Topic Introduction

The Nuclear Matrix: Fractionation Techniques and Analysis

Rosemary H.C. Wilson,^{1,2,3} Emma L. Hesketh,¹ and Dawn Coverley¹

¹*Department of Biology, University of York, York YO10 5DD, United Kingdom*

The first descriptions of an insoluble nuclear structure appeared more than 70 years ago, but it is only in recent years that a sophisticated picture of its significance has begun to emerge. Here we introduce multiple methods for the study of the nuclear matrix.

A BRIEF HISTORY

Descriptions of a protein fraction that is resistant to extraction under high salt conditions were first made in 1942 (reviewed in Pederson 1998). However, the term “nuclear matrix” was first used in 1974 to refer to those proteins resistant to extraction with 2 M NaCl (Berezney and Coffey 1974). Nuclear matrix has become widely adopted as an overarching term for the proteins that resist aggressive methods of extraction, and we use it as such here. The 2 M NaCl method has been criticized because of its potential to cause aggregation of proteins. This led to the development of more refined extraction methods, including the use of lithium 3,5-diiodosalicylate, which was first used by Mirkovitch et al. (1984) to reveal a protein fraction termed the nuclear scaffold, and extraction after encapsulation in agarose under physiologically relevant salt concentrations (Jackson and Cook 1988) to reveal a sub-structure known as the nuclear skeleton (or nucleoskeleton). Further variations and refinement of these techniques have also been used (reviewed in Martelli et al. 2002). A modification of the original nuclear matrix method was developed by Capco et al. (1982), which reduced the potential for aggregation by using more physiologically relevant buffers with lower salt (0.5 M NaCl) and used nucleases (DNase I or other enzymes) to digest chromatin into small diffusible fragments. This was termed the “in situ nuclear matrix” as the cytoskeleton is also maintained under these conditions. The structure resistant to extraction with 2 M NaCl has subsequently been termed the “core nuclear matrix.”

Over time, these methods and their derivatives have led to the understanding that the nuclear matrix consists of core components that are consistently present, such as matrisins, lamins, hnRNPs, and other “structural” proteins, and conditional proteins that are recruited into the nuclear matrix to facilitate specific processes (Mika and Rost 2005). These include components of the DNA replication machinery (reviewed in Wilson and Coverley 2013), transcription machinery (Jackson and Cook 1985), DNA repair (Qiao et al. 2001; Boisvert et al. 2005; Campalans et al. 2007), splicing (Zeitlin et al. 1987; Jagatheesan et al. 1999) and chromatin remodeling (Reyes et al. 1997), as well as a catalogue of proteins identified by proteomic analysis (e.g., Albrethsen et al. 2009). In most cases, the functional significance of their immobilization remains an area of study; nevertheless, a number of nuclear matrix proteins are already gaining credibility as clinically useful biomarkers (Keese et al. 1999; Subong et al. 1999; Van Le et al. 2004; Higgins et al. 2012).

²Present address: Wellcome Trust Centre for Human Genetics, Oxford OX3 7BN, United Kingdom.

³Correspondence: rosemary.wilson@well.ox.ac.uk

© 2016 Cold Spring Harbor Laboratory Press

Cite this introduction as *Cold Spring Harb Protoc*; doi:10.1101/pdb.top074518

A SIMPLE PICTURE?

Despite the range of approaches used, some controversies have remained and reviews of the evidence for and against the nuclear matrix have come to conflicting conclusions (Pederson 1998; Hancock 2000; Nickerson 2001; Martelli et al. 2002). It has proven difficult to visualize the filamentous structure revealed by electron microscopy using immunofluorescence light microscopy, which typically reveals punctate foci for most of the nuclear matrix proteins that have been described. However, it should be borne in mind that the proteins that fractionate with the nuclear matrix and are identified by proteomic analysis may in fact make up highly dynamic “local” matrices rather than one large static structure (Martelli et al. 2002).

One fundamental biological reason for the debate surrounding the existence and nature of the nuclear matrix may in fact be that it exists in different forms in different cell types and may even be absent in some instances. We and others have shown that the composition of the nuclear matrix varies dramatically with differentiation and disease status and that some proteins are actively recruited as part of normal cellular transitions (Getzenberg 1994; Zink et al. 2004; Munkley et al. 2011; Varma and Mishra 2011). Thus, a lot of work previously undertaken on cancer cell lines, embryonic cells, or *Xenopus* eggs must now be interpreted in this light to avoid clouding the picture in normal somatic cells (Munkley et al. 2011). There remains a need for further studies of the nuclear matrix, including comparative analyses. For a description of two related protocols that identify nuclear matrix proteins by immunofluorescence and immunoblotting, see Protocol: **Preparation of the Nuclear Matrix for Parallel Microscopy and Biochemical Analyses** (Wilson et al. 2014).

APPROACHES TO FUNCTIONAL ANALYSIS

A summary of the methods available for the analysis of the nuclear matrix is presented in Figure 1.

FIGURE 1. Schematic diagram showing the methods that are available for analysis of the nuclear matrix (NM) and their functional significance.

Electron Microscopy

The resolution attained with the electron microscope has allowed detailed visualization of the fibrillar protein network within the nucleus of higher eukaryotic cells (Capco et al. 1982; Fey et al. 1986; Jackson and Cook 1988). The nuclear matrix has been viewed after RNase digestion (Berezney and Coffey 1974), DNase digestion (Capco et al. 1982), and removal of chromatin by electroelution (Jackson and Cook 1988) and in a range of buffer conditions designed to minimize artifacts (Mirkovitch et al. 1984; Jackson and Cook 1988; Nickerson et al. 1997; Engelhardt 1999; Wan et al. 1999). The nuclear matrix has also been viewed in paraformaldehyde-fixed sections of unextracted nuclei, identifying protein-rich interchromosomal areas consistent with the description of a nuclear matrix (Hendzel et al. 1999).

Proteomic Analysis

Studies designed to identify the component parts of the nuclear matrix were compiled in a database of nuclear matrix proteins, termed NMPdb (Mika and Rost 2005). Since then, large-scale proteomic screens have been undertaken that compare nuclear matrix components enriched in tumor cells and at different developmental stages (Albrethsen et al. 2009, 2010; Varma and Mishra 2011).

Analysis of Attached DNA

The proteinaceous structure isolated by nuclear matrix extraction protocols is associated with residual DNA as well as RNA. Attachment of DNA was first observed by electron microscopy in the 1970s (Paulson and Laemmli 1977). Various methods have since been used to study the attached DNA, including digestion of chromatin loops with restriction enzymes, DNase I, or topoisomerase II to reveal the attachment points (Mirkovitch et al. 1984; Djeliova et al. 2001; Linnemann et al. 2009; Rivera-Mulia et al. 2011), followed by isolation and sequencing, labeling by FISH, or incorporation of nucleotide analogs. Regions of DNA that remain attached after extraction with high salt are termed matrix-attached regions and those that remain after extraction for the scaffold or skeleton are known, respectively, as scaffold-attached regions or skeleton-attached sequences. The possible functional significance of these classes of attachment has been reviewed previously (Wilson and Coverley 2013).

Related procedures (e.g., maximum fluorescence halo radius), in which histones and loosely attached proteins are extracted but chromatin is undigested, have been used to study the DNA loops that emanate from the attachment points on the nuclear matrix (Vogelstein et al. 1980; Gerdes et al. 1994; Lemaitre et al. 2005; Guillou et al. 2010). A method that combines nuclear matrix extraction with chromosome conformation capture (3C) has also recently been developed and is termed M3C (Gavrilov et al. 2010).

A Protocol Using Cytoskeletal (CSK) Buffer

The method we prefer to use for the analysis of nuclear-matrix-related functions is described in Protocol: **Preparation of the Nuclear Matrix for Parallel Microscopy and Biochemical Analyses** (Wilson et al. 2014). This protocol involves extraction with nonionic detergent (Triton X-100) to remove membranes and soluble proteins under physiologically relevant salt concentrations, followed by extraction with 0.5 M NaCl, digestion with DNase I, and removal of fragmented DNA. Complementary analysis by immunoblotting and immunofluorescence reveals information regarding isoforms and spatial organization. This protocol uses CSK buffer to stabilize the cytoskeleton and nuclear matrix in relatively gentle conditions. CSK was first used by Lenk et al. (1977) and later by Capco et al. (1982) for electron microscopy. Others have used it for immunodetection of individual nuclear matrix components (Nickerson et al. 1992; Grondin et al. 1996; Huang et al. 2004; Boisvert et al. 2005; Ainscough et al. 2007; Campalans et al. 2007), identification of protein domains required for nuclear matrix binding (Ainscough et al. 2007), analysis of temporally regulated recruitment (Fujita 1999; Fujita et al. 2002; Miccoli et al. 2003; Samaniego et al. 2006; Sree et al. 2012), and comparison of recruitment between developmental or differentiation stages and between disease states (Munkley

et al. 2011). The benefits of this method include relatively gentle buffer conditions, the potential to generate robust results by using imaging and biochemical analysis in parallel, and its flexibility to incorporate high salt to reveal the core nuclear matrix or pretreatment with a protein–protein cross-linker to reveal those proteins only weakly associated with the nuclear matrix. It can also be combined with other cellular manipulations to address specific questions, such as determination of cell synchrony protocols, depletion by RNAi, and expression of ectopic proteins. Together, these offer the potential to uncover the functional relevance of recruitment to the nuclear matrix.

ACKNOWLEDGMENTS

Work in this laboratory is supported by the Biotechnology and Biological Sciences Research Council, United Kingdom and Yorkshire Cancer Research, United Kingdom.

REFERENCES

- Ainscough JF, Rahman FA, Sercombe H, Sedo A, Gerlach B, Coverley D. 2007. C-terminal domains deliver the DNA replication factor Ciz1 to the nuclear matrix. *J Cell Sci* **120**: 115–124.
- Albrethsen J, Knol JC, Jimenez CR. 2009. Unravelling the nuclear matrix proteome. *J Proteomics* **72**: 71–81.
- Albrethsen J, Knol JC, Piersma SR, Pham TV, de Wit M, Mongera S, Carvalho B, Verheul HM, Fijneman RJ, Meijer GA, et al. 2010. Subnuclear proteomics in colorectal cancer: Identification of proteins enriched in the nuclear matrix fraction and regulation in adenoma to carcinoma progression. *Mol Cell Proteomics* **9**: 988–1005.
- Berezney R, Coffey DS. 1974. Identification of a nuclear protein matrix. *Biochem Biophys Res Commun* **60**: 1410–1417.
- Boisvert FM, Hendzel MJ, Masson JY, Richard S. 2005. Methylation of MRE11 regulates its nuclear compartmentalization. *Cell Cycle* **4**: 981–989.
- Campalans A, Amouroux R, Bravard A, Epe B, Radicella JP. 2007. UVA irradiation induces relocalisation of the DNA repair protein hOGG1 to nuclear speckles. *J Cell Sci* **120**: 23–32.
- Capco DG, Wan KM, Penman S. 1982. The nuclear matrix: Three-dimensional architecture and protein composition. *Cell* **29**: 847–858.
- Djeliova V, Russev G, Anachkova B. 2001. Distribution of DNA replication origins between matrix-attached and loop DNA in mammalian cells. *J Cell Biochem* **80**: 353–359.
- Engelhardt M. 1999. Demonstration of a DNase-sensitive network associated with the nuclear pore complexes in rat liver nuclei. *Chromosoma* **108**: 64–71.
- Fey EG, Krochmalnic G, Penman S. 1986. The nonchromatin substructures of the nucleus: The ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionation and resinless section electron microscopy. *J Cell Biol* **102**: 1654–1665.
- Fujita M. 1999. Cell cycle regulation of DNA replication initiation proteins in mammalian cells. *Front Biosci* **4**: D816–D823.
- Fujita M, Ishimi Y, Nakamura H, Kiyono T, Tsurumi T. 2002. Nuclear organization of DNA replication initiation proteins in mammalian cells. *J Biol Chem* **277**: 10354–10361.
- Gavrilov AA, Zukher IS, Philonenko ES, Razin SV, Iarovaia OV. 2010. Mapping of the nuclear matrix-bound chromatin hubs by a new M3C experimental procedure. *Nucleic Acids Res* **38**: 8051–8060.
- Gerdes MG, Carter KC, Moen PT Jr, Lawrence JB. 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 RH. 1994. Nuclear matrix and the regulation of gene expression: Tissue specificity. *J Cell Biochem* **55**: 22–31.
- Grondin B, Bazinet M, Aubry M. 1996. The KRAB zinc finger gene ZNF74 encodes an RNA-binding protein tightly associated with the nuclear matrix. *J Biol Chem* **271**: 15458–15467.
- Guillou E, Ibarra A, Coulon V, Casado-Vela J, Rico D, Casal I, Schwob E, Losada A, Mendez J. 2010. Cohesin organizes chromatin loops at DNA replication factories. *Genes Dev* **24**: 2812–2822.
- Hancock R. 2000. A new look at the nuclear matrix. *Chromosoma* **109**: 219–225.
- Hendzel MJ, Boisvert F, Bazett-Jones DP. 1999. Direct visualization of a protein nuclear architecture. *Mol Biol Cell* **10**: 2051–2062.
- Higgins G, Roper KM, Watson IJ, Blackhall FH, Rom WN, Pass HI, Ainscough JF, Coverley D. 2012. Variant Ciz1 is a circulating biomarker for early-stage lung cancer. *Proc Natl Acad Sci* **109**: E3128–E3135.
- Huang JY, Shen BJ, Tsai WH, Lee SC. 2004. Functional interaction between nuclear matrix-associated HBXAP and NF- κ B. *Exp Cell Res* **298**: 133–143.
- Jackson DA, Cook PR. 1985. Transcription occurs at a nucleoskeleton. *EMBO J* **4**: 919–925.
- Jackson DA, Cook PR. 1988. Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat. *EMBO J* **7**: 3667–3677.
- Jagatheesan G, Thanumalayan S, Muralikrishna B, Rangaraj N, Karande AA, Parnik VK. 1999. Colocalization of intranuclear lamin foci with RNA splicing factors. *J Cell Sci* **112**: 4651–4661.
- Keesee SK, Meyer JL, Hutchinson ML, Cibas ES, Sheets EE, Marchese J, Oprea A, Potz D, Wu YJ. 1999. Preclinical feasibility study of NMP179, a nuclear matrix protein marker for cervical dysplasia. *Acta Cytol* **43**: 1015–1022.
- Lemaitre JM, Danis E, Pasero P, Vassetzky Y, Mechali M. 2005. Mitotic remodeling of the replicon and chromosome structure. *Cell* **123**: 787–801.
- Lenk R, Ransom L, Kaufmann Y, Penman S. 1977. A cytoskeletal structure with associated polyribosomes obtained from HeLa cells. *Cell* **10**: 67–78.
- Linnemann AK, Platts AE, Krawetz SA. 2009. Differential nuclear scaffold/matrix attachment marks expressed genes. *Hum Mol Genet* **18**: 645–654.
- Martelli AM, Falcieri E, Zweyer M, Bortul R, Tabellini G, Cappellini A, Cocco L, Manzoli L. 2002. The controversial nuclear matrix: A balanced point of view. *Histol Histopathol* **17**: 1193–1205.
- Miccoli L, Biard DSF, Frouin I, Harper F, Maga G, Angulo JF. 2003. Selective interactions of human kin17 and RPA proteins with chromatin and the nuclear matrix in a DNA damage- and cell cycle-regulated manner. *Nucleic Acids Res* **31**: 4162–4175.
- Mika S, Rost B. 2005. NMPdb: Database of nuclear matrix proteins. *Nucleic Acids Res* **33**: D160–D163.
- Mirkovitch J, Mirault ME, Laemmli UK. 1984. Organization of the higher-order chromatin loop: Specific DNA attachment sites on nuclear scaffold. *Cell* **39**: 223–232.
- Munkley J, Copeland NA, Moignard V, Knight JR, Greaves E, Ramsbottom SA, Pownall ME, Southgate J, Ainscough JF, Coverley D. 2011. Cyclin E is recruited to the nuclear matrix during differentiation, but is not recruited in cancer cells. *Nucleic Acids Res* **39**: 2671–2677.
- Nickerson J. 2001. Experimental observations of a nuclear matrix. *J Cell Sci* **114**: 463–474.
- Nickerson JA, Krochmalnic G, Wan KM, Turner CD, 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.
- Nickerson JA, Krockmalnic G, Wan KM, Penman S. 1997. The nuclear matrix revealed by eluting chromatin from a cross-linked nucleus. *Proc Natl Acad Sci* 94: 4446–4450.
- Paulson JR, Laemmli UK. 1977. The structure of histone-depleted metaphase chromosomes. *Cell* 12: 817–828.
- Pederson T. 1998. Thinking about a nuclear matrix. *J Mol Biol* 277: 147–159.
- Qiao F, Moss A, Kupfer GM. 2001. Fanconi anemia proteins localize to chromatin and the nuclear matrix in a DNA damage- and cell cycle-regulated manner. *J Biol Chem* 276: 23391–23396.
- Reyes JC, Muchardt C, Yaniv M. 1997. Components of the human SWI/SNF complex are enriched in active chromatin and are associated with the nuclear matrix. *J Cell Biol* 137: 263–274.
- Rivera-Mulia JC, Hernandez-Munoz R, Martinez F, Aranda-Anzaldo A. 2011. DNA moves sequentially towards the nuclear matrix during DNA replication in vivo. *BMC Cell Biol* 12: 3.
- Samaniego R, Jeong SY, de la Torre C, Meier I, de la Espina SMD. 2006. CK2 phosphorylation weakens 90 kDa MFP1 association to the nuclear matrix in *Allium cepa*. *J Exp Bot* 57: 113–124.
- Sree NK, Anesh R, Radha V. 2012. Dynamic changes in nuclear localization of a DNA-binding protein tyrosine phosphatase TCPTP in response to DNA damage and replication arrest. *Cell Biol Toxicol* 28: 409–419.
- Subong EN, Shue MJ, Epstein JI, Briggman JV, Chan PK, Partin AW. 1999. Monoclonal antibody to prostate cancer nuclear matrix protein (PRO:4–216) recognizes nucleophosmin/B23. *Prostate* 39: 298–304.
- Van Le TS, Myers J, Konety BR, Barder T, Getzenberg RH. 2004. Functional characterization of the bladder cancer marker, BLCA-4. *Clin Cancer Res* 10: 1384–1391.
- Varma P, Mishra RK. 2011. Dynamics of nuclear matrix proteome during embryonic development in *Drosophila melanogaster*. *J Biosci* 36: 439–459.
- Vogelstein B, Pardoll DM, Coffey DS. 1980. Supercoiled loops and eucaryotic DNA replicaton. *Cell* 22: 79–85.
- Wan KM, Nickerson JA, Krockmalnic G, Penman S. 1999. The nuclear matrix prepared by amine modification. *Proc Natl Acad Sci* 96: 933–938.
- Wilson RH, Coverley D. 2013. Relationship between DNA replication and the nuclear matrix. *Genes Cells* 18: 17–31.
- Wilson RHC, Hesketh EL, Coverley D. 2014. Preparation of the nuclear matrix for parallel microscopy and biochemical analyses. *Cold Spring Harbor Protoc* doi: 10.1101/pdb.prot083758.
- Zeitlin S, Parent A, Silverstein S, Efstratiadis A. 1987. Pre-mRNA splicing and the nuclear matrix. *Mol Cell Biol* 7: 111–120.
- Zink D, Fischer AH, Nickerson JA. 2004. Nuclear structure in cancer cells. *Nature Rev Cancer* 4: 677–687.

Cold Spring Harbor Protocols

The Nuclear Matrix: Fractionation Techniques and Analysis

Rosemary H.C. Wilson, Emma L. Hesketh and Dawn Coverley

Cold Spring Harb Protoc; doi: 10.1101/pdb.top074518

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

[Cell Biology, general](#) (1382 articles)
[Preparation of Cellular and Subcellular Extracts](#) (104 articles)
[Proteins and Proteomics, general](#) (575 articles)
[Subcellular Fractionation](#) (88 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>