

CHAPTER 3

Subnuclear Trafficking and the Nuclear Matrix

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Introduction

The nuclear matrix is the nuclear substructure that remains after the majority of DNA and soluble and chromatin-bound proteins have been removed from the nucleus.¹⁻³ Electron micrographs show that the animal nuclear matrix consists of the nuclear pore complexes embedded in the nuclear lamina, and a network of internal 10 nm filaments, into which granular structures and the nucleoli are embedded.^{4, 5} In two-dimensional protein gels of nuclear matrix preparations, more than 200 polypeptides can be distinguished, but only few components of the nuclear matrix have been cloned.^{6, 7} Best studied from animal systems are the nuclear lamins, a group of intermediate filament proteins that form the lamina, a filamentous protein meshwork that lines the nuclear envelope and is connected to the nuclear pore complexes. The nuclear lamins are attached to the inner envelope membrane by farnesylation and interactions with membrane-associated proteins.⁸⁻¹⁰

The nuclear matrix specifically binds to DNA fragments called matrix attachment regions (MARs). MARs are large, AT-rich DNA fragments with little sequence similarity, and are predicted to form the bases of chromatin loops attached to the nuclear matrix during interphase.¹¹ MARs bind to nuclear matrix preparations across species borders, and have been implicated in reducing position effects and increasing expression of transgenes in animals and plants.¹²⁻¹⁴ Several MAR-binding proteins have been identified, which are components of the nuclear matrix.¹⁵⁻²² In addition, proteins involved in transcription, splicing and RNA processing have been found to be associated with the nuclear matrix,²³⁻²⁵ and significantly the respective processes have been shown to take place at specific sites of an isolated nuclear matrix fraction.²⁶⁻²⁸ Together, the available data suggest that the nuclear matrix represents a core nuclear structure that is involved in chromatin organization and in different aspects of nucleic acid metabolism.

However, the nuclear matrix as a static, cytoskeleton-like structure is still an issue of intense debate (see for example see refs. 29, 30). The major objections are (1) that the procedures used to isolate the matrix might cause precipitation artifacts that we view as nuclear matrix fibers and (2) that proteins forming the interior matrix (as opposed to the lamins that form the outer shell) remain to be identified. It is probably equally possible that the observed specific subnuclear organization and the spatial "addresses" of chromatin domains, proteins, and protein complexes are caused by dynamic soluble interactions or by the association with a (either dynamic or static) solid-state structure. In any case, the information for specific subnuclear positioning exists, and it will be well worth addressing to what degree this information contributes to the biological functions of the respective molecules.

This Chapter does not focus on the association of DNA with the nuclear matrix, the function of nuclear matrix attachment regions, or the proteins binding to MARs. Instead, it investigates the information presently available about signals involved in the intranuclear targeting of proteins, either to the nuclear matrix or to specific subnuclear domains.

Of the several arguments that can be made for the functional importance of specific subnuclear protein targeting—and a role for the nuclear matrix in that targeting—three points will be discussed here. First, several proteins have been found to contain specific signals for their association with the nuclear matrix or for their targeting to specific subnuclear sites. These nuclear matrix-targeting signals (NMTSs) sometimes overlap with other functional domains, like DNA-binding domains or nuclear localization signals (NLSs). However, at least in some cases they could be separated from these functions, showing that nuclear matrix association is independent from DNA binding and that targeting to the nuclear matrix requires a signal in addition to the nuclear import signal. In some cases, the NMTS can confer nuclear matrix targeting to a heterologous protein, and in at least one case it aids to the activity of a heterologous transcription factor, thereby suggesting a functional significance associated with the subnuclear targeting of the protein.

Second, the association of proteins with the nuclear matrix and with specific subnuclear sites has been found to be a regulated process in at least some cases, indicating that it is likely of biological significance beyond a simple “sticking together” of cellular components. And finally, the disruption of the specific subnuclear targeting of some nuclear proteins has been found associated with human diseases caused by chromosome translocations. These are strong indications that spatial information is required for the proper functioning of the respective proteins and that subnuclear mislocalization can have a severe impact on their function.

Nuclear Matrix Targeting Signals

For a number of nuclear proteins that have been found either associated with the nuclear matrix, or localized in specific subnuclear domains, amino acid sequences have been identified that are necessary and sufficient for this localization. They range from small peptide motifs capable to confer nuclear matrix localization to a heterologous protein to larger portions of the protein, which in an additive or synergistic way contribute to nuclear matrix association. The following gives an overview over the motifs mapped in different nuclear proteins, including transcription factors, DNA- and RNA-binding proteins, viral proteins, kinases, and kinase adapters. Figure 1 shows a compilation of the locations and sequences of these motifs. As discussed below, there is presently no consensus sequence that can be derived from their comparison.

Steroid Receptors

Steroid receptor binding to the nuclear matrix was first described in the 1980s and was an early realization of a potential functional association of a regulatory protein with a structural component of the nucleus.^{29, 30} The domain necessary for association with the nuclear matrix has been narrowed down in the human glucocorticoid receptor (hGR) and the human androgen receptor (hAR).³¹ Both proteins consist of an N-terminal domain involved in activation, a central DNA-binding domain (DBD) followed by a tau2 transactivation domain, and a C-terminal steroid-binding domain. While in these early studies the domain required for nuclear matrix attachment was localized to the C-terminal steroid binding domain in hAR, both the DNA-binding domain and the C-terminal domain of hGR were found to be required.

A more detailed mapping of the NMTS of the hGR was performed by Tang et al.,³² who showed that the DBD in combination with the C-terminal tau2 transactivation domain constitute an NMTS of the hGR. Neither the DBD nor the tau2 domain alone was sufficient for nuclear matrix binding and the tau2 domain alone could not confer nuclear matrix binding to the heterologous GAL4 DNA-binding domain. Transactivation and nuclear matrix binding

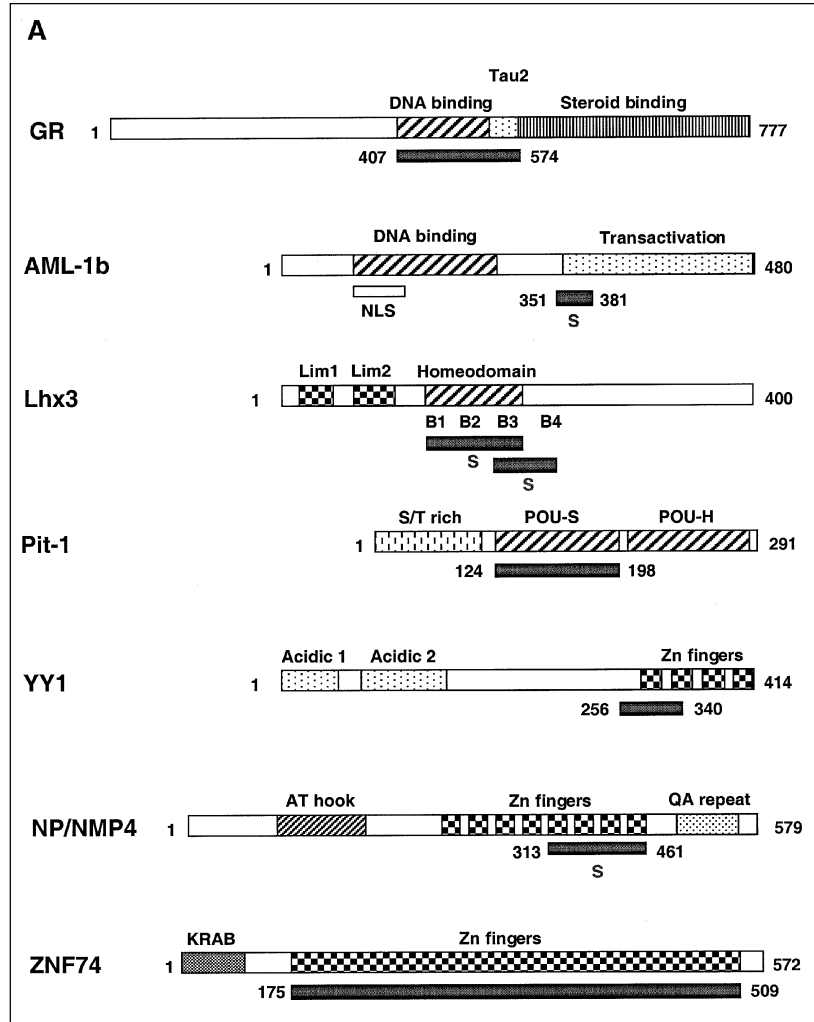


Figure 1A. Comparison of the location and size of nuclear matrix targeting signals. Transcription factors. NMTSs are indicated as bars underneath the schematic representation of the proteins. "S" indicates that the NMTS has been shown to be sufficient for nuclear matrix targeting. Tau2, tau2 transactivation domain; NLS, nuclear localization signal; Lim1, Lim 2, Lim domains; POU-S, POU-specific domain; POU-H, POU-type homeodomain; Zn fingers, zinc fingers; QA repeat, glutamine-alanine repeat; KRAB, Kruppel-associated box; PKA, PKA-binding domain. For detailed information, see text. Proteins and domains are only approximately drawn to scale.

could be uncoupled by point mutagenesis, for example in the S573A mutant, which still binds to the nuclear matrix but shows much reduced transcriptional activation. However, the two functions overlap, as is demonstrated e.g., with a L553G/L554G double mutant, which abolishes both nuclear matrix targeting and transactivation. These data show that the 29 amino acid (aa) tau2 domain probably contains two interaction surfaces—one for transcriptional activation and one for the binding of a nuclear matrix acceptor protein—and that nuclear matrix binding is not sufficient for transactivation by hGR.³³

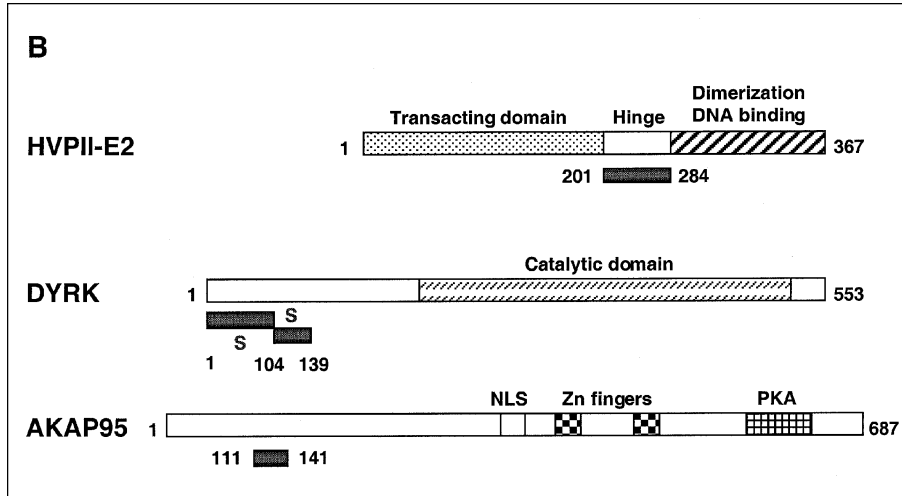


Figure 1B. Comparison of the location and size of nuclear matrix targeting signals. Other nuclear proteins. NMTSs are indicated as bars underneath the schematic representation of the proteins. "S" indicates that the NMTS has been shown to be sufficient for nuclear matrix targeting. Tau2, tau2 transactivation domain; NLS, nuclear localization signal; Lim1, Lim 2, Lim domains; POU-S, POU-specific domain; POU-H, POU-type homeodomain; Zn fingers, zinc fingers; QA repeat, glutamine-alanine repeat; KRAB, Kruppel-associated box; PKA, PKA-binding domain. For detailed information, see text. Proteins and domains are only approximately drawn to scale.

In contrast to the tau2 domain, the hGR DBD appears to function as an NMTS in a heterologous context insofar as fusion of the DBD with the Vp16 activation domain reconstitutes a functional NMTS.³² However, the Vp16 tau2 activation domain shares some structural relatedness to the GR tau2 domain, indicating that their combination might be required in the fusion protein too.

Candidates for Nuclear Matrix Acceptor Proteins

One intriguing—and presently unanswered—question is by which interactions an NMTS targets a protein to the nuclear matrix. Acceptor molecules on the nuclear matrix have been postulated which could be proteins, nucleic acids, or both. In the case of the steroid receptors, several candidates for interaction partners have been identified. However, in no case has a specific interaction been shown to be required for the subnuclear targeting of a protein.

GRIP120, a protein identified as a factor that interacts with GR, was found to be identical to hnRNP U, which belongs to the heterogeneous nuclear ribonucleoproteins, abundant proteins in the eukaryotic nucleus.³⁴ hnRNP U has in turn been found identical to SAF-A, a protein originally identified by its ability to bind to matrix attachment region (MAR) DNA, and a component of the nuclear matrix.³⁵ Indirect immunofluorescence microscopy demonstrated that GR and hnRNP U co-localize in nuclear speckles and large clusters. The C-terminal domain (aa 517-806) of hnRNP U and the C-terminal half of GR are sufficient for functional interaction of the two proteins.³⁴ Because the DBD-tau2 region has been identified as the NMTS of GR, it is therefore possible that hnRNP U constitutes an adapter that associates GR with the nuclear matrix. Overexpression of hnRNP U interferes with glucocorticoid induction and the C-terminal domains of both proteins are sufficient for mediating this effect.

Interestingly, the estrogen receptor (ER) has been shown to interact with another nuclear matrix protein, SAF-B/HAP (Scaffold attachment factor B/hnRNP A1 associated protein).³⁶

In *in vitro* binding assays, SAF-B/HAP binds to both the DBD and the hinge domain of ER. Co-immunoprecipitation showed that the binding occurs *in vivo* in cell lines. SAF-B/HAP was like SAF-A originally isolated as a protein that binds to matrix attachment region DNA and is localized in the nuclear matrix.¹⁹ Subsequently, it was shown that it is identical to hnRNP A1 associated protein, which is itself a bona fide hnRNP protein.³⁷ SAF-B has recently been shown to interact with RNA polymerase II (Pol II) and with SR proteins *in vitro* and *in vivo* and forms a ternary complex with SR proteins and MAR-DNA *in vitro*. Overexpression of SAF-B represses the expression of a MAR-flanked reporter gene. Oesterreich et al³⁶ have shown that SAT-B overexpression also decreases the ability of ER to activate transcription and that the ER-DBD is necessary for the repressive effects of SAT-B.³⁶

Together, the data connect both GR and ER to proteins that bind to MARs and are components of ribonucleoprotein complexes. While the picture is far from complete, it suggests a connection between the targeting of steroid receptors to the nuclear matrix, the recruitment of Pol II, and a modulation of receptor-induced gene expression by nuclear matrix proteins.

Yang et al³⁸ have identified by yeast two hybrid screening another acceptor candidate, the protein Hic-5, which binds specifically to the hinge-tau2 domain of GR. Hic-5 (hydrogen peroxide-inducible clone 5) is a previously identified protein that localizes both to focal adhesion points and to the nuclear matrix. Hic-5 stimulates transactivation by several nuclear receptors in combination with the coactivator GRIP1. In addition, it acts as a coactivator for the isolated DBD-tau2 domain of GR, but not the DBD alone. The C-terminus of Hic-5, containing seven zinc fingers, is required for both binding of GR and association to the nuclear matrix. However, in contrast to GR, Hic-5 binding to the nuclear matrix was not altered by ATP depletion, indicating that its NM binding is not simply caused by association with nuclear matrix-bound GR.³⁸ Hic-5 could therefore be primarily associated with a structural component of the nuclear matrix, where it would bind GR through an interaction of the zinc finger domain with the GR tau2 domain, thereby promoting transactivation.

AML/CBF- α Transcription Factors

The probably best characterized NMTS is that of the bone-specific transcription factor AML1b. The AML/CBF- α *run1* transcription factors are key regulators of hematopoietic and bone tissue-specific gene expression. AML1 and AML1b are two splicing variants, of which only AML1b is transcriptionally active. AML1b, but not AML1 binds to the nuclear matrix. Nuclear matrix association is independent of DNA-binding, shown by point mutations in the *run1* DNA binding-domain, which no longer bind DNA, but still associate with the nuclear matrix. The region necessary for nuclear matrix localization was narrowed down to a 31 aa segment localized near the C-terminus, which is different from the NLS and resides in a region absent in AML1.³⁹ The 31 aa NMTS is sufficient to target the heterologous protein GAL4 to the nuclear matrix. The NMTS is closely associated with the AML1b activation domain and fusing it to the GAL4 DNA-binding leads to transactivation of a genomically integrated GAL4-responsive reporter gene. Epitope tagged AML1b colocalizes with a subset of hyperphosphorylated Pol II in specific nuclear foci which are linked to the nuclear matrix.⁴⁰ This colocalization depends on active transcription and requires the DNA-binding function of the *run1* DNA-binding domain of AML1b.

The crystal structure of the AML1b NMTS fused to glutathione S-transferase has been solved at 2.7 Å resolution⁴¹ and interpreted with respect to its predicted function in nuclear matrix targeting. It consists of two loop domains, which are connected by a flexible hinge region. The two loops of the NMTS could interact with a putative protein or nucleic acid acceptor in the nuclear matrix, a hypothesis supported by mutagenesis studies.^{41,42} The 31 aa NMTS is conserved among AML1b homologs in human, mouse, rat and chicken. A similar motif is also present in the related transcription factors AML2 and AML3 from human and

mouse. Both proteins were also found associated with the nuclear matrix.⁴¹ A C-terminal deletion mutant of AML3, lacking 150 aa including the NMTS-homologous sequence is not retained in the nuclear matrix, indicating that the NMTS is functional in AML3 too.

Homeodomain Proteins

Different homeodomain-containing transcription factors have been found associated with the nuclear matrix. Lhx3 is a LIM homeodomain transcription factor that is essential for pituitary organogenesis and motor neuron specification. Lhx3 is found both in the nucleoplasm and associated with the nuclear matrix, as measured by in situ nuclear matrix extraction. The protein contains three nuclear localization signals within the homeodomain and one additional one at the C-terminus (B1, B2, B3, and B4). The 60 aa homeodomain (containing regions B1, B2, and B3) is sufficient for nuclear matrix targeting. A second, overlapping domain that also confers association with the nuclear matrix was mapped to a fragment of 28 aa which contains the B3 and B4 domains.⁴³ This domain is sufficient to target Green Fluorescent Protein to the nuclear matrix, which makes it an interesting tool for nuclear matrix studies due to its small size.

Interestingly, Pit-1, a homeodomain factor that cooperates with Lhx3 in the activation of several pituitary-specific genes, is also a nuclear matrix-associated protein.⁴⁴ Pit-1 has a bipartite DNA-binding domain known as the POU domain. It consists of a POU-specific domain and a POU-type homeodomain. The 66 aa POU-specific domain constitutes the domain necessary and sufficient for nuclear matrix targeting.⁴⁴ Nuclear matrix-association and DNA binding can be functionally uncoupled, because pointmutants that no longer bind DNA have been shown to remain fully nuclear matrix associated. Both the homeodomain of Lhx3 and the POU-specific domain of Pit-1 adopt a helical structure with basic sequences located at both ends of the domain.⁴³ Whether these structural features are related to the recognition of acceptor molecules on the nuclear matrix is presently not known. It is however interesting to speculate that the nuclear matrix-association of two functionally cooperating transcription factors might be involved in their biological activity. Another POU homeodomain protein, Oct-1 is also present in the nuclear matrix.⁴⁵ Oct-1 partitions, like Pit-1, between soluble and insoluble nuclear fractions. The domain necessary for nuclear matrix association of Oct-1 has not yet been identified. Interestingly, Oct-1 has also been shown to co-localize with lamin B, a component of the nuclear lamina (reviewed in ref. 48). In aging cells, Oct-1 has been found to depart from its location at the nuclear periphery, and this departure correlates with reduced repression of a collagenase gene by Oct-1.⁴⁶ These results suggest that Oct-1 is active as a repressor only when located at the nuclear periphery, and imply a connection between association with sub-nuclear structural elements and regulated function in gene expression.

Zinc Finger Transcription Factors

Yin-Yang 1 (YY1) is a 414 aa zinc finger-containing transcription factor, which acts both as activator and repressor. It is identical to NMP1, a protein found originally as a nuclear-matrix associated protein, which partitions between the nuclear matrix and a 0.4 M salt extract.⁴⁷ Its activation domain has been mapped to the N-terminus, while the repression domain overlaps with the zinc-finger DNA-binding domain at the C-terminus. McNeil et al⁴⁸ show that the C-terminal domain (aa 201 – 414) is necessary for high-affinity interaction with the nuclear matrix, while the N-terminus (aa 1 – 256) shows only weak retention.⁴⁸ Bushmeyer et al⁴⁹ have narrowed down the NMTS further to the region of aa 256 – 340. It is at present not known whether this relatively well defined NMTS is sufficient to target a heterologous protein to the nuclear matrix.

The NP/NMP4 transcription factors are nuclear matrix-associated proteins that contain from five to eight C-terminal Cys₂-His₂ Zinc fingers and an N-terminal AT-hook domain.⁵⁰

Some NP/NMP4 in-frame splice variants have been proposed to be architectural transcription factors which increase the basal activity of the rat type I collagen $\alpha 1(I)$ polypeptide chain promoter in osteoblast-like cells. They bind to the minor groove of a poly(dT) consensus sequence and bend DNA. The isoform 11H is predominantly located in two non-nucleolar foci inside the nucleus, with smaller amounts diffusely distributed in the non-nucleolar part of the nucleus. The zinc finger domain is necessary and sufficient for this localization. Extracting cells transiently transfected with GFP-NP/NMP4 fusions, it has been shown that the amino terminus plus the AT-hook domain could be extracted, while the full-length GFP fusion protein and the zinc finger fusion protein were retained and diffusely distributed in the nuclear matrix. Because the nuclear matrix fraction showed no evidence for residual DNA by DAPI staining, it was concluded that the zinc-finger mediated nuclear matrix targeting of NP/NMP4 does not require DNA binding. The minimal domain sufficient for nuclear matrix targeting is a 148 aa fragment containing zinc fingers four to eight.

ZNF74 is a developmentally expressed zinc finger gene of the Kruppel-associated box (KRAB) multifinger subfamily and is encoded by a candidate gene for DiGeorge syndrome.^{51,52} Grondin et al⁵³ have shown that the zinc finger nucleic acid binding domain is a multifunctional domain which also acts as nuclear matrix targeting sequence and is involved in protein-protein interactions. ZNF74 interacts with its zinc finger domain with the hyperphosphorylated form of the large subunit of RNA polymerase II (pol I α), but not with the hypophosphorylated form.⁵³ In immunofluorescence experiments, ZNF74 co-localized with pol I α and with the SC35 splicing factor in subnuclear domains.

The smallest region sufficient for association with the nuclear matrix was narrowed down to the zinc finger domain between aa 175 and 509. It is presently not known if a smaller region of ZNF74 still tightly binds to the nuclear matrix, or if the entire zinc finger domain is required. Since the binding occurs after extensive DNase and RNase treatment of the nuclear matrix fraction, the authors concluded that it is independent of the nucleic acid-binding affinity of the zinc finger domain, and therefore most likely mediated by protein-protein interactions. In a search for protein-protein interaction partners, and therefore possible nuclear matrix adapters, only binding to pol I α was discovered. The two proteins interact *in vivo* as well as in the absence of nucleic acids and the binding depends on the hyperphosphorylation of pol I α . This result suggests that ZNF74 is not present in preinitiation complexes but rather associates with elongating RNA polymerase II. Whether and how this complex is associated with the nuclear matrix awaits further investigation. Interestingly, a nuclear matrix protein that interacts with the phosphorylated C-terminal domain of RNA polymerase II has been identified by Patturajan et al⁵⁴ and several reports have demonstrated that Pol II itself is associated with the nuclear matrix.⁵⁵⁻⁵⁷

Viral Proteins

The Epstein-Barr virus (EBV) nuclear antigen leader protein (EBNA-LP) is the first viral gene product together with EBNA-2 to be expressed after infection of B-cells. EBNA-LP is a nuclear matrix-associated protein and has been suggested to play an important role in EBV-induced transformation.⁵⁸ The protein has been shown to bind to p53 and to Rb *in vitro* and co-localizes with ND10 nuclear domains, indicating a specific spatial sequestering inside the nucleus. To investigate the biological significance of EBNA-LP nuclear matrix binding, Yokoyama et al⁵⁸ have attempted to map the nuclear matrix targeting domain and correlate it with the ability of EBNA-LP to co-activate EBNA-2 dependent transactivation. EBNA-LP consists of four W1W2 repeat domains flanked by a Y1Y2 domain. The affinity of nuclear matrix binding is reduced as W1W2 repeats are deleted, indicating that their copy number is involved in high-affinity binding. Fine mapping indicated that the 10 amino acid segment EPRRVRRRVL in the W2 domain is involved in NM binding. However, as substitution of this motif also

destroys the NLS of EBNA-LP, leading to accumulation of the protein in the cytoplasm, no clear NLS-independent NMTS could be identified in this study. The mutant protein fails to act as a co-activator, which might be explained by its inability to enter the nucleus.

The papillomavirus E2 protein is a site-specific DNA-binding protein, which functions as the primary origin of replication-recognition protein. In addition, it is involved in regulating transcription from the native viral promoter. The protein is localized in distinct subnuclear foci, which correlate with the replication compartments. It consists of an N-terminal trans-acting domain, a central hinge-domain, and a C-terminal protein-dimerization and DNA-binding domain.⁵⁹ The hinge domain confers strong nuclear localization, while the N-terminus and the C-terminus alone are localized both in the nucleus and in the cytoplasm. Both the hinge and the N-terminus also bind to the nuclear matrix, but with reduced affinity compared to the full-length protein. A cluster of basic amino acids in the hinge domain is required for both nuclear and nuclear matrix localization. As in the case of EBNA-LP, the two functions can not be separated, because the mutated protein no longer enters the nucleus. A truncated hinge region (aa 216 – 255), which contains the basic motif and flanking proline and glycine residues leads to a diffuse nuclear localization, but can not be recovered from a nuclear matrix fraction, indicating that the fragment is not sufficient for the nuclear matrix association observed with the full-length hinge domain. The 82 amino acid hinge domain is therefore the smallest fragment shown to be both necessary and sufficient for nuclear matrix targeting of the HVP-11 protein.

Kinases and Kinase Adapters

DYRK1 is a member of a family of dual-specificity protein kinases involved in brain development. Mutants of the *Drosophila* homolog *minibrain* have reduced numbers of neurons in some brain areas and show specific behavioral defects.⁶⁰ The human homolog maps to the “Down’s syndrome critical region” on chromosome 21, but its specific function in neuronal development is not yet known. The mammalian isoform DYRK1a is a nuclear localized protein and the NLS has been mapped to positions 105 to 139. Becker et al⁶¹ have shown that the region between position 1 and 104 is necessary for the specific, punctate nuclear localization pattern of DYRK1a, and that a deletion fragment containing this region only is as tightly nuclear matrix-bound as the full-length protein. A deletion fragment spanning aa 105 to 139 is also partially localized in the nuclear matrix fraction, indicating that the position of potential NMTS sequences in DYRK1a is more complex.⁶¹

A-kinase anchoring protein (AKAP) 95 is a zinc-finger protein, which binds and anchors cAMP-dependent protein kinase (PKA). The protein is found in the nuclear matrix of a wide variety of mammalian cells.^{62,63} The zinc finger domain required for DNA binding and a C-terminal amphipathic helix that serves as an anchoring domain for PKA are both located in the C-terminal half of the 687 amino acid protein. The N-terminal 386 amino acids contain both the NLS and a domain required for nuclear matrix targeting.⁶⁴ The NMTS was further narrowed down to a domain between amino acid 111 and 141, which is highly conserved in rat, mouse and human AKAP95 and similar to a sequence found in the nuclear matrix protein ZAN75, and in the genetic neighbor of AKAP95, NAKAP95.⁶⁴ The AKAP95 NMTS is independent from both the DNA-binding and the PKA-binding domains, indicating that an additional interaction partner is required for the association with the nuclear matrix.

A candidate for such an acceptor is p68 RNA helicase (p68 RH), which was identified as an AKAP95-binding protein in a yeast two-hybrid screen. The AKAP95 fragment from aa 109 to 201 is sufficient for binding the C-terminal domain of p68 RH, indicating that neither the DNA-binding nor the PKA-binding domain is involved. There is a close correlation between the AKAP95 NMTS (aa 111-141) and the p68 RH-binding domain (aa 109-201), p68 RH has been found to be a nuclear matrix-localized protein, and AKAP95 and p68 RH could be co-immunoprecipitated from nuclear fractions.⁶⁴ Together, these findings make p68 RH a

potential nuclear matrix-localized acceptor of AKAP95. p68 RH has been previously shown to be associated with cAMP-responsive element binding protein (CREB)-binding protein CBP. Association of AKAP95 with a nuclear matrix-bound complex of p68 RH/CREB/CBP/DNA would therefore be a plausible mechanism to position PKA for its establishes function in CBP/CREB-mediated gene regulation.⁶⁴

Comparison of NMTSs in Different Nuclear Proteins

Figure 1 shows an overview over the position and size of the NMTSs discussed in this Chapter. The regions identified to be either necessary or sufficient for nuclear matrix targeting reach from small, ca. 30 aa motifs to large portions of a protein (e.g., in ZNF74). In the case of transcription factors, there are several cases where they overlap or are identical with the DNA-binding domains. However, as nuclear matrix preparations are usually extensively DNase treated, several authors conclude that nuclear matrix binding is a feature of these domains that is separable from DNA-binding, and therefore probably caused by protein-protein interactions.

The smallest identified peptides sufficient for nuclear matrix targeting are those in AML1b (31 aa), the B3/B4 domain of Lhx3 (28 aa), and the second NMTS mapped in DYRK (34 aa), which confers partial nuclear matrix localization and overlaps with the NLS. Another well-defined region necessary for nuclear matrix localization is the 32 aa fragment from aa 111 to aa 141 in AKAP95. This fragment has so far not been shown to be sufficient for nuclear matrix targeting.

The sequences of the AML1b and AKAP95 NMTS are both well conserved within a family of related proteins, including both their homologs from different species such as human, mouse, rat and chicken, and functionally closely related proteins within the same species.^{39,64} However, no consensus can be derived by comparing NMTS sequences of functionally unrelated proteins. This feature of NMTSs might indicate that different proteins use different interaction surfaces on the nuclear matrix, either by binding to different acceptor proteins, or by interacting with different domains of the same ubiquitous nuclear matrix proteins.

To advance our understanding of the nature of NMTSs, interaction partners for the best defined NMTS sequences need to be identified, and experiments need to be designed to show that specific protein-protein interactions are required for the observed targeting events. In addition, more structural information of the respective protein domains—such as the crystal structure of the AML1b NMTS—will allow comparisons beyond the alignment of primary sequences and might uncover presently unknown structural similarities between these domains.

Regulated Nuclear Matrix Interaction

Only a very small number of regulated subnuclear targeting events have been described. Interestingly, one involves the light-regulated subnuclear trafficking of two plant photoreceptors, which have been shown to interact *in vivo* at their targeting sites. These examples provide first evidence that specific subnuclear targeting might serve as a previously unappreciated mechanism regulating the function of nuclear proteins. This is an area of functional cell biology that clearly awaits further investigation.

It has been shown early that the association of steroid receptors with the nuclear matrix requires the presence of the hormone.^{29,30} In addition, the nuclear matrix of ATP depleted cells binds a significantly higher fraction of GRs, while binding equal amounts of SV40 large tumor antigen, indicating that the observation is not caused by an unspecific “collapsing” effect of nuclear material.⁶⁵ This retention is reversible by addition of ATP. The data implicate that ATP is required for a step that actively dissociates GR from its acceptor on the nuclear matrix.

A GR-GFP fusion protein has been tested for nuclear import in response to hormone binding. While GR-GFP translocated to the nucleus in the presence of dexamethasone, progesterone, and the glucocorticoid antagonist RU486, a striking difference in the subnuclear distribution of the fusion protein was observed. In dexamethasone-treated cells GR-GFP was

predominantly located at bright small foci within the nucleus. In contrast, treatment with progesterone lead to a diffuse nuclear localization, while RU486-treated cells showed a diffuse pattern with regions of condensation in a reticular pattern. These data indicate that while all three hormones were sufficient for the activation of nuclear import of GR-GFP, the specific subnuclear trafficking of the three complexes was regulated differentially.⁶⁶ Similar results were obtained with a human estrogen receptor-GFP fusion protein.⁶⁷

Protein kinase CKII is a highly conserved ubiquitous messenger-independent serine/threonine protein kinase, which is localized in the nucleus and the cytoplasm and which is involved in growth and differentiation events. It has been shown to be located in the nuclear matrix and phosphorylates substrates such as topoisomerase II and RNA polymerases.⁶⁸ The association of CKII with the nuclear matrix has been found to be influenced by androgen and growth factor stimuli in rat prostate cells.⁶⁹ Androgen deprivation leads to a progressive decline in NM-bound CKII while androgen treatment leads to an increase. In addition, three growth factors stimulated the association of CKII with the nuclear matrix.

An interesting case of regulated association with the nuclear matrix is that of the retinoblastoma gene product Rb. Rb is a 110 kD tumor suppressor protein that interacts with several viral oncoproteins that are associated with the nuclear matrix. Rb itself is nuclear matrix-bound, but only specifically during G1 phase, while no nuclear matrix association was detected during S phase. The form bound during G1 is hypophosphorylated and was found at the nuclear periphery as well as in dense fibrogranular masses in immunogold labeling experiments with isolated nuclear matrix fractions.⁷⁰ Interestingly, a novel nuclear matrix protein (NRM/B) has been identified that specifically binds to the hypophosphorylated form of Rb.⁷¹ NRB/P is a kelch-domain protein and its expression is limited to neuronal tissue. While it might therefore be an exciting candidate for a nuclear matrix acceptor of Rb, it will also be interesting to find nuclear matrix proteins with affinity for Rb which are less limited to specific cell types.

Subnuclear targeting events and the association of functional proteins with the nuclear matrix are at present a practically uninvestigated field in plant molecular biology. A small number of nuclear matrix-associated and MAR-binding proteins have been identified,^{20-22,72-74} but the association of transcription factors and other gene expression-modulating proteins with the nuclear matrix has not yet been studied. Strikingly, and coming from an entirely different angle of investigation, groups studying the biological activity of plant photoreceptors have recently provided first evidence that subnuclear partitioning might both happen and be of functional consequence in plants too. When a fusion of the red-light photoreceptor phytochrome B with GFP was monitored in dark-grown and light-grown plants, the fusion protein was found to be localized in the cytoplasm in the dark, but in the nucleus in the light.⁷⁵ Strikingly, the protein was not diffusely distributed within the nucleus, but accumulated in "speckles" of comparable size and distribution to some of the nuclear bodies well characterized in animals, but so far barely investigated in plants.⁷⁶⁻⁸¹

Cryptochrome 2 is a plant blue-light photoreceptor that has also been shown to be located in the nucleus^{82, 83}. Mas et al⁸⁴ have demonstrated that cryptochrome 2 changes its subnuclear localization in response to blue light irradiation. While the protein has a diffuse localization in the dark, it accumulates in speckles after brief blue-light irradiation. Moreover, after treatment with both blue and red light, phytochrome B and cryptochrome 2 co-localize in some nuclear speckles and FRET experiments indicate that the two proteins directly interact. Both photoreceptors are implied in the activation of gene expression. It will be of great interest to see if their interaction takes place on the plant nuclear matrix, if the observed speckles can be identified with respect to known nuclear bodies, and what protein domains and interaction partners of cryptochrome 2 are involved in its light-regulated subnuclear trafficking.

Compromised Subnuclear Localization and Disease

Numerous cytogenetic abnormalities that involve the bone-specific transcription factor AML1 have been identified in acute myelogenous leukemia. In the frequent 8;21 translocation, a fusion protein between AML1b and ETO is created that lacks the C-terminus of AML1b, including the NMTS. The AML1/ETO fusion is still targeted to subnuclear sites, but interestingly they differ from the binding sites of AML1b. Instead, the AML1/ETO protein is redirected by the ETO component to alternative nuclear-matrix associated foci.⁸⁵ These findings indicate that modifications in the subnuclear trafficking of transcription factors can disrupt their gene regulatory function and that within the nuclear matrix specific functional subdomains for anchored transcription factors can be defined.

Similarly, the putative transcription factor ALL1 (also called MLL and HRX) normally shows a punctate subnuclear distribution, which is conferred by distinct elements in the N-terminus of the protein.⁸⁶ ALL1 is a 430 kD polypeptide, which contains two putative DNA-binding domains, an amino terminal AT-hook motif and two zinc finger regions near the middle of the protein. In the t(1;11) (p32-q23) translocation, which has been described in rare cases of acute myelogenous leukemia,⁸⁶ the amino-terminal domain of ALL1 is fused to the carboxy-terminal domain of eps15, a ubiquitously expressed epidermal growth factor receptor substrate, which is localized in the cytoplasm. The ALL1-eps15 fusion protein is localized in the nucleus, but is targeted to different, smaller domains than wildtype ALL1, indicating that the protein fusion created a novel targeting address different from both wildtype proteins.⁸⁶

Interestingly, yet another translocation fusing ALL1 to a heterologous protein and causing acute leukemia creates a fusion between ALL1 and a histone acetyltransferase.⁸⁷ Although no localization data exist for this fusion protein, it is tempting to speculate that mistargeting of a functional histone acetyltransferase through fusion with ALL1 might deregulate chromatin structure and gene expression patterns, thereby promoting leukemia.

Besides acute leukemia, subnuclear mislocalization has also been implied in a neurodegenerative disorder, spinocerebellar ataxia type 1 (SCA1). It is caused by expansion of a polyglutamine tract in the *SCA1* gene, coding for ataxin-1. The subnuclear localization patterns of wildtype and mutant ataxin-1 have been compared.⁸⁸ Wildtype ataxin-1 localizes to several nuclear structures of about 0.5 μm diameter, while mutant ataxin-1 was found in a single, large 2 μm structure. PML bodies are specific nuclear structures associated with the nuclear matrix, which contain the marker PML protein. Colocalization experiments showed that mutant ataxin-1 sequestered the PML protein to the 2 μm body and altered the distribution and appearance of PML bodies.⁸⁸ Both wildtype and mutant ataxin-1 were found bound to the nuclear matrix in cerebral tissue, emphasizing that the interactions between ataxin-1 and PML, which might lead to the observed mislocalizations, are associated with the nuclear matrix.

Concluding Remarks

The case has been made for the presence of sequences different from nuclear localization signals that determine the fate of a protein once inside the nucleus. The number of examples where such signals have been studied in detail is still small, and it is too early to draw conclusions about their similarity or multiplicity. If the described signals act like other targeting domains, they will most likely function by providing a surface for protein-protein interactions. If transcription factors come with signals for specific subnuclear "addresses", and if their disruption can compromise transcription factor function, then one ought to think about what those addresses are, and how they relate to the position and/or compartmentalization of the promoters regulated by these factors. The prevailing evidence for the association of specific chromatin regions (MARs) with the nuclear matrix, and the positive effect MARs have on the transcription of flanking genes has led to the model that the association of genes with the nuclear matrix increases their ability to be expressed, possibly by providing a more "open" chromatin environment. It

might be equally attractive to think about another mechanism, by which association with the nuclear matrix of both promoters and transcription factors might increase the probability of productive assembly of transcription initiation complexes. The fact that “transcriptosomes” appear to have specific locations in the nucleus, and that they can function on the isolated nuclear matrix encourages to think about how such complexes might assemble in specific places. It will be highly informative to investigate whether three-way interactions between genes, sequence-specific transcription factors, and nuclear matrix components play a role in their assembly.

References

1. Berezney R, Coffey DS. Identification of a nuclear protein matrix. *Biochem Biophys Res Commun* 1974; 60:1410-1417.
2. Nickerson JA, Krockmalnic G, Wan KM et al. The nuclear matrix revealed by eluting chromatin from a cross-linked nucleus. *Proc Natl Acad Sci USA* 1997; 94:4446-4450.
3. Wan KM, Nickerson JA, Krockmalnic G et al. The nuclear matrix prepared by amine modification. *Proc Natl Acad Sci USA* 1999; 96:933-938.
4. Verheijen R, van Venrooij W, Ramaekers F. The nuclear matrix: structure and composition. *J Cell Sci* 1988; 90:11-36.
5. Penman S. Rethinking cell structure. *Proc Natl Acad Sci USA* 1995; 92:5251-5257.
6. Fey EG, Penman S. Nuclear matrix proteins reflect cell type of origin in cultured human cells. *Proc Natl Acad Sci USA* 1988; 85:121-125.
7. Stuurman N, Meijne AM, van der Pol AJ et al. The nuclear matrix from cells of different origin. Evidence for a common set of matrix proteins. *J Biol Chem* 1990; 265:5460-5465.
8. McKeon FD, Kirschner MW, Caput D. Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. *Nature* 1986; 319:463-468.
9. Furukawa K, Pante N, Aebi U et al. Cloning of a cDNA for lamina-associated polypeptide 2 (LAP2) and identification of regions that specify targeting to the nuclear envelope. *EMBO J* 1995; 14:1626-1636.
10. Wilson KL. The nuclear envelope, muscular dystrophy and gene expression. *Trends Cell Biol* 2000; 10:125-129.
11. Gasser SM, Laemmli UK. A glimpse at chromosomal order. *Trends Genet* 1987; 3:16-22.
12. Phi-Van L, von Kries JP, Ostertag W et al. The chicken lysozyme 5' matrix attachment region increases transcription from a heterologous promoter in heterologous cells and dampens position effects on the expression of transfected genes. *Mol Cell Biol* 1990; 10:2302-2307.
13. Allen GC, Hall GE, Jr., Childs LC et al. Scaffold attachment regions increase reporter gene expression in stably transformed plant cells. *Plant Cell* 1993; 5:603-613.
14. Allen GC, Hall G, Jr., Michalowski S et al. High-level transgene expression in plant cells: Effects of a strong scaffold attachment region from tobacco. *Plant Cell* 1996; 8:899-913.
15. von Kries JP, Buhrmester H, Stratling WH. A matrix/scaffold attachment region binding protein: Identification, purification, and mode of binding. *Cell* 1991; 64:123-135.
16. Dickinson LA, Joh T, Kohwi Y et al. A tissue-specific MAR/SAR DNA-binding protein with unusual binding site recognition. *Cell* 1992; 70:631-645.
17. Dickinson LA and Kohwi-Shigematsu T. Nucleolin is a matrix attachment region DNA-binding protein that specifically recognizes a region with high base-unpairing potential. *Mol Cell Biol* 1995; 15:456-465.
18. Gohring F, Schwab BL, Nicotera P et al. The novel SAR-binding domain of scaffold attachment factor A (SAF-A) is a target in apoptotic nuclear breakdown. *EMBO J* 1997; 16:7361-7371.
19. Renz A, Fackelmayer FO. Purification and molecular cloning of the scaffold attachment factor B (SAF-B), a novel human nuclear protein that specifically binds to S/MAR-DNA. *Nucleic Acids Res* 1996; 24:843-849.
20. Meier I, Phelan T, Gruissem W et al. MFP1, a novel plant filament-like protein with affinity for matrix attachment region DNA. *Plant Cell* 1996; 8:2105-2115.
21. Morisawa G, Han-Yama A, Moda I et al. AHM1, a novel type of nuclear matrix-localized, MAR binding protein with a single AT hook and a J domain-homologous region. *Plant Cell* 2000; 12:1903-1916.

22. Hatton D, Gray JC. Two MAR DNA-binding proteins of the pea nuclear matrix identify a new class of DNA-binding proteins. *Plant J* 1999; 18:417-429.
23. van Wijnen AJ, Bidwell JP, Fey EG et al. Nuclear matrix association of multiple sequence-specific DNA binding activities related to SP-1, ATF, CCAAT, C/EBP, OCT-1, and AP-1. *Biochemistry* 1993; 32:8397-8402.
24. Zeng C, McNeil S, Pockwinse S et al. Intranuclear targeting of AML/CBFalpha regulatory factors to nuclear matrix-associated transcriptional domains. *Proc Natl Acad Sci USA* 1998; 95:1585-1589.
25. Guo B, Odgren PR, van Wijnen AJ et al. The nuclear matrix protein NMP-1 is the transcription factor YY1. *Proc Natl Acad Sci USA* 1995; 92:10526-10530.
26. Blencowe BJ, Nickerson JA, Issner R et al. Association of nuclear matrix antigens with exon-containing splicing complexes. *J Cell Biol* 1994; 127:593-607.
27. Spector DL, Schrier WH, Busch H. Immunoelectron microscopic localization of snRNPs. *Biol Cell* 1983; 49:1-10.
28. Wei X, Somanathan S, Samarabandu J et al. Three-dimensional visualization of transcription sites and their association with splicing factor-rich nuclear speckles. *J Cell Biol* 1999; 146:543-558.
29. Barrack ER and Coffey DS. The specific binding of estrogens and androgens to the nuclear matrix of sex hormone responsive tissues. *J Biol Chem* 1980; 255:7265-7275.
30. Barrack E. *Specific association of androgen receptors and estrogen receptors with the nuclear matrix: Summary and perspectives*. In: Moudgil V, ed. *Recent Advances in Steroid Hormone Action*. Berlin: Walther de Gruiter, 1987:85-107.
31. van Steensel B, Jenster G, Damm K et al. Domains of the human androgen receptor and glucocorticoid receptor involved in binding to the nuclear matrix. *J Cell Biochem* 1995; 57:465-478.
32. Tang Y, Getzenberg RH, Vietmeier BN et al. The DNA-binding and tau2 transactivation domains of the rat glucocorticoid receptor constitute a nuclear matrix-targeting signal. *Mol Endocrinol* 1998; 12:1420-1431.
33. DeFranco DB, Guerrero J. Nuclear matrix targeting of steroid receptors: specific signal sequences and acceptor proteins. *Crit Rev Eukaryot Gene Expr* 2000; 10:39-44.
34. Eggert M, Michel J, Schneider S et al. The glucocorticoid receptor is associated with the RNA-binding nuclear matrix protein hnRNP U. *J Biol Chem* 1997; 272:28471-28478.
35. Romig H, Fackelmayer FO, Renz A et al. Characterization of SAF-A, a novel nuclear DNA binding protein from HeLa cells with high affinity for nuclear matrix/scaffold attachment DNA elements. *Embo J* 1992; 11:3431-3440.
36. Oesterreich S, Zhang Q, Hopp T et al. Tamoxifen-bound estrogen receptor (ER) strongly interacts with the nuclear matrix protein HET/SAF-B, a novel inhibitor of ER-mediated transactivation. *Mol Endocrinol* 2000; 14:369-381.
37. Weighardt F, Cobianchi F, Cartegni L et al. A novel hnRNP protein (HAP/SAF-B) enters a subset of hnRNP complexes and relocates in nuclear granules in response to heat shock. *J Cell Sci* 1999; 112:1465-1476.
38. Yang L, Guerrero J, Hong H et al. Interaction of the tau2 transcriptional activation domain of glucocorticoid receptor with a novel steroid receptor coactivator, Hic- 5, which localizes to both focal adhesions and the nuclear matrix. *Mol Biol Cell* 2000; 11:2007-2018.
39. Zeng C, van Wijnen AJ, Stein JL et al. Identification of a nuclear matrix targeting signal in the leukemia and bone-related AML/CBF-alpha transcription factors. *Proc Natl Acad Sci USA* 1997; 94:6746-6751.
40. Zeng C, McNeil S, Pockwinse S et al. Intranuclear targeting of AML/CBFalpha regulatory factors to nuclear matrix-associated transcriptional domains. *Proc Natl Acad Sci USA* 1998; 95:1585-1589.
41. Tang L, Guo B, Javed A et al. Crystal structure of the nuclear matrix targeting signal of the transcription factor acute myelogenous leukemia-1/polyoma enhancer-binding protein 2alphaB/core binding factor alpha2. *J Biol Chem* 1999; 274:33580-33586.
42. Chen LF, Ito K, Murakami Y et al. The capacity of polyomavirus enhancer binding protein 2alphaB (AML1/Cbfa2) to stimulate polyomavirus DNA replication is related to its affinity for the nuclear matrix. *Mol Cell Biol* 1998; 18:4165-4176.
43. Parker GE, Sandoval RM, Feister HA et al. The homeodomain coordinates nuclear entry of the Lhx3 neuroendocrine transcription factor and association with the nuclear matrix. *J Biol Chem* 2000; 275:23891-23898.

44. Mancini MG, Liu B, Sharp ZD et al. Subnuclear partitioning and functional regulation of the Pit-1 transcription factor. *J Cell Biochem* 1999; 72:322-338.
45. Kim MK, Lesoon-Wood LA, Weintraub BD et al. A soluble transcription factor, Oct-1, is also found in the insoluble nuclear matrix and possesses silencing activity in its alanine-rich domain. *Mol Cell Biol* 1996; 16:4366-4377.
46. Imai S, Nishibayashi S, Takao K et al. Dissociation of Oct-1 from the nuclear peripheral structure induces the cellular aging-associated collagenase gene expression. *Mol Biol Cell* 1997; 8:2407-2419.
47. Guo B, Odgren PR, van Wijnen AJ et al. The nuclear matrix protein NMP-1 is the transcription factor YY1. *Proc Natl Acad Sci USA* 1995; 92:10526-10530.
48. McNeil S, Guo B, Stein JL et al. Targeting of the YY1 transcription factor to the nucleolus and the nuclear matrix in situ: the C-terminus is a principal determinant for nuclear trafficking. *J Cell Biochem* 1998; 68:500-510.
49. Bushmeyer SM, Atchison ML. Identification of YY1 sequences necessary for association with the nuclear matrix and for transcriptional repression functions. *J Cell Biochem* 1998; 68:484-499.
50. Feister HA, Torrungruang K, Thunyakitpisal P et al. NP/NMP4 transcription factors have distinct osteoblast nuclear matrix subdomains. *J Cell Biochem* 2000; 79:506-517.
51. Ravassard P, Cote F, Grondin B et al. ZNF74, a gene deleted in DiGeorge syndrome, is expressed in human neural crest-derived tissues and foregut endoderm epithelia. *Genomics* 1999; 62:82-85.
52. Cote F, Boisvert FM, Grondin B et al. Alternative promoter usage and splicing of ZNF74 multifinger gene produce protein isoforms with a different repressor activity and nuclear partitioning. *DNA Cell Biol* 2001; 20:159-173.
53. Grondin B, Cote F, Bazinet M et al. Direct interaction of the KRAB/Cys2-His2 zinc finger protein ZNF74 with a hyperphosphorylated form of the RNA polymerase II largest subunit. *J Biol Chem* 1997; 272:27877-27885.
54. Patturajan M, Wei X, Berezney R et al. A nuclear matrix protein interacts with the phosphorylated C-terminal domain of RNA polymerase II. *Mol Cell Biol* 1998; 18:2406-2415.
55. Vincent M, Lauriault P, Dubois MF et al. The nuclear matrix protein p255 is a highly phosphorylated form of RNA polymerase II largest subunit which associates with spliceosomes. *Nucleic Acids Res* 1996; 24:4649-4652.
56. Mortillaro MJ, Blencowe BJ, Wei X et al. A hyperphosphorylated form of the large subunit of RNA polymerase II is associated with splicing complexes and the nuclear matrix. *Proc Natl Acad Sci USA* 1996; 93:8253-8257.
57. Chabot B, Bisotto S, Vincent M. The nuclear matrix phosphoprotein p255 associates with splicing complexes as part of the [U4/U6.U5] tri-snRNP particle. *Nucleic Acids Res* 1995; 23:3206-3213.
58. Yokoyama A, Kawaguchi Y, Kitabayashi I et al. The conserved domain CR2 of Epstein-Barr virus nuclear antigen leader protein is responsible not only for nuclear matrix association but also for nuclear localization. *Virology* 2001; 279:401-413.
59. Zou N, Lin BY, Duan F et al. The hinge of the human papillomavirus type 11 E2 protein contains major determinants for nuclear localization and nuclear matrix association. *J Virol* 2000; 74:3761-3770.
60. Tejedor F, Zhu XR, Kaltenbach E et al. minibrain: A new protein kinase family involved in postembryonic neurogenesis in *Drosophila*. *Neuron* 1995; 14:287-301.
61. Becker W, Weber Y, Wetzel K et al. Sequence characteristics, subcellular localization, and substrate specificity of DYRK-related kinases, a novel family of dual specificity protein kinases. *J Biol Chem* 1998; 273:25893-25902.
62. Eide T, Coghlan V, Orstavik S et al. Molecular cloning, chromosomal localization, and cell cycle-dependent subcellular distribution of the A-kinase anchoring protein, AKAP95. *Exp Cell Res* 1998; 238:305-316.
63. Coghlan VM, Langeberg LK, Fernandez A et al. Cloning and characterization of AKAP 95, a nuclear protein that associates with the regulatory subunit of type II cAMP-dependent protein kinase. *J Biol Chem* 1994; 269:7658-7665.
64. Akileswaran L, Taraska JW, Sayer JA et al. A-kinase-anchoring Protein AKAP95 Is Targeted to the Nuclear Matrix and Associates with p68 RNA Helicase. *J Biol Chem* 2001; 276:17448-17454.
65. Tang Y, DeFranco DB. ATP-dependent release of glucocorticoid receptors from the nuclear matrix. *Mol Cell Biol* 1996; 16:1989-2001.

66. Htun H, Barsony J, Renyi I et al. Visualization of glucocorticoid receptor translocation and intranuclear organization in living cells with a green fluorescent protein chimera. *Proc Natl Acad Sci USA* 1996; 93:4845-4850.
67. Htun H, Holth LT, Walker D et al. Direct visualization of the human estrogen receptor alpha reveals a role for ligand in the nuclear distribution of the receptor. *Mol Biol Cell* 1999; 10:471-486.
68. Ahmed K. Nuclear matrix and protein kinase CK2 signaling. *Crit Rev Eukaryot Gene Expr* 1999; 9:329-336.
69. Guo C, Yu S, Davis AT et al. Nuclear matrix targeting of the protein kinase CK2 signal as a common downstream response to androgen or growth factor stimulation of prostate cancer cells. *Cancer Res* 1999; 59:1146-1151.
70. Mancini MA, Shan B, Nickerson JA et al. The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein. *Proc Natl Acad Sci USA* 1994; 91:418-422.
71. Kim TA, Lim J, Ota S et al. NRP/B, a novel nuclear matrix protein, associates with p110(RB) and is involved in neuronal differentiation. *J Cell Biol* 1998; 141:553-566.
72. Masuda K, Xu ZJ, Takahashi S et al. Peripheral framework of carrot cell nucleus contains a novel protein predicted to exhibit a long alpha-helical domain. *Exp Cell Res* 1997; 232:173-181.
73. Gindullis F, Meier I. Matrix attachment region binding protein MFP1 is localized in discrete domains at the nuclear envelope. *Plant Cell* 1999; 11:1117-1128.
74. Gindullis F, Peffer NJ, Meier I. MAF1, a novel plant protein interacting with matrix attachment region binding protein MFP1, is located at the nuclear envelope. *Plant Cell* 1999; 11:1755-1768.
75. Yamaguchi R, Nakamura M, Mochizuki N et al. Light-dependent translocation of a phytochrome B-GFP fusion protein to the nucleus in transgenic Arabidopsis. *J Cell Biol* 1999; 145:437-445.
76. Boudonck K, Dolan L, Shaw PJ. The movement of coiled bodies visualized in living plant cells by the green fluorescent protein. *Mol Biol Cell* 1999; 10:2297-2307.
77. Gall JG, Bellini M, Wu Z et al. Assembly of the nuclear transcription and processing machinery: Cajal bodies (coiled bodies) and transcriptosomes. *Mol Biol Cell* 1999; 10:4385-4402.
78. Gall JG. A role for Cajal bodies in assembly of the nuclear transcription machinery. *FEBS Lett* 2001; 498:164-167.
79. Matera AG. Nuclear bodies: Multifaceted subdomains of the interchromatin space. *Trends Cell Biol* 1999; 9:302-309.
80. Maul GG, Negorev D, Bell P et al. Review: properties and assembly mechanisms of ND10, PML bodies, or PODs. *J Struct Biol* 2000; 129:278-287.
81. Seeler JS, Dejean A. The PML nuclear bodies: actors or extras? *Curr Opin Genet Dev* 1999; 9:362-367.
82. Kleiner O, Kircher S, Harter K et al. Nuclear localization of the Arabidopsis blue light receptor cryptochrome 2. *Plant J* 1999; 19:289-296.
83. Guo H, Duong H, Ma N et al. The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. *Plant J* 1999; 19:279-287.
84. Mas P, Devlin PF, Panda S et al. Functional interaction of phytochrome B and cryptochrome 2. *Nature* 2000; 408:207-211.
85. McNeil S, Zeng C, Harrington KS et al. The t(8;21) chromosomal translocation in acute myelogenous leukemia modifies intranuclear targeting of the AML1/CBFalpha2 transcription factor. *Proc Natl Acad Sci USA* 1999; 96:14882-14887.
86. Yano T, Nakamura T, Blechman J et al. Nuclear punctate distribution of ALL-1 is conferred by distinct elements at the N terminus of the protein. *Proc Natl Acad Sci USA* 1997; 94:7286-7291.
87. Sobulo OM, Borrow J, Tomek R et al. MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3). *Proc Natl Acad Sci USA* 1997; 94:8732-8737.
88. Skinner PJ, Koshy BT, Cummings CJ et al. Ataxin-1 with an expanded glutamine tract alters nuclear matrix-associated structures. *Nature* 1997; 389:971-974.