J. Mol. Biol. (1998) **277**, 147–159

JMB

REVIEW

Thinking About a Nuclear Matrix

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The possible existence in eukaryotic cells of an internal, non-chromatin nuclear structural framework that facilitates gene readout as a set of spatially concerted reactions has become a popular but controversial theater of investigation. This article endeavors to present a circumspect review of the nuclear matrix concept as we presently know it, framed around two contrasting hypotheses: (1) that an internal nuclear framework actively enhances gene expression (in much the same way the cytoskeleton mediates cell locomotion, mitosis and intracellular vesicular traffic) *versus* (2) that the interphase chromosomes have fixed, inherited positions and that the DNA replication, transcripton and RNA processing machinery diffusionally arrives at sites of gene readout, with some aspects of nuclear structure thus being more a result than a cause of gene expression. On balance, the available information suggests that interactions among various gene expression machines may contribute to isolated nuclear matrix preparations. Some components of isolated nuclear matrix preparations may also reflect induced or reconfigured protein–protein associations. The protein characterization and ultrastructural analysis of the isolated nuclear matrix has advanced significantly in recent years, although controversies remain. Important new clues are now coming in from promising contemporary lines of research that report on nuclear structure in living cells.

Keywords: nuclear structure; nuclear proteins; gene expression; RNA processing; configuration of interphase chromosomes

Introduction

The component processes of cell physiology, all initially discovered in cell extracts, are envisioned to proceed as concerted reactions in facilitating environments of ordered intracellular structure. One of the most well understood exemplifications of this is the sliding filament model of skeletal muscle contraction (Hanson & Huxley, 1953; Huxley & Hanson, 1954), in which the underlying framework consists of both stable (Z-line) and motile elements (the dynamically tilting actomyosin cross-bridges). This structural organization facilitates the sarcomere's conversion of chemical to mechanical energy and the resultant generation of force, but also presents, in the electron microscope, an engaging image in which cellular architecture and function are compellingly seen as one.

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Abbreviations used: MARs, matrix attachment regions; SARs, scaffold attachment regions; RNP, ribonucleoprotein; FRAP, fluorescence recovery after photobleaching.

† Miescher was first by the narrowest of margins, due to the dilatory behavior of his Tübingen laboratory boss and journal editor, Felix Hoppe-Seyler, who delayed publication of Miescher's work until he had himself repeated and confirmed the findings (Hoppe–Seyler, 1871; Portugal & Cohen, 1972).

The Nucleus, Then and Now

The nucleus has for over 125 years been known to be an extremely durable component of cells and tissue. The first isolation of nuclei, by Friedrich Miescher†, from white blood cells embedded in discarded pus-soaked surgical bandages, involved a combination of limited proteolysis (in the form of an extract of pig stomach, i.e. crude pepsin) and acid digestion of cells followed by ether extraction

0022–2836/98/120147–13 $25.00/0/mb971618

(Miescher, 1871). The fact that cytologically intact nuclei resulted from such an extraordinarily harsh procedure is not only remarkable, but also informative: Miescher's results suggested that there is a durable nuclear structure (whether based on the chromatin *per se* or conceivably something else). A subsequent century of experience has confirmed that nuclei withstand the strong hydrodynamic shear, compression and friction generated during cell or tissue homogenization, also involving extremes of osmotic pressure in some methods (Dounce, 1955; Maggio *et al*., 1963a; Penman, 1966; Blobel & Potter, 1966; Pederson, 1997). Moreover, once isolated, nuclei are virtually impossible to break open mechanically, except by sonication (Muramatsu *et al*., 1962; Maggio *et al*., 1963b; Pederson, 1972; Bhorjee & Pederson, 1972; 1973; Pederson, 1974a;b).

The Nuclear Matrix Arrives

Studies between 1942 and 1963 revealed the existence of a class of proteins in the nucleus that resist extraction by 1.0 to 2.0 M NaCl (Mayer & Gulick, 1942; Zbarskii & Debov, 1948; Braun & Ernst, 1960; Georgiev & Chentsov, 1962; Zbarsky *et al*., 1962; Smetana *et al*., 1963). A similar fraction of 1.0 to 2.0 M NaCl non-extractable proteins was also described in isolated mitotic chromosomes (Mirsky & Ris, 1947). Light and electron microscopic observations of 1.0 to 2.0 M NaCl-extracted nuclei revealed a considerable retention of overall nuclear shape, with the internal remnants of nucleoli and heterochromatin clearly demarcated (Braun & Ernst, 1960; Georgiev & Chentsov, 1962; Zbarsky *et al*., 1962; Smetana *et al*., 1963; Shankar *et al*., 1967). The terms ''residual protein fraction'' and ''nuclear network'' were appropriately used at that time to describe this salt-extracted biochemical fraction of the nucleus and its ultrastructural appearance, respectively.

In 1974, there appeared a paper describing virtually the same type of preparation as reported in the earlier studies, but introducing the term ''nuclear matrix'' (Berezney & Coffey, 1974). There are at least three reasons that this term caught on so well. This paper was one of the first on the subject to appear in a biochemically-oriented journal, allowing a mostly biochemist readership to encounter the nuclear matrix, both as a term and a concept, for the first time. Second, a follow-up publication (Berezney & Coffey, 1975) was likely to have substantially broadened awareness of the new term, nuclear matrix, since very little previous work in this field had appeared in wide-readership journals. But most important was the fact that these investigators explicitly speculated that the nuclear matrix was a critical, facilitating element in nuclear function. It is extraordinary, from the standpoint of this field's epistemological development, that in none of the many previous studies (which after all produced a nuclear fraction not materially different in composition or cytological appearance than the 1974 version) did any of the several authors elect to speculate on function. The popularity of the idea of a functional nuclear matrix has continued for two decades (Berezney & Jeon, 1995), springing from Berezney and Coffey's initial daring to propose a functional connection.

The Nuclear Matrix as a Cell Fraction

After the initial reports that launched the nuclear matrix both as a name and a functional concept (Berezney & Coffey, 1974; 1975), there followed numerous studies of this extracted nuclear preparation which revealed its selective retention not only of certain proteins and RNA species, but also of distinct DNA sequence elements (Berezney & Jeon, 1995). This latter phenomenon, dealing with specific DNA sequence elements that remain bound to the isolated, extracted nuclear preparations, and known as matrix attachment regions (MARs) or scaffold associated regions (SARs), has led to a very active and productive area of research within the overall nuclear matrix field (Berezney & Jeon, 1995). Moreover, several actively transcribed genes were found to be retained in nuclear matrix preparations (Berezney & Jeon, 1995), although this was not found to be so in other instances (for a thoughtfully discussed case see Small *et al*., 1985). From all these studies, there was no question whatsoever that, once prepared, the nuclear matrix bound to a distinctive (but surprisingly complex) subset of nuclear proteins, RNA species and (experimentally nuclease-excised) DNA sequence elements. However, this set of observations obviously did not, and still today does not, address the pre-existence of the nuclear matrix *in vivo* in the first place.

A slight detour occurred in the evolving biochemical definition of the nuclear matrix when it was found that certain proteins in these preparations polymerized upon sulfhydryl group oxidation (Cobbs & Shelton, 1978; Kaufmann *et al*., 1981). However, a subsequent comprehensive investigation of this issue revealed that protein sulfhydryl-disulfide shifts, while certainly at play in the various nuclear matrix procedures, are not a significant factor in the ultrastructure or protein composition of the preparations obtained (Belgrader *et al*., 1991). More recent work has identified some of the proteins that form intermolecular disulfide stabilization of isolated nuclear matrix preparations (Stuurman *et al*., 1992b).

In 1982, a modification of the initial nuclear matrix preparation methods was introduced (Capco *et al*., 1982) employing $(NH_4)_2SO_4$ instead of NaCl, but at nearly the same ionic strength (0.25 M $(NH_4)_2SO_4$ *versus*. 1.0 M NaCl, $\mu = 0.75$ and 1.0, respectively). This protocol had its roots in earlier studies in which 0.4 M $(NH_4)_2SO_4$ was used

to selectively extract from HeLa cell nuclei a subfraction of nuclear RNA (Price *et al*., 1974) generating a chromatin-depleted preparation which ultrastructurally represented essentially the nuclear lamina (Herman *et al*., 1978). The nuclear matrix preparations obtained with 0.25 M $(NH_4)_2SO_4$ were subsequently studied with and without the use of RNase digestion, yielding structures termed the RNP-depleted and RNP-containing nuclear matrices (Capco *et al*., 1982), the latter fraction resembling the ''ribonucleoprotein nuclear network'' that employed a different nuclear extraction method (Smetana *et al*., 1963; Shankar *et al*., 1967). The newer preparations were observed by electron microscopy as unembedded whole mounts (Capco *et al*., 1982) or as resinless sections (Fey *et al*., 1986), presenting images that revealed an extensively anastomosed network of filaments.

Concerns about the high ionic strength employed in the initial nuclear matrix work led to two alternative procedures. In one (Mirkovitch *et al*., 1984), nuclei were exposed sequentially to DNase and then to a low concentration (typically 25 mM) of lithium 3,5-diiodosalicylate (LIS). This procedure was developed primarily in the context of studying DNA-matrix attachment regions, based on this group's (unsettling) finding that such attachments are disturbed in the high ionic strength nuclear matrix isolation procedures (Mirkovitch *et al*., 1984). The LIS procedure yielded a nuclear matrix preparation that ultrastructurally resembled conventional ones but this result was dependent on ''stabilization'' steps in which, prior to DNase digestion and LIS extraction, the nuclei had to be incubated at 37°C (notorious for deranging and rearranging nuclear structure), or at 4°C with either $CaCl_2$ or $CuSO_4$ (Mirkovitch *et al*., 1984). The $CuSO_4$ cocktail stemmed from earlier work by this group (Lebkowski & Laemmli, 1982), suggesting that metaphase chromosomes are organized around a Cu^{2+}-containing metalloprotein scaffold (Lewis & Laemmli, 1982). The major metal in mammalian chromosomes is iron (Robbins & Pederson, 1970), and the Cu^{2+} metalloprotein chromosome backbone idea (Lewis & Laemmli, 1982) remains an open issue. A recent comprehensive study has called into serious question the use of Cu^{2+}-based and other ''stabilization'' steps in nuclear matrix isolation (Neri *et al*., 1997).

The second alternative procedure (Jackson & Cook, 1986) involved the nifty encapsulation of mammalian cells in gelled agarose spheres, in which growth and metabolism continue (at 37°C) due to the large pore size of the solid agarose, which readily allows access to the cells by nutrients and growth factors in the medium. Extraction of the agarose-encapsulated cells by detergent, followed by restriction endonuclease digestion of chromatin and electrophoretic removal of the (matrix-unattached) cleavage products results in an extensively anasomatized internal nuclear network of filaments (Jackson & Cook, 1988) similar to that reported for matrix preparations obtained by high ionic strength methods (Capco *et al*., 1982). The procedure involving agarose encapsulation of cells has typically been carried out using an isotonic buffer, known to lead to chromatin aggregation due presumably to the imperfect balancing of electrolytes (including divalent cations) in such buffers with respect to the actual intranuclear milieu. This notwithstanding, the general ultrastructural similarity of the nucleoskeletons prepared by the agarose encapsulation method (Jackson & Cook, 1988) and those prepared with high ionic strength (Capco *et al*., 1982) is noteworthy.

Looking at the Nuclear Matrix

The presentation of the nuclear matrix in whole mount preparations or resinless sections, both in its RNP-containing and RNP-depleted form, constitutes the current ultrastructural frontier in this field (Capco *et al*., 1982; Fey *et al*., 1986; Jackson & Cook, 1988). Before addressing the ultrastructure of nuclear matrix preparations, what is seen in between chromatin in cell nuclei? Electron micrographs of sectioned cell nuclei stained with uranyl acetate or the ribonucleoprotein-selective Bernhard staining method reveal that the spaces between chromatin contain two types of ribonucleoprotein elements, called perichromatin fibrils and interchromatin granule clusters, that have subsequently been functionally connected to sites of pre-mRNA transcription and processing (Spector, 1993). Perichromatin fibrils are typically 3 to 5 nm in diameter, but can reach ~20 nm, and are often irregularly coiled (Monneron & Bernhard, 1966). Interchromatin granule clusters consist of 20 to 25 nm diameter particles interconnected by anasomotized fibrils ranging from 3 to 8 nm in diameter (Monneron & Bernhard, 1966), but are distinct from perichromatin fibrils.

In RNP-containing nuclear matrix preparations visualized by resinless section electron microscopy (Fey *et al*., 1986; Jackson & Cook, 1988), numerous spherical structures having diameters of 20 to 25 nm can be seen associated with the various filaments and it is possible that some of these represent interchromatin granules. However, with respect to the filaments themselves, they seem to be more abundant and more heterogeneous in diameter, particularly in the RNP-depleted nuclear matrix (Berezney & Coffey, 1974; Small *et al*., 1985), than are perichromatin fibrils or any other interchromatin elements observed by conventional electron microscopy. An earlier whole mount (plastic embedment) electron microscopy study of mouse liver nuclear matrix (RNP-depleted) preparations revealed two classes of filaments, having diameters of 2 to 3 nm and 10 to 30 nm (Comings & Okada, 1976). As has been emphasized (Wolosewick, 1980; Penman, 1995), the electron absorptive property of standard plastic embedment media limits the contrast achieved in conventional thin section electron microscopy. Nonetheless, one might reasonably

expect the extensive filament system observed in the isolated nuclear matrix to be frequently caught, in sectioned nuclei, in cross, oblique or longitudinal section. But the numerous ultrastructural studies of sectioned cell nuclei do not convey the expected various transections of the (presumably abundant) 9 to 13 nm average diameter filament system thought to comprise the nuclear matrix *in situ*.

A related issue is the protein composition of the isolated nuclear matrix. These preparations contain both hnRNP proteins (Pederson, 1983) as well as numerous other polypeptides identified primarily by their molecular weights. Although antibodies to these various matrix proteins can of course often be shown to decorate elements of the isolated nuclear matrix itself, there are few instances in which such antibodies reveal a nucleoplasmic network by immunofluorescence or by immuno-electron microscopy of unextracted cells (for examples see Bhorjee *et al*., 1983; Nickerson *et al*., 1990). Obviously, one would want to see, in expansions of this line of work, a more extensive catalog of proteins which are present in the isolated matrix on the one hand, and can be shown to be present in a nucleoplasmic network system in unextracted cell nuclei on the other. The need is especially pressing with respect to the core filament components of the isolated nuclear matrix which comprise that majority of the preparation, whereas the majority of (non-hnRNP) protein species studied to date localize to particles and granular elements of the isolated matrix. These latter components may be gene expression-relevant entities but it is the protein composition of the core filaments of the isolated matrix that remains at such an unsatisfying state at present.

The Nucleus is Mainly Nucleoprotein with Vulnerable and Re-arrangeable Bonds

All nuclear matrix preparation methods necessarily involve the removal of chromatin and, in some methods, RNA as well. DNA and RNA are the major anions forming electrostatic bonds with the cationic groups of many (probably most) nuclear proteins. Removal of nucleic acid will therefore provide opportunities for rearrangement of some proteins as well as aggregation, as is in fact observed when the RNA of ribosomes (Palade & Siekevitz, 1956; Tashiro, 1958; Madison & Dickman, 1963) or nuclear ribonucleoprotein (hnRNP) particles (Lothstein *et al*., 1985) is digested. These protein rearrangement and aggregation phenomena observed with ribosomes and nuclear hnRNP particles certainly warrant consideration in nuclear matrix isolation endeavors. In the case of those nuclear matrix isolation procedures in which high ionic strength is also involved, another factor comes into play. Cationic protein sites previously electrostatically bonded to nucleic acid phosphodiester bonds (isoelectric point ~3.0) will undergo anion exchange with the vast molar excess of solvent Cl^- or SO_4^{2-} ions presented by the high ionic strength NaCl or $(NH_4)_2SO_4$ solutions used in the standard, high salt nuclear matrix preparation methods. This global nuclear protein *gegenion* phenomenon can be expected to profoundly change the interactivity of proteins previously resident in the native nucleoprotein, irrespective of other, new protein–protein association opportunities that may be created by the removal of the nucleic acid from non-electrostatic sites of protein–nucleic acid interaction. This same ionic strength factor will also influence van der Waals interactions among proteins. At elevated ionic strength the van der Waals forces between hydrophobic amino acid groups of proteins and water decrease whereas the strength of van der Waal's interactions among proteins increases (Kauzmann, 1959). An example of this phenomenon in the present context is the response of isolated chromatin to moderate ionic strength, *viz*. 0.6 M NaCl, which leads to an extensive redistribution of the remaining histone proteins along the DNA (Varshavsky & Ilyin, 1974). There are also precedents in which nucleic acid-bound proteins adopt radically different structures once freed. For example, when it is bound to human immunodeficiency virus RNA the *rev* protein is organized into oligomeric structures with an average diameter of 60 nm, whereas the same protein in the absence of RNA forms an extensive gel consisting of filaments ~14 nm in diameter (Heaphy *et al*., 1991). Such phenomena must be borne in mind when considering the isolated nuclear matrix, particularly in those preparations from which the RNA has been depleted.

Of course, it is very difficult to predict on purely theoretical grounds the extent to which, for any nucleoprotein complex, the combined removal of nucleic acid and a high solvent ionic strength will induce extensive protein–protein rearrangements or aggregation since, even if one knew for each of the many proteins its primary sequence, post-translational modifications, secondary structure (in the assembled nucleoprotein) and its detailed intermolecular contact map with other proteins in the complex, formally predicting the new associations that would occur upon nuclease digestion at either physiological or elevated ionic strength essentially amounts to a combined intramolecular and intermolecular version of the protein folding (refolding) problem for a very large number of proteins considered together, which is beyond present analytical or theoretical capabilities (Finkelstein, 1997).

The Contribution of Gene Readout Machines to Nuclear Organization

The nuclear matrix preparations generated with ammonium sulfate and no RNase digestion created

something of a paradigm shift. Many who had been regarding the nuclear matrix as a marginalized concept became intrigued, or converted, because of the (entirely rational) idea that ribonucleoproteins, constituting after all abundant elements of the nuclear mass, are likely to be functionally integrated elements of the nuclear architecture being revealed. Moreover, several groups reported in 1982 and 1983 that pre-mRNA and splicing intermediates are retained in these RNP-containing nuclear matrix preparations (Ben Ze'ev *et al*., 1982; Ross *et al*., 1982; Ciejek *et al*., 1982; Mariman *et al*., 1982; Gallinaro *et al*., 1983; Ben Ze'ev & Aloni, 1983), and subsequent work also revealed the presence of pre-mRNA splicing activity (Zeitlin *et al*., 1987) and splicing co-factor proteins (Smith *et al*., 1989; Blencowe *et al*, 1994, 1995) in nuclear matrix preparations.

Two recent studies relating to RNA polymerase II have provided an instructive and potentially important way of envisioning dynamic nuclear structure functionally related to gene expression. Mortillaro *et al*. (1996) reported that the hyperphosphorylated form of the largest subunit of RNA polymerase II is associated with nuclear sites at which pre-mRNA splicing factors are concentrated and, more pertinent as regards the present discussion, was selectively retained in extracted nuclear matrix preparations. A few months later, Vincent *et al*. (1996) reported studies of a nuclear protein, p225, that they had previously defined (Bisotto *et al*., 1995) as a component of ribonucleoprotein-depleted nuclear matrix preparations. p225 turned out to be the largest subunit of RNA polymerase II, and it was further observed that this protein was present in nuclear matrix preparations uniquely in its hyperphosphorylated form, in complete agreement with the results of Mortillaro *et al*. Hyperphosphorylation of the largest subunit of RNA polymerase II is a transient modification, functionally linked to the most active phase of this enzyme (Dahmus, 1996), and hyperphosphorylation–dephosphorylation cycles are known to occur for numerous other nuclear proteins involved in DNA replication, transcription and RNA processing. For example, the associations of several proteins with pre-mRNA and spliceosomes have been linked to their phosphorylation state (Mermoud *et al*., 1992; Mayrand *et al*., 1993, 1996; Tazi *et al*., 1993; Gui *et al*., 1994; Colwill *et al*., 1996; Misteli & Spector, 1996; Fung *et al*., 1997). Hyperphosphorylation-dephosphorylation can of course markedly change the folding and potential for a given protein's interaction with other proteins.

It seems very likely that at least one way (and conceivably a major way) in which various nuclear proteins become functionally recruited into heterotypic complexes at sites of gene replication, repair or transcription is by virtue of regulated, transient covalent modifications (phosphorylation, acetylation, etc.) that increase their equilibrium association constants for one another, and possibly also for their nucleic acid templates. Depending on the particular chemical bonds involved, at least some of these new or enhanced protein–protein affinities would be predicted to resist dissociation by elevated ionic strength. Thus, some components of the isolated nuclear matrix preparation may be viewed as a cell biological analogue of the classical ''salting out'' protein separation phenomenon (Edsall & Gutfreund, 1983) taking place on all of the many transient protein–protein associations and dissociations occurring in the cell nucleus at the moment of its isolation and extraction. Such interactions may also extend to the machine–machine level, thus extending the spatial reach of this phenomenon within the nucleus. For example, recent studies have demonstrated physical interactions among the transcriptional, polyadenylation and splicing machinery (Du & Warren, 1997; McCracken *et al*., 1997; Zeng *et al*., 1997; Yue *et al*., 1997) and also between the DNA repair and transcription machinery (Maldonado *et al*., 1996). To the extent that some of these recently described inter-machine associations can be expected to survive or be enhanced by elevated ionic strength, the potential exists for a rather extensive scale to the resulting stabilized structures, perhaps in the range of 25 to 100 nm. Viewed this way, some elements of the isolated nuclear matrix may not reflect so much the pejorative ''salt precipitation artifact'' as its initial and continuing opponents have so often claimed, but rather a meaningful ''chemical footprint'' of the transient protein–protein and nucleic acid–protein associations that are at play in the many dynamic nuclear machines that mediate gene readout. This idea is compatible with the ultrastructural presentation of the RNP-containing isolated nuclear matrix which reveals a large number of granular particles of heterogeneous diameter (Fey *et al*., 1986; Jackson & Cook, 1988; Stuurman *et al*., 1992a; Nickerson *et al*., 1997) that could well represent the remnants of gene readout machines.

Is a Non-Chromatin Nuclear Infrastructure Theoretically Even Necessary?

The overall arrangement of individual chromosomes in the interphase nucleus is heritable in time and relatively fixed in space (Comings, 1980; Hochstrasser *et al*., 1986; Hochstrasser & Sedat, 1986; Manuelidis & Borden, 1988; Manuelidis, 1990; Marshall *et al*., 1996, 1997). Although it is tempting to use this fact to argue that there must therefore be an internal organizing framework within the nucleus, it is also possible that the fixed chromosome locations, based on specific chromatin–nuclear envelope (and perhaps chromatin–chromatin) binding sites, themselves constitute this nuclear structure, with soluble replication or transcription/RNA processing factors readily reaching active targets and the requi-

site machines assembled at each chromosomal site. This rather different view of nuclear organization is widely held by many investigators at the present time (sometimes called the nuclear matrix opponents, or worse names) and is compatible, *inter alia*, with the recent discovery of high affinity interactions among the transcription, splicing and polyadenylation machinery (Du and Warren, 1997; McCracken *et al*., 1997; Zeng *et al*., 1997; Yue *et al*., 1997). The fact that manually isolated chromosomes retain, despite the hydrodynamic forces that attend their preparation, mRNA splicing factors in the absence of any nuclear matrix component (Sass & Pederson, 1984; Gall & Callan, 1989; Wu *et al*., 1991) indicates that the association of gene expression machinery with active genes does not require a nucleoplasmic structural framework. This consideration is not, in and of itself, a compelling extinction of the nuclear matrix concept, but simply sharpens the specific cell biological picture that should be envisioned.

Can a Nuclear Matrix be Seen *In Vivo*?

Biochemical and ultrastructural methods applied to the cell should, ideally, generate coherent and cross-confirming results. As regards the nucleus, a particularly striking example of this congruence was the discovery of the nucleosome structure of chromatin, which was first revealed by electron microscopy of spread native chromatin (Olins & Olins, 1974), subsequently defined at the molecular level (Kornberg & Thomas, 1974) and then corroborated *in situ* by a biophysical–chemical method (Hanson *et al*., 1976). The *in vivo* confirmation of the nucleosome structure of chromatin by psoralen-mediated selective crosslinking of inter-nucleosomal DNA (Hanson *et al*., 1976) in living cells represents an elegant ''gold standard'' type of reporter experiment. Is it possible to apply comparably penetrating *in situ* methods to reveal a nuclear matrix?

Two recent studies employing fluorescent nuclear proteins may offer the first glimpses of a non-chromatin, internal nuclear structure in living cells. Images taken in living mammalian cells reveal that a green fluorescent protein-tagged splicing factor (SF2/ASF) in many instances leaves its nuclear sites of high accumulation as discrete packages moving along what appear to be relatively straight tracks (Misteli *et al*., 1997; additional video data available at http://www.cshl.org/labs/spector). These apparently straight routes are compatible with the existence of a nuclear filament system serving as a track. Alternatively, it is possible that whenever the intranuclear movement of anything is observed over a reasonably short path length, it will inevitably appear as a linear route, due simply to the geometry of the interchromatin space (Zachar *et al*., 1993; Zimowska *et al*., 1997). This same consideration applies to apparently linear, short arrays of pre-mRNA transcripts in the nucleus that have been visualized, at least for certain genes under some conditions (Lawrence *et al*., 1989; Murti *et al*., 1993; Xing *et al*., 1995; Dirks *et al*., 1995; Ishov *et al*., 1997). Nevertheless, the GFP-tagged splicing factor movements (Misteli *et al*., 1997) do provide suggestive evidence for some kind of non-chromatin nuclear organization.

In a second recent investigation, two centrosome-nucleus shuttling proteins, CP60 and CP190, were observed by immunofluorescence within the nuclei of *Drosophila* cellular blastoderm stage embryos as nucleoplasmic networks (Oegema *et al*., 1997). The CP60 and CP190 containing intranuclear networks were found to be spatially non-overlapping and neither was coincident with DNA staining. These patterns of CP60 and CP190 may well represent a nuclear matrix *per se* or, alternatively, a high concentration of monomeric proteins in the interchromatin spaces (see also Zimowski *et al*., 1997). The latter possibility is reduced by the observation that the CP60 and CP190 interphase networks persisted after nuclear envelope breakdown as cells entered metaphase. The same type of intranuclear network system was seen when fluorescently tagged CP60 and CP190 was injected into nuclei followed by 3-D wide-field fluorescence microscopy observation of living embryos (Oegema *et al*., 1997; K. Oegema, W. F. Marshall and J. W. Sedat, personal communication of unpublished results). These types of investigations, conducted in nuclei of living cells, and now further catalyzed by the availability of green (and other color) fluorescent proteins and recent advances in fluorescence microscopy and image processing methods, are *precisely* what the nuclear matrix field has lacked up to now, and the results of future, expanded studies along these lines are eagerly awaited.

Conventional and Non-conventional Cytoplasmic Filament Proteins in the Nucleus

Another way to think about the existence of a real nuclear matrix *in vivo* is to ask if nuclei contain any members of the well-characterized cytoplasmic filament protein systems, *viz*. tubulin, actin, myosin, or intermediate filament proteins, since one presumed function of the nuclear matrix is intranuclear transport. With the exception of cells which undergo a ''closed'' mitosis in which the spindle forms within the nucleus (various fungi, protozoa and algae; Kubai, 1975), there is little if any tubulin and no convincing evidence for microtubules inside the nucleus. In contrast, there is now a substantial body of evidence for the presence of nuclear F-actin in a variety of cell types (Clark & Merriam, 1977; Fukui, 1978; Fukui & Katsumaru, 1979; Clark & Rosenbaum, 1979; Osborn & Weber, 1980; Welch & Suhan, 1985; De Boni, 1994; Yan

et al., 1997) as well as high affinity nuclear actin-binding proteins (Rimm & Pollard, 1989; Ankenbauer *et al*., 1989) and nuclear myosin (Hauser *et al*., 1975; Berrios & Fisher, 1986; Hagen *et al*., 1986; Rimm & Pollard, 1989; Nowak *et al*., 1997). The functional significance of nuclear actin, myosin and actin-binding proteins has remained elusive, however. One report suggested a role of nuclear actin in transcription (Scheer *et al*., 1984) but, surprisingly, seems not to have been pursued. More recently, mRNA splicing factors have been co-localized with nuclear actin (Sahlas *et al*., 1993). These types of studies seeking to link nuclear actin or myosin to gene expression have been surprisingly sparse and warrant far more attention, not only as potential elements of a nuclear matrix but also as a possible mechanochemical component of RNA export or other intranuclear transport phenomena. It is worth mentioning here in passing that pancreatic DNase binds F-actin (Lazarides & Lindberg, 1974) and promotes F-actin depolymerization (Hitchcock *et al*., 1976). To the extent that F-actin may contribute to a genuine nuclear matrix *in situ*, it is possible that the use of DNase I in many nuclear isolation procedures might promote F-actin disassembly.

As regards the presence of intermediate (10 nm) filament proteins in the nucleus, a large fraction of the filaments seen in resinless section images of RNP-containing nuclear matrix preparations are 10 to 11 nm in diameter (Jackson & Cook, 1988; He *et al*., 1990; Hozák *et al*., 1995). In one report, it was observed that some of these 10-11 nm nuclear filaments react with an antibody against lamin A (Hozák *et al*., 1995), which is a relative (like all the nuclear lamins) of the intermediate filament protein family. The initial idea that nuclear lamins are restricted to the nuclear periphery became established because in that region of the nucleus these proteins are polymerized into an intermediate filament-like coiled : coiled arrangement, i.e. the nuclear lamina *per se*. The finding that lamins are also present as discrete foci in the nucleoplasm (Goldman *et al*., 1992; Bridger *et al*., 1993; Moir *et al*., 1994) represents one of the potentially most important recent clues as regards the enduringly veiled concept of an actual nuclear matrix in situ. If nucleoplasmic lamins undergo dynamic assembly-disassembly *in vivo*, which is certainly plausible based on the available evidence (Goldman *et al*., 1992; Moir *et al*., 1994; Schmidt *et al*., 1994), they might well contribute to authentic internal nuclear structure. It is also noteworthy that many intermediate filament proteins have binding affinity for nucleic acids (although a great many proteins do) and also share some amino acid sequence homologies with transcription factors (Traub & Shoeman, 1994).

New Approaches to Nuclear Structure

It has become possible to measure in the nucleus of living cells the movement of various molecules and thereby deduce how they are diffusing or being conveyed through the non-chromatin space of the nucleoplasm, reflecting the microscopic structure of that space, i.e. the nucleoplasmic ground substance. Fluorescence recovery after photobleaching (FRAP) has been employed to study the movement of dextrans and Ficolls in the nucleus of living mammalian cells, and the results suggest the existence of a domain in the nucleus in which these polysaccharides (molecular mass 4×10^3 to 2×10^6 Da) apparently move by simple diffusion (Seksek *et al*., 1997). In another study the FRAP-measured movement of (non-DNA bound) ethidium bromide dye inside the nucleus of living cells was also found to be consistent with diffusion (Abney *et al*., 1997). Fluorescence correlation spectroscopy (FCS) and FRAP studies have recently revealed that oligodeoxynucleotides move within the nucleus of living mammalian cells at rates similar to those measured in aqueous solution (J. C. Politz, E. B. Brown, D. E. Wolf and T. Pederson, unpublished results). Moreover, certain nucleolar RNA species (U3, U8 and RNase MRP RNAs) are observed to move to nucleoli within 30 to 60 seconds after microinjection into the nucleoplasm of living mammalian cells (Jacobson *et al*., 1995; Jacobson & Pederson, 1997; 1998). These various recent studies suggest the existence of apparently isotropic zones of relatively unstructured nucleoplasm in which molecules can move without collisional impedance. It is important to note that the bleachspot diameter (and volume) used in the FRAP experiments (Seksek *et al*., 1997; Abney *et al*., 1997), and the confocal volumes employed in our FCS studies, were large enough to include elements of a nuclear matrix if it is assumed, as is strongly conveyed by the ultrastructural images of isolated matrix preparations (Capco *et al*., 1982; Jackson & Cook, 1988), that the matrix is extensively and consistently present throughout the total interchromatin space, i.e. there are no matrix-less pockets. (However, the possibility that these studies of intranuclear molecular movement reflect the transient existence of small domains in which the matrix filament system is dynamically depolymerized or disassembled at a given point in space and time must be borne in mind.) These recent biophysical studies are likely to represent the beginning of a new era of studying nuclear structure *in vivo*.

Cross-linking of protein–protein or protein–nucleic acid interactions by exposure of living cells to chemical or physical agents that induce the formation of non-perturbing covalent diadducts is an approach to native macromolecular interactions that has had some previous success. For example, exposure of intact mammalian erythrocytes to the 11 Å bridging, primary amine reactive protein cross-linker dimethyl-3,3′-dithiobispropionimidate

(Wang & Richards, 1974) captures intermolecular protein : protein contacts in the tetrameric hemoglobin molecule (Wang & Richards, 1975). Photochemical crosslinking (254 nm) of proteins to RNA has been accomplished in living mammalian cells and used to elucidate aspects of native nuclear ribonucleoprotein structure *in vivo* (Mayrand & Pederson, 1981; Mayrand *et al*., 1981; Economidis & Pederson, 1983). Recently a nuclear matrix preparation was obtained from formaldehyde-fixed cells that ultrastructurally resembles standard nuclear matrix preparations (Nickerson *et al*., 1997). Further *in situ* protein–protein and protein–nucleic acid cross-linking strategies, conducted on living cells, warrant strong encouragement as the nuclear matrix field moves forward.

Finally, it is to be recalled that one of the most important advances in the field of eukaryotic gene expression, *viz*. the ultrastructural visualization of active genes, involved the daring to spread open nuclear contents under controlled conditions (Miller & Beatty, 1969; Miller & Bakken, 1972). In a field (the nuclear matrix) that has encountered harsh criticism for alleged artifacts, it may seem curious to suggest subcellular systems. But splaying nuclei to a moderate degree, intermediate between that of an explosion of chromatin fibers on the one hand and the highly compact native nucleus on the other may hold promise for revealing a nuclear matrix, for example by new cryo-electron microscopy methods involving passage of the cooled specimens through a vitreous ice phase (Dubochet *et al*., 1982, 1988). Such splayed preparations might also allow visualization of fluorescent transcript movements along filamentous elements of the released infrastructure, ideally captured on glass or some other optically favorable surface, which might also open the door to analysis of the mechanochemical basis of RNA movement, a presumed major function of a nuclear matrix as it is envisioned.

Conclusion

The presence of two meters of DNA and approximately three times as much protein mass in the ~500 μm^3 volume of a typical mammalian cell nucleus generates a crowded macromolecular environment and therefore confounds our ability to define, much less observe, the inter-chromatin space. Intuitively, it would seem that molecular dynamics and the classical particle transport phenomena of physical chemistry would be subject to enormous viscoelastic constraints in a volume so crowded in the first instance by nucleoprotein (chromatin, spliceosomes and nucleoli). But, alas, we do not really know if this is the case (and the recent biophysical studies discussed earlier suggest it is not). A Maxwell's demon inspecting a specific functional site in the nucleus, such as a nascent ribosome in the nucleolus or an active spliceosome in the nucleoplasm, would surely report a functionally organized array of protein : protein and RNA–protein interactions. In the nuclear matrix field, however, this Maxwellian surveyor has essentially been asked to inspect the limiting space in between these functional domains of gene expression and, in addition, the demon has been forced to operate at a disadvantage, *viz*. the territory has been subjected to extensive nuclease digestion, in most cases also at very high ionic strength.

Returning our thoughts to the sliding filament model of muscle contraction that was presented as a scientific-intellectual ideal at the outset of this review, even this textbook concept is, in the formal sense, still only a hypothesis, and remains the continuing subject of surprising controversy and intense efforts. Its most persuasive evidence to date comes to us as a set of *in vivo* data, collected by time-resolved X-ray diffraction from live muscle fibers using the very high energy beams that can be delivered from the storage rings of synchroton accelerators (Huxley, 1996). Analogues (obviously not literally identical experiments) of this kind of elegant approach conducted on the cell nucleus in living cells would now seem the most promising way to advance the nuclear structure frontier. These new efforts to understand the structure of the cell nucleus call to mind a statement (quoted in Tanford, 1978) made by one of America's greatest chemists, Josiah Willard Gibbs (who gave us the enabling concept of the chemical potential). Gibbs said, ''One of the principal objects of theoretical research in any department of knowledge is to find the point of view from which the subject appears in its greatest simplicity.'' The current era of work on nuclear structure could not hoist a more appropriate banner.

Acknowledgments

This article is warmly dedicated to Paul Doty, who was a source of considerable encouragement when the author first embarked on the study of nuclear proteins. Constructive comments on drafts of the manuscript were provided by Bruce Alberts, John Edsall, Irving Klotz, Laszlo Lorand, Masayasu Nomura, and Alexander Varshavsky. I am grateful to Andrea Werme for skillful word processing, and to David Tabatadze for helping me translate the Zbarskii and Debov (1948) paper. I am supported by N. I. H. grant GM-21595-22.

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Edited by J. Karn

(Received 20 October 1997; received in revised form 15 December 1997; accepted 19 December 1997)