only one skeleton. Again, by analogy with the cytoplasm, the nucleus may contain several. Identification of these local matrices will probably be one of the most intriguing challenges for future morphological and functional analysis of the nucleus.

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