Histol Histopathol (1996) 11: 1035-1048

Histology and Histopathology

Invited Review

The nuclear matrix: a critical appraisal

A.M. Martelli[1], L. Cocco[2], B.M. Riederer[3] and L.M. Neri[4,5]

[1]Department of Human Morphology, Università di Trieste, Trieste, [2]Institute of Anatomy, University of Bologna, Bologna, Italy, [3]Institute of Anatomy, Université de Lausanne, Lausanne, Switzerland, [4]Institute of Anatomy, University of Ferrara, Ferrara, Italy, [5]C.N.R. Institute of Normal and Pathological Cytomorphology, c/o I.O.R., Bologna, Italy

Summary. It is becoming increasingly clear that the cell nucleus is a highly structurized 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;

Offprint requests to: Dr. Alberto M. Martelli, Dipartimento di Morfologia Umana Normale, Università di Trieste, via Manzoni 16, I-34138 Trieste, Italy

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 m 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 uncertainity 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 nucleases 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 2M 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 0.25M $(NH_4)_2SO_4$) may lead to a better preservation of the morphology without significantly affecting the efficiency of protein extraction (e.g. Belgrader et al., 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 Laemmli and associates (Mirkovitch et al., 1984) described the use of an ionic detergent, lithium diiodosalicylate (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 for a brief time to heat *in vitro* (37 °C or 42 °C) or to millimolar concentrations (typically 0.5mM) Cu^{++} (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; Izuarralde 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 should be components of the inner matrix network (Izuarralde et al., 1988). So far, a few nuclear proteins that can 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 (Melan 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 Pardoll et al., 1980; Vogelstein et al., 1980; Tubo et al., 1987; Tubo and Berezney, 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 immuno-labelling 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 lamins (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

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 $(NH_4)_2SO_4$ 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 rybonucleoside complex, is necessary for isolating intact structures. 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 $(NH_4)_2SO_4$) 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 Bohmannn et al., 1995a), and containing the small nuclear ribonucleoproteins (snRNPs) U1, U2, U4, U5 and U6, the autoantigen p80 coilin, 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 Korioth 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 antiserum.

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-ehtylmaleimide 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

(Stuurman 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 Eeekelen 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 divalent 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, Evan 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 si 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 aminoacids (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 polyanions, 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/numatrin 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

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

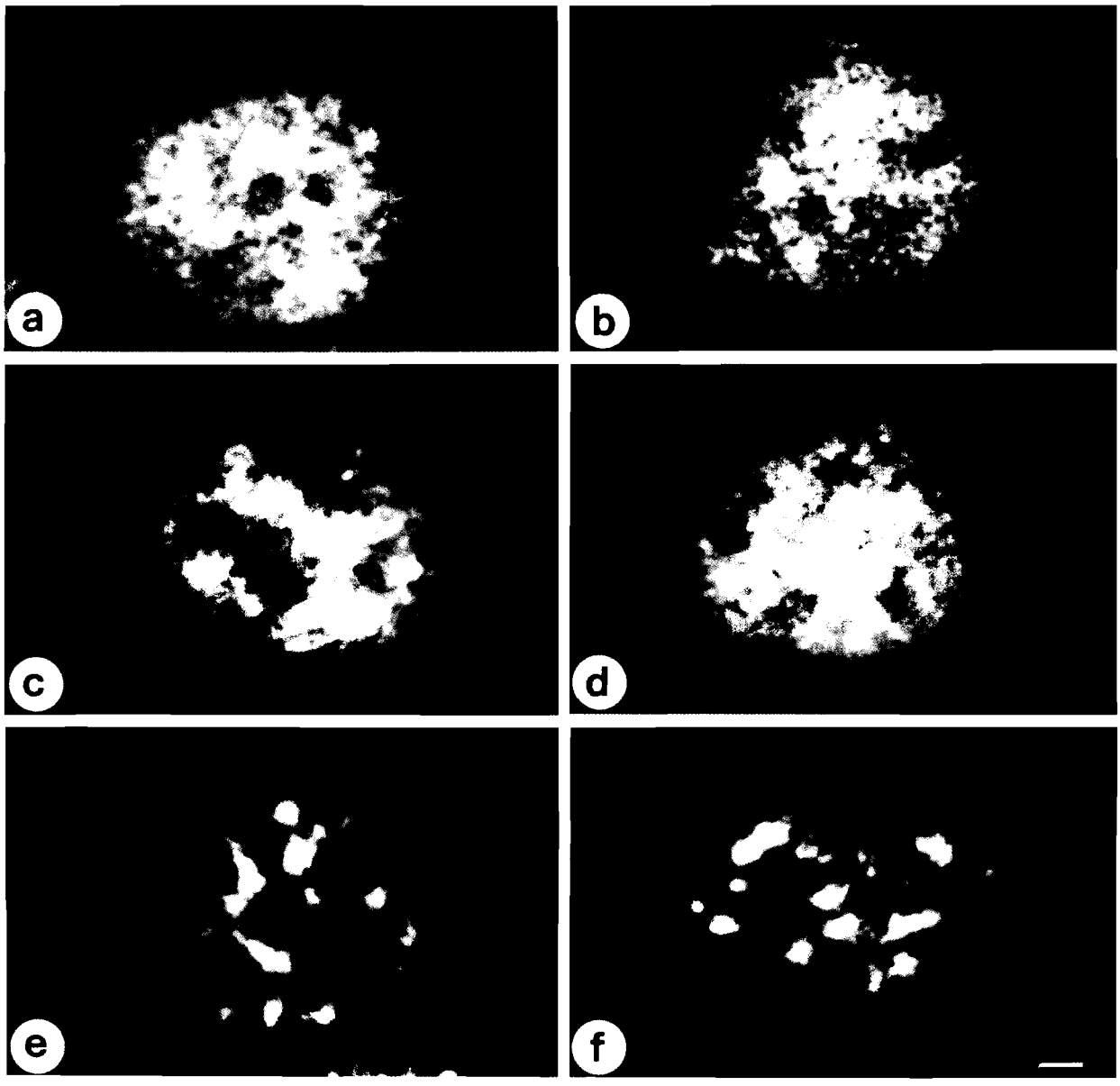

Fig. 1. Immunofluorescent staining of intact K562 human erythroleukemia cells (a, c, e) and isolated nuclei (b, d, f) reacted with antibody to NuMA protein (**a**,b), RNP constituent (**c,d**) and 105-kDa splicing component (**e,f**). In b and d nuclei were incubated for 20 min at 37 °C in a buffer containing 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; Keesee et al., 1991). Stuurman et al. (1990) have investigated the NaTT-stabilized matrix protein composition of different cell types from several mammalian species. Silver staining combined with two-dimensional separations have revealed several hundreds of polypeptides. The electrophoretic profiles differed depending on the cell types being studied. The authors referred to these proteins as the «minimal matrix». Nakayasu and Berezney (1991) have identified several abundant proteins that are ubiquitous in matrix preparations obtained from different cells and tissues and have denominated these polypeptides as «nuclear matrins». Polyclonal antibodies, staining an inner nuclear fibrogranular network, have been raised against nuclear matrins. Some of the matrins have been cloned and sequenced. Matrin F/G are two proteins with a molecular weight of 75-kDa and 65-kDa, respectively, containing approximately 50% hydrophobic amino acids and exhibiting two putative Cys-Cys zinc finger DNA binding motifs (Hakes and Berezney, 1991b). Interestingly enough, these two proteins are capable of binding DNA in a 2M NaCl-resistant fashion that, however, is apparent only when incubation is performed at 37 °C (Hakes and Berezney, 1991a). A 225-kDa protein (matrin 3) contains a highly acidic domain (Belgrader et al., 1991b), a feature shared by other nuclear proteins (Earnshaw, 1987).

Fields and Shaper (1988) identified a major 62-kDa matrix polypeptide as a component of metaphase chromosomes. This observation underlies the similarity existing between the nuclear matrix and the scaffold prepared from metaphase chromosomes (Pieck et al., 1987), also because topoisomerase II is an abundant component of both types of structures (see Earnshaw and Laemmli, 1983; Earnshaw et al., 1985; Boy de la Tour and Laemmli, 1988). In this connection, it is worth noting that several nuclear matrix proteins are functional components of the mitotic spindle (e.g. Kallajoki et al., 1991; Wan et al., 1994; Liao et al., 1995).

A very abundant component of the NaTT-stabilized matrix is protein B23/numatrin, a 38-kDa nucleolar polypeptide acting as a shuttle protein playing a role in transport from cytoplasm to the nucleus (Fields et al., 1986; Feuerstein et al., 1988; Hernández-Verdun, 1991; Martelli et al., 1995). Concerning proteins that bind MARs, a few of them have been identified, including topoisomerase II, a constituent of the matrix isolated from different cell types (e.g. Berrios et al., 1985; Adachi et al., 1989; Kaufmann and Shaper, 1991; 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 (Ludérus 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 Scheuermann (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-μ 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 B_1 (Ludérus 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 lamins A and C are DNA-binding proteins, and bind MAR sequences (Hakes and Berezney, 1991a; Ludérus et al., 1992, 1994). Very recently, Yanagisawa et al. (1996) have identified a 114-kDa MAR-binding protein that is expressed only in human breast carcinomas and not in either normal and 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), fibronectin (Zerlauth et al., 1988), keratin-like proteins (Aligué 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.,

1995; Merrimann 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 (Kaufmann 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 $(NH_4)_2SO_4$) 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; Izuarralde 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 (Izuarralde 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 Vassetzky, 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 phospho-inositide kinases, diacylglycerol kinase and phospho-lipase C (PLC). Ultrastructural observations 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 ß1 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 (RNA and 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 uniformously-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.

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. Waitz and Loidl, 1988; Lang et al., 1993) or even Dinoflagellates (Minguez 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 epithelial cell line LLC-PK_1 different extraction protocols do not yield final structures differing in a substantial way (either biochemically or immunologically) when the *in situ* procedure (Waitz 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 haemolymphopoietic 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*.

Acknowledgements. This work was supported by the Italian CNR grant PF ACRO, an Italian MURST 60% grant to the University of Trieste and Fondi AIRC 1995. We thank Giovanna Baldini for the illustrations.

References

Adachi Y., Kas E. and Laemmli U.K. (1989). Preferential, cooperative binding of DNA topoisomerase II to scaffold-associated regions. EMBO J. 13, 3997-4006.

Adolph K. (1980). Organization of chromosomes in HeLa cells: isolation of histone-depleted nuclei and nuclear scaffolds. J. Cell Sci. 42, 291-294.

Aligué R., Bastos R., Serratosa J., Enrich C., James P., Pujades C. and Bachs O. (1990). Increase in 55-kDa keratin-like protein in the nuclear matrix of rat liver cells during proliferative activation. Exp. Cell Res. 186, 346-353.

Amati B.B. and Gasser S. (1988). Chromosomal ARS and CEN elements bind specifically to the yeast nuclear scaffold. Cell 54, 967-978.

Ascoli C.A. and Maul G.G. (1991). Identification of a novel nuclear domain. J. Cell Biol. 112, 785-795.

Becker J. and Craig E.A. (1994). Heat-shock proteins as molecular chaperones. Eur. J. Biochem. 219, 11-23.

Belgrader P., Siegel A.J. and Berezney R. (1991a). A comprehensive study on the isolation and characterization of the HeLa S3 nuclear matrix. J. Cell Sci. 98, 281-291.

Belgrader P., Dey R. and Berezney R. (1991b). Molecular cloning of matrin 3. A 125-kilodalton protein of the nuclear matrix contains an extensive acidic domain. J. Biol. Chem. 266, 9893-9899.

Berezney R. (1979). Effect of protease inhibitors on matrix proteins and the association of replicating DNA. Exp. Cell Res. 123, 411-414.

Berezney R. (1984). Organization and functions of the nuclear matrix. In: Chromosomal nonhistone proteins. Vol IV. Hnilica L.S. (ed). CRC Press. Boca Raton. pp 119-180.

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

Berezney R. and Coffey D.S. (1974). Identification of a nuclear protein matrix. Biochem. Biophys. Res. Commun. 60, 1410-1417.

Berezney R. and Coffey D.S. (1977). Nuclear matrix. isolation and characterization of a framework structure from rat liver nuclei. J. Cell Biol. 73, 616-637.

Berrios S. and Fisher P.A. (1988). Thermal stabilization of putative karyoskeletal protein-enriched fractions from *Saccharomyces cerevisiae*. Mol. Cell Biol. 8, 4573-4575.

Berrios M., Osheroff N. and Fisher P.A. (1985). In situ localization of DNA topoisomerase II, a major polypeptide component of the Drosophila nuclear matrix fraction. Proc. Natl. Acad. Sci. USA 82, 4142-4146.

Bertagnolo V., Mazzoni M., Ricci D., Carini C., Neri L.M., Previati M. and Capitani S. (1995). Identification of PI-PLC ß1, γ1 and δ1 in rat liver: subcellular distribution and relationship to inositol lipid nuclear signalling. Cell Signal. 7, 669-678.

Beren A. Guan Y.H., Peart J., Cooper C and Shaw P. (1991). Monoclonal antibodies to plant nuclear matrix reveal intermediate filament-related components within the nucleus. J. Cell Sci. 98, 293-302.

Bisotto S., Lauriault P., Duval M. and Vincent M. (1995). Colocalization of a high molecular mass phosphoprotein of the nuclear matrix (p255) with spliceosomes. J. Cell Sci. 108, 1873-1882.

Blencowe B.J., Nickerson J.A., Issner R., Penman S. and Sharp P.A. (1994). Association of nuclear matrix antigens with exon-containing splicing complexes. J. Cell Biol. 127, 593-607.

Bohman K., Ferreira J., Santana N., Weis K. and Lamond A.I. (1995a) Molecular analysis of the coiled body. J. Cell Sci. Suppl. 19, 107-113.

Bohmann K., Ferreira J.A. and Lamond A.I. (1995b). Mutational analysis of p80 coilin indicates a functional interaction between coiled bodies and the nucleolus. J. Cell Biol. 131, 817-831,

Boy de la Tour E. and Laemmli U.K. (1988). The metaphase scaffold is helically folded: sister chromatids have predominantly opposite helical handedness. Cell 55, 937-944.

Boyle W.J. and Baluda M.A. (1987). Subnuclear association of the v-myb oncogene product and actin are dependent on ionic strength during nuclear isolation. Mol. Cell Biol. 7, 3345-3348.

Brasch K. and Ochs R.L. (1992). Nuclear bodies (NBs): a newly «rediscovered» organelle. Exp. Cell Res. 202, 211-223.

Capco D.G., Wan K.M. and Penman S. (1982). The nuclear matrix: three-dimensional architecture and protein composition. Cell 29, 847-858.

Capitani S., Girard P.R., Mazzei G.J., Kuo J.F., Berezney R. and Manzoli F.A. (1987). Immunochemical characterization of protein kinase C in rat liver nuclei and subnuclear fractions. Biochem. Biophys. Res. Commun. 142, 367-375.

Cárdenas M.E., Laroche T. and Gasser S.M. (1990). The composition and morphology of yeast nuclear scaffolds. J. Cell Sci. 96, 439-450.

Carter K.C., Taneja K.L. and Lawrence J.B. (1991). Discrete nuclear domains of poly(A)RNA and their relationship to the functional organization of the nucleus. J. Cell Biol. 115, 1191-1202.

Carvalho T., Seeler J.B., Ohman K., Jordan P., Pettersson U., Akusjarvi G., Carmo-Fonseca M. and Dejean A. (1995). Targeting of adenovirus E1A and E4-ORF3 proteins to nuclear matrix-associated PML bodies. J. Cell Biol. 131, 45-56.

Clevenger C.V. and Epstein A.L. (1984). Identification of a nuclear protein component of interchromatin granules using a monoclonal antibody and immunogold electron microscopy. Exp. Cell Res. 151, 194-207.

Cocco L., Martelli A.M. and Gilmour R.S. (1994). Inositol lipid cycle and the nucleus. Cell Signal. 6, 481-485.

Cook P.R. (1988). The nucleoskeleton: artefact, passive framework or active site? J. Cell Sci. 90, 1-6.

Cook P.R. (1995). A chromomeric model for nuclear and chromosome structure. J. Cell Sci. 108, 2927-2935.

Dessev G.N. (1994). Nuclear envelope structure. Cur. Opin. Cell Biol. 6, 430-435.

De Thé H., Lavau C., Marchio A., Chomienne C., Degos L. and Dejean A. (1991). The PML.RARα fusion mRNA generated by the t(15;17) translocation of acute promyelocytic leukemia encodes a functionally altered RAR. Cell 76, 333-343.

Djondjurov L., Ivanova E., Markov D., Bardarov S. and Sachsenmaier W. (1986). Is the nucler matrix the site of DNA replication in eukaryotic cells? Exp. Cell Res. 164, 79-96.

Dickinson P., Cook P.R. and Jackson D.A. (1990). Active RNA plymerase I is fixed within the nucleus of HeLa cells. EMBO J. 9, 2207-2214.

Dickinson L.A., Joh T., Kohwi Y. and Kohwi-Shigematsu T. (1992). A tissue-specific MAR/SAR DNA binding protein with unusual binding site recognition. Cell 70, 631-645.

van Driel R., Humbel B. and de Jong L. (1991). The nucleus: a black box being opened. J. Cell Biochem. 47, 311-316.

Durfee T., Mancini M.A., Jones D., Elledge S.J. and Lee W.H. (1994). The amino-terminal region of the retinoblastoma gene product binds a novel nuclear matrix protein that co-localizes to centers for RNA processing. J. Cell Biol. 127, 609-622.

Dworetzky S.I., Fey E.G., Penman S., Lian J.B., Stein J.L. and Stein G.S. (1990). Progressive changes in the protein composition of nuclear matrix during rat osteoblast differentiation. Proc. Natl. Acad. Sci. USA 87, 4605-4609.

van Eeekelen C.A.G., Salden M.H.L., Habets W.J.A., van de Putte L.B.A. and van Venrooij W.J. (1982). On the existence of an internal nuclear protein structure in HeLa cells. Exp. Cell Res. 141, 181-190.

Earnshaw W.C. (1987). Anionic regions in nuclear proteins. J. Cell Biol. 105, 1479-1482.

Earnshaw W.C. and Laemmli U.K. (1983). Architecture of metaphase chromosomes and chromose scaffolds. J. Cell Biol. 96, 84-93.

Earnshaw W.C., Halligan B., Cooke C.A., Heck M.S. and Liu L.F. (1985). Topoisomerase II is a structural component of mitotic chromosome scaffold. J. Cell Biol. 100, 1716-1725.

Eberharter A., Grabher A., Gstraunthaler G. and Loidl P. (1993). Nuclear matrix of the lower eukaryote *Physarum polycephalum* and the mammalian epithelial LLC-PK_1 cell line. A comprehensive investigation of different procedures. Eur. J. Biochem. 212, 573-580.

Eggert H. and Jack R.S. (1991). An ectopic copy of the Drosophila ftz associated SAR neither reorganizes local chromatin structure nor hinders elution of a chromatin fragment from isolated nuclei. EMBO J. 10, 1237-1243.

Evan G.I. and Hancock D.C. (1985). Studies on the interaction of the human c-myc protein with cell nuclei: $p62^{cmyc}$ as a member of a discrete subset of nuclear protein. Cell 43, 253-261.

Fackelmayer F.D., Dahm K., Renz A., Ramsperger U. and Richter A. (1994). Nucleic-acid-binding properties of hnRNP-U/SAF-A, a nuclear-matrix protein which binds DNA and RNA *in vivo* and *in vitro*. Eur. J. Biochem. 221, 749-757.

Ferreira J.A., Carmo-Fonseca M. and Lamond A.I. (1994). Differential interaction of splicing snRNPs with coiled bodies and interchromatin granules during mitosis and assembly of daughter cell nuclei. J. Cell Biol. 126, 11-23.

Feuerstein N., Spiegel S. and Mond J.J. (1988). The nuclear matrix protein, numatrin (B23), is associated with growth factor-induced mitogenesis in Swiss 3T3 fibroblasts and with T lymphocyte

proliferation stimulated by lectins and anti-T cell antigen receptor antibody. J. Cell Biol. 107, 1629-1642.

Fey E.G. and Penman S. (1988). Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc. Natl. Acad. Sci. USA 85, 121-125.

Fields A.P. and Shaper J.H. (1988). A major 62-kD intranuclear matrix polypeptide is a component of metaphase chromosomes. J. Cell Biol. 107, 833-840.

Fields A.P., Kaufmann S.H. and Shaper J.H. (1986). Analysis of the internal nuclear matrix. Oligomers of a 38 kD nucleolar polypeptide stabilized by disulfide bonds. Exp. Cell Res. 164, 139-153.

Fisher P.A., Lin L., McConnell M., Greeleaf A., Lee J.-M. and Smith D.E. (1989). Heat shock-induced appearance of RNA Polymerase II In karyoskeletal protein-enriched (nuclear «matrix») fractions correlates with transcriptional shutdown in *Drosophila melanogaster*. J. Biol. Chem. 264, 3464-3469.

Frey M.R. and Matera A.G. (1995). Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells. Proc. Natl. Acad. Sci. USA 92, 5915-5919.

Gerdes M.G., Carter K.C., Moen P.T. and Lawrence J.B. (1994). Dynamic changes in the higher-level chromatin organization of specific sequences revealed by in situ hybridization to nuclear halos. J. Cell Biol. 126, 289-304.

Getzenberg R.H., Pienta K.J., Huang E.Y.W., Murphy B.C. and Coffey D.S. (1991a). Modifications of the intermediate filament and nuclear matrix networks by the extracellular matrix. Biochem. Biophys. Res. Commun. 179, 340-344.

Getzenberg R.H., Pienta K.J., Huang E.Y.W. and Coffey D.S. (1991b). Identification of nuclear matrix proteins in the cancer and normal rat prostate. Cancer Res. 51, 6514-6520.

de Graaf A., Meijne A.M.L., van Renswoude J.B.M., Humbel B.M., van Bergen en Henegouwen P.M.P., de Jong L., van Driel R., Verkleij A.J. (1992). Heat shock-induced redistribution of a 160-kDa nuclear matrix protein. Exp. Cell Res. 202, 243-251.

Guo B., Odgren P.R., van Wijnen A.J., Last T.J., Nickerson J., Penman S., Lian J.B., Stein J.L. and Stein G.S. (1995). The nuclear matrix protein NMP-1 is the transcription factor YY1. Proc. Natl. Acad. Sci. USA 92, 10526-10530.

Hakes D.J. and Berezney R. (1991a). Molecular cloning of matrin F/G: a DNA binding protein of the nuclear matrix that contains putative zinc finger motifs. Proc. Natl. Acad. Sci. USA 88, 6186-6190.

Hakes D.J. and Berezney R. (1991b). DNA binding properties of the nuclear matrix and individual nuclear matrix proteins. Evidence for salt-resistant DNA binding sites. J. Biol. Chem. 266, 11131-11140.

Hartl F.-U., Hlodan R. and Langer T. (1994). Molecular chaperones in protein folding: the art of avoiding sticky situations. Trends Biochem. Sci. 19, 20-25.

Hassan A.B. and Cook P.R. (1993). Visualization of replication sites in unfixed human cells. J. Cell Sci. 105, 541-550.

He D., Nickerson J.A. and Penman S. (1990). Core filaments of the nuclear matrix. 110, 569-580.

He D., Martin T. and Penman S. (1991). Localization of heterogeneous nuclear ribonucleoprotein in the interphase nuclear matrix core filaments and on perichromosomal filaments at mitosis. Proc. Natl. Acad. Sci. USA 88, 7469-7473.

Hendzel M.J., Delcuve G.P. and Davie J.R. (1991). Histone deacetylase is a component of the internal nuclear matrix. J. Biol. Chem. 266, 21936-21942.

Hernández-Verdun D. (1991). The nucleolus today. J. Cell Sci. 99, 465-471.

Hozak P., Hassan A.B., Jackson D.A. and Cook P.R. (1993). Visualization of replication factories attached to a nucleoskeleton. Cell 73, 361-373.

Hozak P., Jackson D.A. and Cook P.R. (1994). Replication factories and nuclear bodies: the ultrastructural characterization of replication sites during the cell cycle. J. Cell Sci. 107, 639-648.

Hozak P., Sasseville A.M.-J., Raymond Y. and Cook P.R. (1995). Lamins proteins form an internal nucleoskeletons as well as a peripheral lamina in human cells. J. Cell Sci. 108, 635-644.

Hughes T.A., Pombo A., McManus J., Hozak P., Jackson D.D. and Cook P.R. (1995). On the structure of replication and transcription factories. J. Cell Sci. Suppl. 19, 59-65.

Humphrey G.W. and Pigiet V. (1987). Protein disulfide crosslinking stabilizes a polyoma large T antigen-host protein complex on the nuclear matrix. Exp. Cell Res. 171, 122-136.

Isomura T., Tamiya-Koizumi K., Suzuki M., Yoshida M., Taniguchi M., Matsuyama M., Ishigaki T., Sakuma S. and Takahashi M. (1992). RFP is a DNA binding protein associated with the nuclear matrix. Nucleic Acids Res. 20, 5305-5310.

Izuarralde E., Mirkovitch J. and Laemmli U.K. (1988). Interaction of DNA with nuclear scaffolds in vitro. J. Mol. Biol. 200, 111-125.

Izuarralde E., Kas E. and Laemmli U.K. (1989). Highly preferential nucleation of histone H1 assembly on scaffold-associated regions. J. Mol. Biol. 210, 573-585.

Jack R.S. and Eggert H. (1992). The elusive nuclear matrix. Eur. J. Biochem. 209, 503-509.

Jackson D.A. and Cook P.R. (1988). Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat. EMBO J. 7, 3667-3677.

Jackson D.A., Yuan J. and Cook P.R. (1988). A gentle method for preparing cyto- and nucleo-skeletons and associated chromatin. J. Cell Sci. 90, 365-378.

Jackson D.A., Dickinson P. and Cook P.R. (1990a). Attachment of DNA to the nucleoskeleton of HeLa cells examined using physiological conditions. Nucleic Acids Res. 18, 4385-4393.

Jackson D.A., Dickinson P. and Cook P.R. (1990b). The size of chromatin loops in HeLa cells. EMBO J. 9, 567-571.

Jackson D.A., Dolle G., Robertson G. and Cook P.R. (1992). The attachments of chromatin loops to the nucleoskeleton. Cell Biol. Int. Rep. 16, 687-696.

Jackson D.A., Balajee A.S., Mullenders L. and Cook P.R. (1994). Sites in human nuclei where DNA damaged by ultraviolet light is repaired: visualization and localization relative to the nucleoskeleton. J. Cell Sci. 107, 1745-1752.

de Jong L., van Driel R., Stuurman N., Meijne A.M.L. and van Renswoude J. (1990). Principles of nuclear organization. Cell Biol. Int. Rep. 14, 1051-1074.

Kallajoki M., Weber K. and Osborn M. (1991). A 210 kDa nuclear matrix protein is a functional part of the mitotic spindle; a microinjection study using SPN monoclonal antibodies. EMBO J. 10, 3351-3362.

Karwan R.M., Laroche T., Wintersberger U., Gasser S.M. and Binder M. (1990). Ribonuclease H(70) is a component of the yeast nuclear scaffold. J. Cell Sci. 96, 451-459.

Kas E., Izuarralde E. and Laemmli U.K. (1989). Specific inhibition of DNA binding to nuclear scaffolds and histone H1 by distamycin. The role of oligo(dA)oligo(dT) tracts. J. Mol. Biol. 210, 587-599.

Kaufmann S.H. and Shaper J.H. (1984). A subset of non-histone nuclear proteins stabilized by the sulfhydryl cross-linking reagent tetrathionate. Polypeptides of the internal nuclear matrix. Exp. Cell

Res. 155, 477-495.

Kaufmann S.H. and Shaper J.H. (1991). Association of topoisomerase II with the hepatoma cell nuclear matrix: the role of intermolecular disulfide band formation. Exp. Cell Res. 192, 511-523.

Kaufmann S.H., Coffey D.S. and Shaper J.H. (1981). Considerations in the isolation of rat liver nuclear matrix, nuclear envelope and pore complex lamina. Exp. Cell Res. 132, 105-123.

Kaufmann S.H., Brunet G., Talbot B., Lamarr D., Dumas C., Shaper J.H. and Poirier G. (1991). Association of poly(ADP-ribose) polymerase with the nuclear matrix: the role of intermolecular disulfide bond formation, RNA retention, and cell type. Exp. Cell Res. 192, 524-535.

Keese S.K., Meneghini M.D., Szaro R.P. and Wu Y.-J. (1991). Nuclear matrix proteins in human colon cancer. Proc. Natl. Acad. Sci. USA 91, 1913-1916.

Kirov N., Djondjurov L. and Tsanev R. (1984). Nuclear matrix and transcriptional activity of the mouse α-globin gene. J. Mol. Biol. 180, 601-614.

Koken M.H.M., Puvion-Dutilleul F., Guillemin M.C., Viron A., Linares-Cruz G., Stuurman N., de Jong L., Szostecki C., Calco C., Chomienne C., Degos L., Puvion E. and De Thé H. (1994). The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. EMBO J. 13, 1073-1083.

Korioth F., Gieffers C., Maul G.G. and Frey J. (1995). Molecular characterization of a NDP52, a novel protein of the nuclear domain 10, which is redistributed upon virus infection and interferon treatment. J. Cell Biol. 130, 1-13.

von Kries J.P., Buck F. and Stratling W.H. (1994). Chicken MAR binding protein p120 is identical to human heterogeneous nuclear ribonucleoprotein (hnRNP) U. Nucleic Acids Res. 22, 1216-1220.

Lang S., Decristoforo T., Waitz W. and Loidl P. (1993). Biochemical and morphological characterization of the nuclear matrix during the synchronous cell cycle of *Physarum polycephalum*. J. Cell Sci. 105, 1121-1130.

Lawrence J.B., Singer R.H. and Marselle L.M. (1989). Highly localized tracks of specific transcripts within interphase nuclei visualized by in situ hybridization. Cell 57, 493-502.

Lebkoswki J.S. and Laemmli U.K. (1982). Non-histone proteins and long-range organization of HeLa interphase DNA. J. Mol. Biol. 156, 325-344.

Lewis C.D., Lebkowski J.S., Daly A.K. and Laemmli U.K. (1984). Interphase nuclear matrix and metaphse scaffolding structures. J. Cell Sci. Suppl. 1, 103-122.

Liao H., Winkfein R.J., Mack G., Rattner J.B. and Yen T.J. (1995). CENP-F is a protein on the nuclear matrix that assembles onto kinetochores at late G2 and is rapidly degraded after mitosis. J. Cell Biol. 130, 507-518.

Littlewood T.D., Hancock D.C. and Evan G.I. (1987). Characterization of a heat shock-induced insoluble complex in the nuclei of cells. J. Cell Sci. 88, 65-72.

Ludérus M.E.E., de Graaf A., Mattia E., den Blaauwen J.L., Grande M.A., de Jong L. and van Driel R. (1992). Binding of attachment regions to lamin B_1. Cell 70, 949-959.

Ludérus M.E.E., den Blauween J.L., de Smit O.J., Compton D.A. and van Driel R. (1994). Binding of matrix attachment regions to lamin polymers involves single-stranded regions and the minor groove. Mol. Cell Biol. 14, 6297-6305.

Mancini M.A., Shan B., Nickerson J.A., Penman S. and Lee W.-H. (1994). The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated proteins. Proc. Natl. Acad. Sci. USA 91, 418-422.

Manfredi Romanini M.G. and Fraschini A. (1996). Facts and paradoxes in current notions of nuclear organization and function. Histol. Histopathol. 11, 513-519.

Manzoli F.A., Martelli A.M., Capitani S., Maraldi N.M., Rizzoli R., Barnabei O. and Cocco L. (1989). Nuclear polyphosphoinositide during cell growth and differentiation. Adv. Enzyme Regul. 28, 25-34.

Maraldi N.M., Marinelli F., Cocco L., Papa S., Santi P. and Manzoli F.A. (1986). Morphometric analysis and topological organization of nuclear matrix in freeze-fractured electron microscopy. Exp. Cell Res. 163, 349-362.

Maraldi N.M., Zlni N., Ognibene A., Martelli A.M., Barbieri M., Mazzotti G. and Manzoli F.A. (1995). Immunocytochemical detection of the intranuclear variations of phosphatidylinositol 4,5-biphosphate amount associated with changes of activity and amount of phospholipase C ß1 in cells exposed to mitogenic or differentiating agents. Biol. Cell 83, 201-210.

Martelli A.M. and Cocco L. (1994). No discrete complexes containing DNA polymerase α activity can be solubilized from the heat-stabilized nuclear matrix prepared from HeLa S3 cells. Cell Biochem. Funct. 12, 37-44.

Martelli A.M., Gilmour R.S., Falcieri E., Manzoli F.A. and Cocco L. (1990). Temperature-dependent association of DNA polymerase α activity with the nuclear matrix. Exp. Cell Res. 190, 227-232.

Martelli A.M., Falcieri E., Gobbi P., Manzoli L., Gilmour R.S. and Cocco L. (1991). Heat-induced stabilization of the nuclear matrix. A biochemical and morphological analysis in mouse erythroleukemia cells. Exp. Cell Res. 196, 216-225.

Martelli A.M., Falcieri E., Gobbi P., Manzoli L., Cataldi A., Rana R.A. and Cocco L. (1992a). Further considerations on the thermal stabilization of the nuclear matrix in mouse erythroleukemia cells. Cell Biol. Int. Rep. 16, 307-317.

Martelli A.M., Gilmour R.S., Bareggi R. and Cocco L. (1992b). The effect of *in vitro* heat exposure on the recovery of nuclear matrix-bound DNA polymerase α activity during the different phases of the cell cycle in synchronized HeLa S3 cells. Exp. Cell Res. 201, 470-476.

Martelli A.M., Billi A.M., Gilmour R.S., Manzoli L., Di Primio R. and Cocco L. (1992c). Mouse and human hemopoietic cell lines of erythroid lineage express lamins A, B and C. Biochem. Biophys. Res. Commun. 185, 271-275.

Martelli A.M., Gilmour R.S., Bertagnolo V., Neri L.M., Manzoli L. and Cocco L. (1992d). Nuclear localization and signalling activity of phosphoinositidase Cß in Swiss 3T3 cells. Nature 358, 342-344.

Martelli A.M., Neri L.M., Billi A.M., Bareggi R. and Cocco L. (1993). Absence of high levels of DNA polymerase α activity in the nuclear matrix prepared from mouse erythroleukemia cells. Cell Biol. Int. Rep. 17, 635-644.

Martelli A.M., Neri L.M., Zamai L., Bareggi R., Manzoli L. and Cocco L. (1994a). 6-iodoacetamidofluorescein labelling to assess the state of sulfhydryl groups after thermal stabilization of isolated nuclei. Histochem. J. 26, 179-188.

Martelli A.M., Bareggi R. and Narducci P. (1994b). Catalytic properties of DNA polymerase α activity associated with the heat-stabilized nuclear matrix prepared from HeLa S3 cells. Cell Biochem. Funct. 12, 129-135.

Martelli A.M., Manzoli L., Rubbini S., Billi A.M., Bareggi R. and Cocco L. (1995). The protein composition of Friend cell nuclear matrix

stabilized by various treatments. Different recovery of nucleolar proteins B23 and C23 and nuclear lamins. Biol. Cell 83, 15-22.

Mazzotti G., Rizzoli R., Galanzi A., Papa S., Vitale M., Falconi M., Neri L.M., Zini N. and Maraldi N.M. (1990). High-resolution detection of newly synthesized DNA by anti-bromodeoxyuridine antibodies identifies specific chromatin domains. J. Histochem. Cytochem. 38, 13-22.

Mazzotti G., Zini N., Rizzi E., Rizzoli R., Galanzi A., Ognibene A., Santi S., Matteucci A., Martelli A.M. and Maraldi N.M. (1995). Immunocytochemical detection of phoshatidylinositol 4,5-biphosphate localization sites within the nucleus. J. Histochem. Cytochem. 43, 181-191.

McConnell M., Whalen A.M., Smith D.E. and Fisher P.A. (1987). Heat shock-induced changes in the structural stability of proteinaceous karyoskeletal elements in vitro and morphological effects in situ. J. Cell Biol. 105, 1087-1098.

McKeon F.D., Kirschner M.W. and Caput D. (1986). Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. Nature 319, 463-468.

Melan M.A. and Sluder G. (1992). Redistribution and differential extraction of soluble proteins in permeabilized cultured cells. Implications for immunofluorescence microscopy. J. Cell Sci. 101, 732-743.

Merrimann H.L., van Wijnen A.J., Hiebert S., Bidwell J.P., Fey E., Lian J., Stein S. and Stein G.S. (1995). The tissue-specific nuclear matrix, NMP-2, is a member of the AML/CBF/PEBP2/Runt domain transcription factor family: interactions with the osteocalcin gene promoter. Biochemistry 34, 13125-13132.

Mínguez A., Franca S. and Moreno Diaz de la Espina S. (1994). Dinoflagellates have a eukaryotic nuclear matrix with lamin-like proteins and topoisomerase II. J. Cell Sci. 107, 2861-2873.

Mirkovitch J., Mirault M.-E. and Laemmli U.K. (1984). Organization of the higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. Cell 39, 223-232.

Moir R.D. and Goldman R.D. (1993). Lamin dynamics. Curr. Opin. Cell Biol. 5, 408-411.

Moir R.D., Montag-Lowy M. and Goldman R.D. (1994). Dynamic properties of nuclear lamins: lamin B is associated with sites of DNA replication. J. Cell Biol. 125, 1201-1212.

Nakayasu H. and Berezney R. (1989). Mapping replicational sites in the eucaryotic cell nucleus. J. Cell Biol. 108, 1-11.

Nakayasu H. and Berezney R. (1991). Nuclear matrins: identification of the major nuclear matrix proteins. Proc. Natl. Acad. Sci. USA 88, 10312-10316.

Neri L.M., Mazzotti G., Capitani S., Maraldi N.M., Cinti C., Baldini N., Rana R. and Martelli A.M. (1992). Nuclear matrix-bound replicational sites detected in situ by 5-bromodeoxyuridine. Histochemistry 98, 19-32.

Neri L.M., Santi S., Marugg R.A., Riederer B.M., Capitani S., Cataldi A. and Martelli A.M. (1994). In vitro heat exposure induces a redistribution of nuclear matrix proteins in K562 erythroleukemia cells. Exp. Cell Res. 213, 275-285.

Neri L.M., Riederer B.M., Marugg R.A., Capitani S. and Martelli A.M. (1995a). Analysis by confocal microscopy of the behaviour of the heat shock protein 70 within the nucleus and of a nuclear matrix polypeptide during prolonged heat shock response in HeLa cells. Exp. Cell Res. 221, 301-310.

Neri L.M., Riederer B.M., Marugg R.A., Capitani S. and Martelli A.M. (1995b). The effect of sodium tetrathionate stabilization on the distribution of three nuclear matrix proteins in human K562 erythroleukemia cells. Histochem. Cell Biol. 104, 29-36.

Nickerson J.A., Krochmalnic G., Wan K.M. and Penman S. (1989). Chromatin architecture and nuclear RNA. Proc. Natl. Acad. Sci. USA 86, 177-181.

Nickerson J.A., Krockmalnic G., Wan K.M., Turner C.D. and Penman S. (1992). A normally masked nuclear matrix antigen that appears at mitosis on cytoskeleton filaments adjoining chromosomes, centrioles, and midbodies. J. Cell Biol. 116, 977-987.

Ochs R.L. and Smetana K. (1991). Detection of fibrillarin in nucleolar remnants and the nucleolar matrix. Exp. Cell Res. 197, 183-190.

Panté N. and Aebi U. (1995). Exploring nuclear pore complex structure and function in molecular detail. J. Cell Sci. Suppl. 19, 1-11.

Panzeter P.L. and Ringer D.P. (1993). DNA precursors are channelled to nuclear matrix DNA replication sites. Biochem. J. 293, 775-779.

Pardoll D.M., Vogelstein B. and Coffey D.S. (1980). A fixed site of DNA replication in eucaryotic cells. Cell 19, 527-536.

Payrastre B., Nievers M., Boonstra J., Breton M., Verkleij A.J. and van Bergen en Henegouwen P.M.P. (1992). A differential location of phosphoinositide kinases, diacylglycerol kinase, and phospholipase C in the nuclear matrix. J. Biol. Chem. 267, 5078-5084.

Pieck A.C.M., Rijken A.A.M. and Wanka F. (1987). Nuclear matrix and chromosome scaffold preparations of in vitro cultured bovine liver cells have two proteins in common. FEBS Lett. 212, 276-280.

Pienta K.J. and Coffey D.S. (1992). Nuclear-cytoskeletal interactions: evidence for physical connections between the nucleus and cell periphery and their alteration by transformation. J. Cell. Biochem. 49, 357-365.

Pienta K.J., Partin A.W. and Coffey D.S. (1989). Cancer as a disease of DNA organization and dynamic cell structure. Cancer Res. 49, 2525-2532.

Pombo A., Ferreira J., Bridge E. and Carmo-Fonseca M. (1994). Adenovirus replication and transcription sites are spatially separated in the nucleus of infected cells. EMBO J. 13, 5075-5085.

Puvion-Dutilleul F., Chelbi-Alix M.K., Koken M., Quignon F., Puvion E. and De Thé H. (1995). Adenovirus infection induces rearrangements in the intranuclear distribution of the nuclear body-associated PML protein. Exp. Cell Res. 218, 9-16.

Razin S.V. and Gromova I.I. (1995). The channels model of nuclear matrix structure. Bioessays 17, 443-450.

Razin S.V. and Vassetzky Y.S. (1992). Domain organization of eukaryotic genome. Cell Biol. Int. Rep. 16, 697-708.

Saunders W.S., Cooke C.A. and Earnshaw W.C. (1991). Compartmentalization within the nucleus: discovery of a novel nuclear region. J. Cell Biol. 115, 919-931.

Smith H.C. and Berezney R. (1982). Nuclear matrix-bound deoxyribonucleic acid synthesis: an in vitro system. Biochemistry 21, 6751-6761.

Smith H.C. and Berezney R. (1983). Dynamic domains of DNA polymerase α in regenerating rat liver. Biochemistry 22, 3042-3046.

Smith H.C., Berezney R., Brewster J.M. and Rekosh D. (1985). Properties of adenoviral DNA bound to the nuclear matrix. Biochemistry 24, 1197-1202.

Smith H.C., Harris S.G., Zillmann M. and Berget S.M. (1989). Evidence that a nuclear matrix protein participates in premessenger RNA splicing. Exp. Cell Res. 182, 521-533.

Staufenbiel M. and Deppert W. (1984). Preparation of nuclear matrices from cultured cells: subfractionation of nuclei in situ. J. Cell Biol. 98, 1886-1894.

van Steensel B., Brink M., van der Meulen K., van Binnendijk E.P., Wansink D.G., de Jong L., de Kloet E.R. and van Driel R. (1995). Localization of the glucocorticoid receptor in discrete clusters in the cell nucleus. J. Cell Sci. 108, 3003-3011.

Stuurman N., Meijne A.M.L., van der Pol A.J., de Jong L., van Driel R. and van Renswoude J. (1990). The nuclear matrix from cells of different origin. Evidence for a common set of matrix proteins. J. Biol. Chem. 265, 5460-5465.

Stuurman N., de Graaf A., Floore A., Josso A., Humbel B., de Jong L. and van Driel R. (1992a). A monoclonal antibody recognizing nuclear matrix-associated nuclear bodies. J. Cell Sci. 101, 773-784.

Stuurman N., Floore A., Colen A., de Jong L. and van Driel R. (1992b). Stabilization of the nuclear matrix by disulfide bridges: identification of matrix polypeptides that form disulfides. Exp. Cell Res. 200, 285-294.

Stuurman N., de Jong L. and van Driel R. (1992c). Nuclear frameworks: concepts and operational definitions. Cell Biol. Int. Rep. 16, 837-852.

Sun J.-M., Chen H.Y. and Davie J.R. (1994). Nuclear factor 1 is a component of the nuclear matrix. J. Cell Biochem. 55, 252-263.

Tamiya-Koizumi K., Umekawa H., Yoshida S., Ishihara H. and Kojima K. (1989). A novel phospholipase A_2 associated with nuclear matrix: stimulation of the activity and modulation of the Ca^{2+} dependency by polyphosphoinositides. Biochem. Biophys. Acta 1002, 182-188.

Tawfic S. and Ahmed K. (1994). Growth stimulus-mediated differential translocation of casein kinase 2 to the nuclear matrix. Evidence based on androgen action in the prostate. J. Biol. Chem. 269, 24615-24620.

Thiry M. (1993). Differential location of nucleic acids within interchromatin granule clusters. Eur. J. Cell Biol. 62, 259-269.

Thiry M. (1994). Cytochemical and immunocytochemical study of coiled bodies in different cultured cell lines. Chromosoma 103, 268-276.

Thiry M. (1995). The interchromatin granules. Histol. Histopathol. 10, 1035-1045.

Tsutsui K., Tsutsui K., Okada S., Watarai S., Seki S., Yasudai T. and Shohmori T. (1993). Identification and characterization of a nuclear scaffold protein that binds the matrix attachment region DNA. J. Biol. Chem. 268, 12886-12894.

Tubo R.A. and Berezney R. (1987a). Pre-replicative association of multiple replicative enzyme activities with the nuclear matrix during rat liver regeneration. J. Biol. Chem. 262, 1148-1154.

Tubo R.A. and Berezney R. (1987b). Identification of 100 and 150 S DNA polymerase α-primase megacomplexes solubilized from the nuclear matrix of regenerating rat liver. J. Biol. Chem. 262, 5857-5865.

Tubo R.A. and Berezney R. (1987c). Nuclear matrix-bound DNA primase. Elucidation of an RNA priming system in nuclear matrix isolated from regenerating rat liver. J. Biol. Chem. 262, 6637-6642.

Tubo R.A., Martelli A.M. and Berezney R. (1987). Enhanced processivity of nuclear matrix bound DNA polymerase α from regenerating rat liver. Biochemistry 26, 5710-5718.

Verheijen R., van Venrooij W. and Ramekers F. (1988). The nuclear matrix: structure and composition. J. Cell Sci. 90, 11-36.

Vishwanatha J.K., Kindal H.K. and Davis R.G. (1992). The role of primer recognition proteins in DNA replication: association with nuclear matrix in HeLa cells. J. Cell Sci. 101, 25-34.

Vogelstein B., Pardoll D.M. and Coffey D.S. (1980). Supercoiled loops and eucaryotic DNA replication. Cell 22, 79-85.

Wansink D.G., Schul W., van der Kraan I., van Steensel B., van Driel R. and de Jong L. (1993). Fluorescent labeling of nascent RNA reveals transcription by RNA polymerase II in domains scattered throughout the nucleus. J. Cell Biol. 122, 283-293.

Wachsberger P.R. and Coss R.A. (1993). Alterations in nuclear matrix ultrastructure of G1 mammalian cells following heat shock: resinless section electron microscopy, biochemical, and immunofluorescence studies. J. Cell. Physiol. 155, 615-634.

Waitz W. and Loidl P. (1988). In situ preparation of the nuclear matrix of *Physarum polycephalum*: ultrastructural and biochemical analysis of different matrix isolation procedures. J. Cell Sci. 90, 621-628.

Wan K., Nickerson J.A., Krockmalnic G. and Penman S. (1994). The B1C8 protein is in the dense assemblies of the nuclear matrix and relocates to the spindle and pericentriolar filaments at mitosis. Proc. Natl. Acad. Sci. USA 91, 594-598.

Wang B., Dickinson L.A., Koivunen E., Ruoslahti E. and Kohwi-Shigematsu T. (1995). A novel matrix attachment region DNA binding motif identified using a random phage peptide library. J. Biol. Chem. 270, 23239-23242.

Wang X. and Traub P. (1991). Resinless section immunogold electron microscopy of karyo-cytoskeletal frameworks of eukaryotic cells cultured in vitro. Absence of a salt-stable nuclear matrix from mouse plasmacytoma MPC-11 cells. J. Cell Sci. 98, 107-122.

Weis K.S., Rambaud S., Lavau C., Jansen J., Carvalho M., Carmo-Fonseca M., Lamond A.I. and Dejean A. (1994). Retinoic acid regulates aberrant nuclear localization of PML-RARα in acute promyelocytic leukemia cells. Cell 76, 345-356.

Yanagisawa J., Ando J., Nakayama J., Kohwi Y. and Kohwi-Shigematsu T. (1996). A matrix attachment region (MAR)-binding activity due to a p114 kilodalton protein is found only in human breast carcinomas and not in normal and benign breast disease tissues. Cancer Res. 56, 457-462.

Yancheva N., Markov D. and Djondjurov L. (1986). Evidence for the existence in the nuclei of Friend cells of a new type of ribonucleoprotein network. J. Cell Sci. 84, 165-182.

Zerlauth G., Wesierska-Gadek J. and Sauermann G. (1988). Fibronectin observed in the nuclear matrix of HeLa tumor cells. J. Cell Sci. 89, 415-421.

Zhang G., Taneja K.L., SInger R.H. and Green M.R. (1994). Localization of pre-mRNA splicing in mammalian nuclei. nature 372, 809-812.

Zini N., Santi S., Ognibene A., Bavelloni A., Neri L.M., Valmori A., Mariani E., Negri C., Astaldi-Ricotti G.C.B. and Maraldi N.M. (1994). Discrete localization of different DNA topoisomerases in HeLa and K562 cell nuclei and subnuclear fractions. Exp. Cell Res. 210, 336-348.

Zini N., Martelli A.M., Neri L.M., Bavelloni A., Sabatelli P., Santi S. and Maraldi N.M. (1995a). Immunocytochemical evaluation of protein kinase C translocation to the inner nuclear matrix in 3T3 mouse fibroblasts after IGF-I treatment. Histochemistry 103, 447-457.

Zini N., Ognibene A., Marmiroli S., Bavelloni A., Maltarello M.C., Faenza I., Valmori A. and Maraldi N.M. (1995b). The intranuclear amount of phospholipase C ß1 decreases following cell differentiation in Friend cells, whereas the γ1 isoform is not affected. Eur. J. Cell Biol. 68, 25-34.

Zong R.-T. and Scheuermann R.H. (1995). Mutually exclusive interaction of a novel matrix attachment region binding protein and the NF-μNR enhancer repressor. Implications for the regulation of immunoglobulin heavy chain expression. J. Biol. Chem. 270, 24010-24018.