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Half a Century of “The Nuclear Matrix”

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A cell fraction that would today be termed “the nuclear matrix” was first described and patented in 1948 by Russian investigators. In 1974 this fraction was rediscovered and promoted as a fundamental organizing principle of eukaryotic gene expression. Yet, convincing evidence for this functional role of the nuclear matrix has been elusive and has recently been further challenged. What do we really know about the nonchromatin elements (if any) of internal nuclear structure? Are there objective reasons (as opposed to thinly veiled disdain) to question experiments that use harsh nuclear extraction steps and precipitation-prone conditions? Are the known biophysical properties of the nucleoplasm *in vivo* consistent with the existence of an extensive network of anastomosing filaments coursing dendritically throughout the interchromatin space? To what extent may the genome itself contribute information for its own quarternary structure in the interphase nucleus? These questions and recent work that bears on the mystique of the nuclear matrix are addressed in this essay. The degree to which gene expression literally depends on nonchromatin nuclear structure as a facilitating organizational format remains an intriguing but unsolved issue in eukaryotic cell biology, and considerable skepticism continues to surround the nuclear matrix fraction as an accurate representation of the *in vivo* situation.

When cell nuclei (or cells) are extracted in certain ways, an extensive array of filaments is observed in the remnant nucleus: “the nuclear matrix.” Onto this framework virtually every step in gene readout has been conceptually draped. Some investigators worshipfully attribute to this envisioned nuclear scaffold the same biochemically enabling attributes that the cytoskeleton (demonstrably) conveys for cell shape, cell locomotion, intracellular vesicular traffic, and chromosome movement during cell division. But many other investigators consider this nuclear matrix fraction to be a global aggregation phenomenon. Few aspects of contemporary eukaryotic cell biology have been more contentious.

These polar views reflect different perspectives on the cell nucleus as a living organelle versus a subcellular platform for hopefully instructive extraction. Some strong opponents of the nuclear matrix have had research experiences with various cells, including living material, that have given them pause about the existence of a nuclear matrix *in vivo*. And, at the same time, some nuclear matrix proponents perhaps have been too ready to suspend disbelief. Meanwhile, a large cast of investigators has waited in the wings as it were, mostly unbiased and just curious, wondering whether there is in fact some kind of nuclear scaffold *in vivo* on which their favorite Eppendorf tube-contained reactions really take place in the cell.

I have recently reviewed the wobbly nuclear matrix concept (Pederson, 1998), and the present article endeavors to bring the subject up to date by integrating key developments that have occurred during the past 2 years. These recent studies include ones that further call into question the biological validity of nuclear matrix preparations and others that reduce the theoretical need to invoke the existence of such a structure. Yet, new avenues of research do not rule out the possibility that there is nonchromatin nuclear structure of some kind, still to be revealed.

Is This Important?

The nucleus of today’s cells has come down over the ~2.5 billion years of eukaryotic evolution with a coselected genome, and everything we know of eukaryotic genomes smacks of a heritable three-dimensional form (Comings, 1980; Manuelidis, 1990; Marshall *et al.*, 1996, 1997). It would be a good thing, as sheer epistemology, to understand this beguiling (and technically bedeviling) organelle in which the genome is arranged. On a more pragmatic level, it is reasonable to ask whether “nuclear matrix research” (as presently defined by its proponents) remains a useful endeavor altogether. At the close of a 1998 meeting, a wrap-up session was about to end when a question arose at a floor microphone from a young investigator. She said she had sat through 5 days of talks and now, at the conclusion of the conference, had a question for the organizers: “What is the nuclear matrix?” Neither organizer had an explicit answer,

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and one commented to the effect that it really does not matter, because this fraction has nevertheless been useful as a way to identify various nuclear proteins (true enough). But knowing the structure of the cell nucleus does matter. Here is the current situation as I see it.

The Nuclear Matrix Turns 50

Although there are antecedents going back more than 125 years (see Pederson, 1998), it is now half a century since the extraction of nuclei with high-salt solutions was observed to produce a residual structure (Zbarskii and Debov, 1948), which was patented (Zbarskii, 1948). Subsequently, this observation of a salt-insoluble nuclear residual structure was confirmed and extended in Houston (Smetana *et al.*, 1963). These preparations were given appropriately circumspect names such as “residual nuclear protein fraction,” but it was not until 1974 that this (same) preparation was given a name that stuck, the nuclear matrix (Berezney and Coffey, 1974). From 1974 to present, the nuclear matrix has lived what might generously be called a charmed life—but considered by some opponents to be a dark life.

The Nuclear Matrix Fraction versus the Interchromatin Space

In its “postmodern” era (i.e., 1980s) the filament system that constitutes the observed nuclear matrix is obtained by deliberate removal of soluble (electron-translucent) proteins to increase contrast, coupled with critical point drying of whole-mount specimens (Capco *et al.*, 1984). The observed nuclear filament network is extensive on both a mass and space-filling basis, with the filaments highly branched in an extensively arborized pattern (Fey *et al.*, 1986). This dendritic pattern and the spatial propinquity of the vertices and number (and apparent mass) of extending filaments per unit volume are inconsistent with the topography of the interchromatin space of the nucleus as observed in living cells. The interchromatin space of living cells appears as a sinusoidal, interconnected system bounded by chromatin contours (Kanda *et al.*, 1998; Zink *et al.*, 1998; Bornfleth *et al.*, 1999; Manders *et al.*, 1999; Politz *et al.*, 1999). Thus, it is not apparent, on geometric grounds and space-filling considerations, how this *in vivo* topography of the interchromatin space could accommodate a crisscrossing network of mostly very straight filaments observed in nuclear matrix preparations. Although this comparison of critical point-dried electron microscopic whole mounts on the one hand and living cells on the other has obvious room for interpretative (and, in the former case, preparative) differences, there is more reason to question the former images than the latter.

Another basis for skepticism about the biological reality of the observed nuclear matrix comes from the ultrastructural landscape of the interchromatin space. Early studies of the cell nucleus in unextracted material revealed, using an EDTA-uranyl acetate staining protocol that highlights ribonucleoprotein (RNP) (Bernhard, 1969), that the interchromatin space contains two major types of structures, termed interchromatin granule clusters (IGCs) and perichromatin fibrils, in an electron-translucent ground substance (Monneron and Bernhard, 1969; Spector, 1993). No filaments or any sort of polymer-appearing structures are typically seen in the interchromatin space of unextracted nuclei using ei-

ther the RNP-highlighting method or standard electron microscopic staining protocols. It has been argued that thin-section electron microscopy produces essentially a surface image because of the electron absorptive properties of the embedment plastic (Penman, 1995), a point that had long been appreciated in electron microscopy, and yet, as I have previously pointed out (Pederson, 1998), it would nonetheless seem that a system of filaments as anastomosing and extensive on a mass basis as is observed in nuclear matrix preparations would display itself in ultrastructural studies of nonextracted cell nuclei as cross, tangential, or longitudinal sections *at least to some degree*.

Another consideration is the claim that the nuclear matrix filaments observed in preparations made without RNase treatment are a ribonucleoprotein network (Fey *et al.*, 1986). This view has been challenged by two recent studies in which electron microscopy-based spectroscopic methods of elemental nitrogen versus phosphorous analysis revealed that the interchromatin space in between IGCs and perichromatin fibrils is *not* nucleoprotein (Vázquez-Nin *et al.*, 1997; Hendzel *et al.*, 1999). This further raises the level of skepticism as to an extensive ribonucleoprotein filament reticulum being present in unextracted nuclei.

If these reservations were not enough, there is more. Nuclear RNA-bound proteins can undergo unexpected rearrangements when dislodged from their usual RNA associations. For example, the human immunodeficiency virus *Rev* protein that binds to viral pre-mRNA transcripts in the nucleus undergoes a surprising spontaneous filament formation when released from its normal RNA binding partner (Heaphy *et al.*, 1991). More specifically with regard to the nuclear matrix, a finding of major relevance is that heterogeneous nuclear RNP proteins, once released from their RNA binding sites, form filaments (Lothstein *et al.*, 1985). These artificial filaments are strikingly similar in their branching and fibrilgranular texture to those observed in the standard nuclear matrix preparations (Tan *et al.*, 2000). This yet further calls into question the degree to which observed nuclear matrix filaments reflect preexisting versus induced structures.

Is Nuclear Isolation Itself an Issue?

The various biochemical issues that arise in nuclear matrix protocols have been evaluated in detail (Cook, 1988; Jack and Eggert, 1992; Pederson, 1998). But the degree to which macromolecular rearrangements can occur during the very isolation of nuclei in the first place, before any nuclear matrix preparation steps, has not been adequately appreciated. Chromatin, classically prepared at low ionic strength ($[\text{NaCl}] \leq 10 \text{ mM}$) (Zubay and Doty, 1959; Marushige and Bonner, 1966; Pederson, 1972; Bhorjee and Pederson, 1973), is insoluble at 0.15 M NaCl (Fredericq, 1971) and can adopt different folded conformations and histone H1-retaining versus histone H1-depleted states within a very narrow range of Na^+ and Mg^{++} concentrations (Clark and Kimura, 1990). Significant alterations of apparent nuclear structure occur when nuclei are isolated in various buffers even without exposure to any nuclear matrix preparation conditions, i.e., high ionic strength or nuclease digestion. For example, a protein that is normally extractable from nuclei in mild ($< 200 \text{ mM}$) NaCl concentrations becomes irreversibly insoluble and unextractable from the nuclei if they are simply

incubated at 37°C (Evan and Hancock, 1985). Similar findings have been reported in numerous subsequent studies (Pfeifer and Riggs, 1991; Neri *et al.*, 1997a–d).

These findings suggest that there may have always been a “blind spot” (or “blind step”) in nuclear matrix research, namely the initial isolation of nuclei. Various nucleic acid–protein short-range interactions within discrete nucleoprotein structures in the nucleus can be demonstrated to exist in living cells before nuclear isolation, for example by photochemical cross-linking conducted *in vivo* (Hanson *et al.*, 1976; Mayrand and Pederson, 1981; Economidis and Pederson, 1983), but in the case of the nuclear matrix it is the long-range, nucleus-filling dimension that is the relevant scale, and this is the domain of preparative artifacts during nuclear isolation that have been described (Evan and Hancock, 1985; Pfeifer and Riggs, 1991; Neri *et al.*, 1997a–d). This issue also obviously bears on studies in which, *after* isolation, nuclei are stabilized in various ways before nuclear matrix preparation (e.g., Mirkovitch *et al.*, 1984; see also Pederson, 1998). In light of the many studies cited above demonstrating that extensive intranuclear rearrangements occur when nuclei are first isolated, experiments involving postisolation fixation of nuclei before nuclear matrix fractionation (e.g., Nickerson *et al.*, 1997) must be interpreted cautiously, notwithstanding that these novel types of experiments are certainly reasonable undertakings.

Could the Genome Itself Harbor Chemical Information Necessary and Sufficient for Its Intranuclear Organization?

A very large fraction of the genome in higher eukaryotes does not code for protein (nor is it part of transcription units, i.e., introns) and has no known function at present. One of the foremost investigators of the nuclear matrix has frequently and correctly reminded us that we do not differ from our chimpanzee relatives in the nucleotide sequences of transcription units but rather in these vast stretches of noncoding DNA. It of course remains possible that there are very different human versus chimpanzee morphogenetic–morphotypic genes that simply have not yet been found (because these genes might not be the most likely to be picked up in various cDNA-based strategies). But, alternatively, it remains possible that the noncoding DNA somehow manages, by folding of the remaining genome, to set up cell type–specific gene expression. Although there may be an inherent flaw of logic in this concept (the genome is invariant, at least in nonlymphoid cells [Pederson, 1999b], so how then does it thus fold variably in different cells to set up distinct gene expression patterns?), pondering the biological function of all this noncoding DNA remains valid nonetheless. This idea would seem to necessarily depend on specific factors, probably proteins, that somehow recognize noncoding DNA and then set up a global 3-D organization within the nucleus. Or perhaps nuclear envelope attachment sites are located within these vast stretches of nontranscription unit DNA, and, after attachment, all else with regard to the interphase 3-D genome organization obligatorily follows.

These kinds of ideas have been generally ignored because the noncoding DNA is so “uninteresting” as sequence (as if we were at present clever enough to be able to detect all “interesting” DNA text, which we certainly are not). At our

present state of knowledge (ignorance) we can only view the noncoding DNA’s information content on the basis of what is absent [e.g., promoters, cap sites, splice sites, terminators, and poly(A) sites]. One very plausible role of all this extra DNA is to create a chemically requisite DNA concentration to optimize the operation of gene regulatory mechanisms, as was first persuasively proposed by Lin and Riggs (1975). But the idea, not mutually exclusive with the model of Lin and Riggs, that noncoding DNA somehow manages to spatially organize the interphase 3-D genome remains intriguing.

RNA Movement in the Interchromatin Space

Recent studies by two groups have addressed the rate, spatial dimensions, and mechanistic basis of RNA movement in the nucleus. These results provide no evidence whatsoever for a nuclear matrix and in fact argue quite strongly against such a system of extensive filaments coursing throughout the interchromatin space.

In an integration of fluorescence correlation spectroscopy (a classical biophysical method for studying molecular kinetics) with fluorescence microscopy, Politz *et al.* (1998) found that the rate of movement of poly(A) RNA in the nucleus of living mammalian cells was similar to the measured movement of a typical size pre-mRNA in aqueous solution. In parallel studies a complementary method, fluorescence recovery after photobleaching, was used to measure the mobility of poly(A) RNA in the nucleus of living cells, and, once again, the results were consistent with diffusion (Politz *et al.*, 1998). This diffusive nature of these poly(A) RNA movements in the nucleus was further indicated by their nondependence on ATP in these living cell experiments (Politz *et al.*, 1998). In a subsequent study a caged fluorescent probe (Politz, 1999) was used to track poly(A) RNA in the nucleus from an initial site out into the surrounding space (Politz *et al.*, 1999). These results were, again, consistent with diffusion, and this was reinforced by additional experiments involving temperature variations (Politz *et al.*, 1999). The conclusion from both of these studies that nuclear poly(A) RNA moves by diffusion (Politz *et al.*, 1998, 1999; Politz and Pederson, 2000; Pederson, 1999a) is consistent with an earlier study of the intranuclear movement of fluorescent dextrans, which indicated free translational diffusion of these molecules (Seksek *et al.*, 1997). Moreover, recent fluorescence recovery after photobleaching studies of the intranuclear trafficking of proteins involved in three different nuclear processes have revealed rapid diffusion similar to that seen in the aforementioned nuclear poly(A) RNA and dextran studies (T. Misteli, personal communication of unpublished results).

Subsequently, a second group reported studies in which a specific pre-mRNA–ribonucleoprotein particle was tracked in the interchromatin space of *Chironomus* salivary gland nuclei (Singh *et al.*, 1999). It was found that this pre-mRNP moves out from its transcription site in all directions as a spatially random process. These two studies were carried out in mammalian versus insect cell nuclei and involved very different methods, yet they led to the same conclusion, namely nuclear RNA moves by diffusion (Daneshmandi, 1999).

Two other recent studies bearing on nuclear mRNA transport concerned specific *Drosophila* embryo mRNAs that specifically localize in the perinuclear cytoplasm on the apical side of the nuclei in which they are synthesized. In one study

this positioning was shown to require the association of a heterogeneous nuclear RNP protein with the transcripts, which then apparently causes them, once in the cytoplasm, to seek a particular site (Lall *et al.*, 1999). These results argue against vectorial export out of the nucleus through the nuclear pores closest to the cytoplasmic localization site. In a second investigation, other mRNA transcripts localizing at a distinct cytoplasmic site were found to emerge from many different intranuclear locations (Wilkie *et al.*, 1999), again consistent with a global distribution of this RNA in the nucleus before export. Although these studies, unlike ours and those of the Karolinska group (Politz *et al.*, 1998, 1999; Daneholt, 1999; Singh *et al.*, 1999), did not directly investigate the intranuclear spatial pattern of mRNA transport, the results do not support models (e.g., Blobel, 1985) in which a specific mRNA tracks to the nearest pore.

What Structure May Lurk in the Nucleoplasmic Ground Substance?

Notwithstanding the controversial evidence for an extensive meshwork of filaments in the interchromatin space, it still behooves us to ask with an open mind whether the electron-translucent nucleoplasm is simply a concentrated sea of individual protein molecules or has, in addition, some formed elements.

In a study of IGCs labeled with a green fluorescent protein-mRNA splicing factor protein (Misteli *et al.*, 1997), real-time observations in living cells revealed that ~80% of the IGCs remain stationary. This might signify tethering to a putative nuclear matrix or to nascent pre-mRNAs extending from their transcription sites to a vicinal IGC. In addition, a small portion of the IGCs were observed to undergo short-range movements (Misteli *et al.*, 1997). Although the observed degree of movement of IGCs can, at present, be taken neither as supporting nor negating the nuclear matrix concept, these important *in vivo* observations prompt one to further ponder whether the interchromatin space is simply a concentrated protein solution or has some degree of preformed structure. A good place to start is nuclear actin.

Nonmuscle actin is ubiquitously present in eukaryotic cells and has been shown to equilibrate between nucleus and cytoplasm in amphibian oocytes (Clark and Merriam, 1977). Evidence for the presence of actin in the nucleus of several other species and cell types has also been reported (Fukui, 1978; Fukui and Katsumaru, 1979; Krohne and Franke, 1980; Osborne and Weber, 1980; Gounon and Karsenti, 1981; Welch and Suhan, 1985; Milanov and DeBoni, 1993; Amankwah and DeBoni, 1994; Yan *et al.*, 1997; Wada *et al.*, 1998; Gonsior *et al.*, 1999). Moreover, a number of actin-binding nuclear proteins have been described (Ankenbauer *et al.*, 1989; Rimm and Pollard, 1989; Nowak *et al.*, 1997; Cairns *et al.*, 1998; Harata *et al.*, 1999). The nuclear actin of *Xenopus* oocytes exists as a gel within the intact nucleus under certain, gentle conditions of germinal vesicle preparation (Clark and Rosenbaum, 1979; J.G. Gall, personal communication of unpublished results) and can be microsurgically extirpated by teasing away the nuclear envelope (Clark and Rosenbaum, 1979). This suggests that one of the abundant nuclear proteins in living amphibian oocytes is on a delicate edge of polymerization.

Recently, monomeric β -actin in the nucleus has emerged in the context of studies of chromatin remodeling during

gene transcription activation. A group of mammalian nuclear proteins termed BAFs has been described that are related to the well-characterized yeast SWI/SNF chromatin remodeling complex (Wang *et al.*, 1996). Biochemical characterization of the nuclear BAF complex from calf thymus and activated mouse lymphocytes revealed that its subunits include both monomeric β -actin as well as a novel actin-related protein (Zhao *et al.*, 1998). The association of actin with the nuclear BAF complex *in vivo* was confirmed in studies using a cell-permeant protein-protein cross-linking agent, and additional data indicated that the BAF complexes also bind to profilin and cofilin, further emphasizing the central role of actin-binding proteins, as well as actin, in this chromatin remodeling complex (Zhao *et al.*, 1998). These results bring to mind an earlier publication in which it was reported that actin antibodies inhibited transcription on lampbrush chromosomes when injected into amphibian oocyte nuclei (Scheer *et al.*, 1984). Whether actin is normally other than monomeric in the nucleus remains unclear. There may be gene transcription site-proximal actin, present as monomers or perhaps short filaments, possibly capped in a transcription-linked regulated mechanism or conceivably dynamically unstable. However, the described extensive, anastomosing nuclear matrix does not appear to be substantially composed of actin by either ultrastructural criteria or polypeptide composition (Pederson, 1998).

Are there any other clues to structure in the nucleoplasmic ground substance, if not as multimicrometer-spanning scaffolds then perhaps at least as shorter-range elements? Here there are recent, encouraging clues.

Nup 153 and *Tpr* are nuclear pore complex-associated proteins that are organized into filaments extending 100–350 nm into the nucleus (Cordes *et al.*, 1993, 1997; Zimowska *et al.*, 1997). Although these filaments do not extend sufficiently deeply or intersectionally into the nucleus to be candidates for the observed extensively anastomosing nuclear matrix, their suggested role in mRNA export (Bangs *et al.*, 1998) nonetheless presents an alternative element of nonchromatin nuclear structure that may facilitate a late step in gene readout, albeit confined to the outer nuclear perimeter.

A second and intriguing group of proteins for careful consideration as elements of internal nuclear structure are the nuclear lamins. These cousins of the cytoplasmic intermediate filaments were originally thought to exist solely as a fence-wire network underneath the nuclear envelope. But subsequent studies have revealed the presence of a population of internal nuclear lamins as well (Goldman *et al.*, 1992; Bridger *et al.*, 1993, 1998; Neri *et al.*, 1999; R. Goldman, personal communication of unpublished results; C. Hutchison, personal communication of unpublished results). Although the oligomerization-polymerization state of these intranuclear lamins is not known, their mobility measured by fluorescence recovery after photobleaching in living cells suggests that they may not be monomeric (Moir *et al.*, 1998). This is a very important subject for further investigation.

These studies of nuclear actin, *Tpr* proteins, and nucleoplasmic lamins remind us that filament-forming protein families are present in cell nuclei. If short arrays of filaments were to nucleate around gene transcription and RNA processing sites, these local "gene expression matrices" might help tether the necessary transcription and RNA processing machinery and yet would not necessarily comprise a nucle-

us-filling, long-range filament system such as the one seen in extracted preparations called the nuclear matrix. Such local structure might be important as an organized framework for final transcript processing and active release of the finished RNA before a diffusion-based transport to the Nup 153/*Tpr* and possibly other filament systems at the nuclear perimeter (Strambio-de-Castillia *et al.*, 1999; Politz and Pederson, 2000).

Nothing in the foregoing considerations rules out the possibility that mRNA might move by diffusion and yet also transiently interact with some sort of structural elements in the interchromatin space. Although these two notions might seem somewhat contradictory, or even mutually exclusive, the issue comes down to the lifetimes of the postulated mRNA-structural element interaction (Politz and Pederson, 2000). A recent electron microscopic tomography study of *Chironomus* Balbiani ring mRNP particles in the nucleoplasm reveals that a portion of these RNPs is in contact with thin fibers (Miralles *et al.*, 2000), even though kinetic analysis of the movement of mRNP in this very same system (using living *Chironomus* salivary gland cells) indicates that the particles overall display random movement that is compatible with diffusion (Daneholt, 1999; Singh *et al.*, 1999). The thin nucleoplasmic fibers observed by Miralles *et al.* (2000) are described by the authors as not resembling the extensive, nucleoplasm-filling meshwork observed in typical nuclear matrix preparations.

CONCLUSION

The biological reality of the nuclear matrix, a challenged subcellular fraction and an ultrastructural entity subject to various interpretations, remains uncertain. The nuclear matrix concept now appears, in retrospect, as something of a mystique. A certain charm has surrounded this idea because of enabling precedents in cell biology wherein function has been elegantly revealed as underlying structure, e.g., the actomyosin sliding filaments and cross-bridges of the sarcomere (Hanson and Huxley, 1953; Huxley and Hanson, 1954; Huxley, 1996; Corrie *et al.*, 1999). But in the nuclear structure field this key link to function was never conclusively made. Nevertheless, we remain in search of nuclear structure. Some new suspects have recently been uncovered and include possible intranuclear lamin-based arrays, the *Tpr* filaments emanating inward from the nuclear pore complexes, as well as the active gene-tethered RNA transcription and processing machinery itself (Pederson, 1998). The remaining and entirely plausible possibility is that nothing contributes as much to nuclear structure as does the genome (i.e., chromatin) itself. This is the simplest hypothesis, consistent with all the observations and, for precisely this reason, should receive all due attention as the nuclear structure field moves on.

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