

Matrin3: connecting gene expression with the nuclear matrix

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Abstract

As indicated by its name, Matrin3 was discovered as a component of the nuclear matrix, an insoluble fibrogranular network that structurally organizes the nucleus. Matrin3 possesses both DNA and RNA binding domains and, consistent with this, has been shown to function at a number of stages in the life-cycle of mRNAs. These numerous activities indicate that Matrin3, and indeed the nuclear matrix, do not just provide a structural framework for nuclear activities, but play direct functional roles in these activities. Here we review the structure, functions, and molecular interactions of Matrin3 and of Matrin3-related proteins, and the pathologies that can arise upon mutation of Matrin3.

Introduction

Matrin3 was first identified as a major component of the nuclear matrix^{1, 2}, which connects the nuclear membrane and the lamin class of intermediate filaments to intra-nuclear structures (see Box 1). The nuclear matrix acts as a scaffold for attachment of chromatin, both active and inactive, binding to specific regions on the chromosomes termed scaffold/matrix attachment regions (S/MARs)³. The S/MARs have been associated with various functions including regulation of transcription^{4, 5}, RNA processing⁶, insulation of genomic domains⁷ and facilitating DNA replication⁸. Thus, attachment of chromatin to the nuclear matrix not only spatially organizes the nucleus, but also contributes to numerous nuclear functions.

Box 1. The nuclear matrix

The nuclear matrix consists of a non-chromatin fibrogranular ribonucleoprotein network located within the nucleus but connecting with the internal surface of the nuclear membrane, interior to the nuclear lamina. Sometimes called the nuclear scaffold or skeleton, historically the nuclear matrix has been defined in two complementary ways. First, and most importantly, electron microscopy allowed direct visualization of an extensive non-chromatin branched fibrogranular ribonucleoprotein network that had not been evident in earlier analyses by light microscopy⁹. Biochemical approaches identified the matrix as a structure resistant to sequential extractions, including high concentrations of salt, non-ionic detergents and digestion with DNase. These treatments remove both soluble complexes such as the spliceosome as well as some insoluble complexes including chromatin. The general structure of the nuclear matrix is insensitive to DNase treatment, emphasizing the identity of the matrix as a separate entity from chromatin, albeit one that is proposed to organize

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3 chromatin loops via interaction at S/MARs. Conversely, the matrix is sensitive to RNase
4 digestion, indicating that ribonucleoprotein complexes are integral to the structure, and
5 presumably functions, of the matrix¹⁰. One of the major challenges in understanding the
6 nuclear matrix is defining the major protein constituents that underlie the observed
7 filamentous structure; proteomic analyses indicate a highly complex composition¹¹, including
8 many RNA binding proteins. The matrix-association of proteins known to be active in various
9 nuclear processes argues for the functional importance of the matrix. Nevertheless it should
10 be noted that the concept of a nuclear matrix is not universally accepted^{12,13}.

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16 MatrIn3 was identified by analysis of the protein complement of the
17 biochemically defined nuclear matrix fraction¹. To characterize the key players of the
18 nuclear matrix, Berezney and colleagues used two-dimensional polyacrylamide gels
19 to separate and identify the major protein components of the extracted nuclear
20 matrix from rat liver cells¹. Together with the lamins A, B and C, other abundant
21 proteins were identified, including MatrIn3, 12, 13, D/E, F/G (Prostaglandin
22 transporter SLC21A2), PSF/SFPQ (at the time named MatrIn4) and nucleophosmin
23 (also known as B-23), as well as several hnRNPs. Subsequently, many other
24 proteins have been found to localize to this structure and have been identified using
25 quantitative proteomic tools¹¹. Taking a complementary approach Sugano and
26 colleagues identified a 130kDa protein, initially named P130 after its gel migration
27 size, which binds to AT-rich sequences found in highly repetitive matrix-associated
28 DNA sequences, and upon protein sequence analysis revealed to be MatrIn3^{14,15}.

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37 Although a major component of the matrix that provides structure and
38 organization to the nucleus, the majority of the 847 amino acids of MatrIn3 comprise
39 intrinsically disordered regions, with the exceptions of two zinc-finger domains and
40 two tandem RNA binding domains¹⁶. The assumption that its matrix localization
41 implies a passive structural role could not be further from the truth; MatrIn3 has been
42 associated with functions ranging from transcription regulation, pre-mRNA splicing,
43 mRNA stability, DNA damage repair to cell proliferation (see below). The
44 involvement of MatrIn3 and other nuclear matrix proteins in these processes
45 suggests that the nuclear matrix might have important roles not only in the spatial
46 organization and compartmentalization, but also in the activity of the specialized
47 machineries involved in these various nuclear functions.

Domain structure and function of Matrin3

Four distinguishable structured domains are present in Matrin3 (Fig. 1A), two DNA binding C₂H₂ zinc-finger domains (ZF) and two RNA binding domains of the RNA recognition motif type (RRM)¹⁶. In addition to these domains there are also a bipartite nuclear localization signal (NLS) and a nuclear export signal (NES)^{16, 17}. Most of the remaining protein sequence is predicted to be disordered, and therefore might be dedicated to mediating protein-protein interactions, as motifs for protein interactions are commonly found in disordered regions¹⁸. One such motif is a PTBP1 RRM interaction motif (PRI), a 7 amino acid motif that mediates interaction with the splicing regulator polypyrimidine tract binding protein (PTBP1) and possibly other proteins¹⁹.

Along with Lamin B, Matrin3 is one of the few nuclear matrix proteins that do not bind to total genomic DNA from rat liver, when analysed for DNA binding by southwestern blot²⁰. Nevertheless, subsequent studies have shown that Matrin3 can bind to at least some DNA sequences¹⁴⁻¹⁶. The two zinc-finger (ZF) domains belong to the U1-like zinc finger family although in contrast to the member that gives name to the family, U1C²¹, the ZF domains in Matrin3 mediate interaction with DNA¹⁶. The two ZF domains show some redundancy as deletion of both ZF1 and ZF2 is required to impair DNA binding, assayed by electrophoretic mobility shift assay (EMSA), and recruitment to chromatin in cells or in biochemically fractionated chromatin¹⁶. The ZF domains cannot compensate binding to RNA when the RRM domains are deleted¹⁶. The ZF domains are possibly associated with roles in chromosome positioning, transcription regulation and/or genic insulation as all these typically involve DNA binding proteins. Deletion of the ZF domains does not affect the general role of Matrin3 in alternative splicing regulation, but it remains possible that it interacts with DNA to regulate specific co-transcriptional RNA processing events¹⁹.

The two RRM domains of Matrin3 are most similar to the RRM domains of PTBP1 and hnRNPL¹⁶, with 37% sequence identity to the first of the four RRM domains of PTBP1. The AUCUU motif was determined as the optimal RNA binding site for the tandem RRMs of Matrin3 by RNA-compete array-based selection as part of a study characterizing the binding sites for 207 RNA binding proteins²². Whether this motif reflects optimal binding to one or both RRMs currently remains unclear, since the 5-mer length of this motif is consistent with interaction of a single RRM

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3 domain²³. There is evidence that some RNA-dependent protein interactions require
4 the second RRM but not the first, suggesting that RRM2 might play the dominant
5 role in RNA binding²⁴. The AUCUU motif is similar to the UUUCU motif bound by
6 PTBP1, which directly interacts with Matrin3 and cooperates with it in alternative
7 splicing regulation. The two tandem RRMs of Matrin3 bind to the regulated RNA
8 targets and are required for its role in splicing regulation and RNA stabilization^{19, 24}.
9 RNA map constructed by mapping Matrin3 iCLIP tags onto the Matrin3 regulated
10 splicing events suggests that *in vivo* RNA binding might involve initial binding to
11 specific sites followed by oligomerization along the RNA²⁵ (see below).
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18 Immunofluorescence microscopy found Matrin3 as a mainly nuclear protein in
19 various mammalian cell lines, where it localizes throughout the nucleoplasm with the
20 exception of the nucleolus^{26, 27}. Nuclear matrix localization of proteins like SATB1,
21 Runx and AML-1 is mediated through a nuclear matrix targeting sequence (NMTS)
22 distinct from the NLS^{28, 29}, but a functionally equivalent motif could not be identified in
23 Matrin3. Although Matrin3 was originally discovered by its presence in nuclear matrix
24 fractions, biochemical fractionation and western blotting show it is also present in
25 soluble nucleoplasmic fractions^{16, 19}, and to some extent also in cytoplasmic
26 fractions, albeit at lower levels than in the nucleus¹⁶. The lack of an identified NMTS
27 in Matrin3 means that it is not yet possible to determine which of its nuclear functions
28 depend on its matrix localization. Shuttling between the nucleus and cytoplasm is
29 mediated by the nuclear export signal (NES) located at the N-terminus, and the bi-
30 partite nuclear localization signal (NLS) located between the second RRM and the
31 second ZF domain^{16, 17} (Fig.1A). Although no evidence for a cytoplasmic role has yet
32 been described, the presence of a NES and some cytoplasmic localization suggest
33 that such a role may exist.
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48 **Molecular interactions of Matrin3**

49 Matrin3 can interact with multiple nuclear proteins, a high proportion of which
50 are involved in RNA binding and/or processing (Table 1). Some of these are
51 important for Matrin3 function, including the interactions with PTBP1, NONO (also
52 known as p54nrb) and PSF (reviewed in³⁰), but the function of other interactions
53 remains unclear. The domains and motifs mediating most direct protein-protein
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3 interactions remain unclear, with one exception being the PRI-mediated interaction
4 with PTBP1¹⁹. In fact, some of the reported interactions might be indirectly bridged
5 by RNA, since they were identified by techniques that do not distinguish between
6 direct and RNA-bridged interactions, including yeast two hybrid (Y2H) and
7 immunoprecipitation (IP) without nuclease treatment (Table 1). Moreover, interaction
8 sites for binding partners may overlap, and their competition for Matrin3 binding
9 might be relevant for the different Matrin3 functions. Some of these interactions may
10 require presence of Matrin3 within the nuclear matrix, which could serve as a
11 scaffold for specific functions. The interaction between Matrin3 and LaminA²⁶ is one
12 such matrix associated interaction. Matrin3 binds to the tail of LaminA and
13 immunofluorescence shows co-localization on the nuclear rim of differentiated
14 C2C12 myotubules, although it is unclear if this is in the nuclear membrane or
15 nuclear matrix. This interaction might be involved in the anchoring of the nuclear
16 envelope bound lamins to the nuclear matrix, thereby ensuring structural integrity of
17 the nucleus.
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31 **Matrin3-related proteins**

32 Mammals possess two Matrin3-related proteins, RBM20 and ZNF638, which
33 share its overall domain organization, including the related ZF and RRM domains
34 (Fig. 1A). While Matrin3 and ZNF638 are widely expressed across different cell
35 types, RBM20 expression is mostly restricted to the foetal and adult heart (Fig 1B).
36 ZNF638 (originally known as NP220) has a pair of RRMs between the two C₂H₂ ZF
37 domains³¹. Sequence identity is highest in the ZF and RRM domains, although
38 RBM20 only has a single RRM domain. RBM20 shares 29% amino acid identity with
39 Matrin3 over 43% of its length. Mapping of the RNA targets of RBM20 by CLIP
40 revealed an optimal UCUU binding motif³², which is remarkably similar to the optimal
41 binding sites for both Matrin3 (AUCUU)²², and PTBP1 (UUUCU/UCUU)^{22, 33}. This is
42 consistent with the fact that the first RRM of PTBP1 recognises YCU motif³⁴ and has
43 a high degree of identity with the RRMs of Matrin3 (40% and 35%) and RBM20
44 (51%). RBM20 and ZNF638 lack the PRI motif found in Matrin3, and so presumably
45 do not interact with the splicing regulator PTBP1. Nevertheless, both proteins were
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3 found to regulate splicing^{35, 36}, and have regions enriched in RS dipeptides, a feature
4 commonly associated with splicing factors and regulators
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8 **Matrin3 function in transcriptional regulation**

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10 A number of functions have been suggested for Matrin3 throughout the life cycle of
11 mRNAs, from the regulation of transcription, alternative splicing, viral RNA export,
12 mRNA stability, to the DNA damage response. The role of Matrin3 in transcriptional
13 regulation originates from the finding that Matrin3 interacts with the regions of DNA
14 that are present within the scaffold/matrix attachment regions (S/MARs)³. These
15 Matrin3-bound DNA regions augmented transcription when placed upstream of a
16 promoter in a reporter plasmid³⁷. Matrin3 binding decreased upon methylation of this
17 DNA region, with a corresponding decrease in transcription, thereby suggesting that
18 Matrin3 can activate transcription³⁸. Matrin3 might not be the only factor involved in
19 transcriptional activation by these DNA regions, given that these regions were
20 isolated from the nuclear matrix scaffold that contain many proteins involved in
21 transcription^{11, 37}. Matrin3 was also found to bind to Pit1, a transcription factor
22 involved in the activation of multiple genes, most notably the pituitary hormone gene.
23 Enhancer-bound Pit1 has to interact with the nuclear matrix to recruit the
24 transcription factors beta-catenin and SATB1 to specific enhancer loci. The
25 interaction of Pit1 with the nuclear matrix and Matrin3, requires beta-catenin and
26 SATB1, and in absence of these factors Pit1 is unable to activate transcription. A
27 disease-causing dominant negative mutation of Pit1 (R271W) disrupts the interaction
28 with beta-catenin and SATB1, and consequent loss of association of enhancer
29 occupied Pit-1 with the nuclear matrix. This interaction loss can be rescued by fusing
30 Pit1 R271W to the nuclear matrix targeting region from hnRNPU/SAF-A, with the
31 remarkable rescue of enhancer activation and recruitment of transcription co-
32 activators such as p300, and consequent transcription activation³⁹.
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49 Transcription is thought to occur in a limited number of “transcription factories”
50 at fixed locations in the nucleus⁴⁰, which implies anchoring to a structural component
51 that limits diffusion. Besides Matrin3, other components of the nuclear matrix impact
52 transcription, positively and negatively. While Lamin B is associated with insulator
53 sequences⁴¹, matrix-associated RNA-binding proteins seem to aid in
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maintaining a transcriptional active environment. The nuclear matrix component hnRNPU is associated with sites of RNA pol II transcription⁴², and expression of a dominant negative mutant of hnRNPU destabilises the matrix in a similar manner as does RNase treatment⁴³. Matrin3 has been found at RNA pol II initiation sites together with hnRNPU, highlighting the involvement of Matrin3 in a network of transcriptional activators⁴⁴.

Matrin3 function in splicing regulation

Generation of alternative spliced mRNAs allows cells to expand their proteome⁴⁵. Transcriptome-wide analysis of changes in alternative splicing upon Matrin3 depletion in HeLa cells revealed that Matrin3 regulates many alternative exons, mostly as a direct repressor but in some cases also as an activator¹⁹. While Matrin3 required its RRM for splicing activation, no evidence of direct binding was found around the enhanced exons, suggesting that this function might be indirect. Matrin3 was identified as one of the main binding partners of the PTBP1 (Table 1), interacting with the second RRM domain of PTBP1¹⁹. This interaction is mediated through a specific small linear motif GILGPPP found between Matrin3's first ZF and the RRM. This sequence conforms to a consensus PRI (PTBP1 RRM Interaction) motif, originally identified in the PTBP1 co-regulator Raver1 and shown to dock onto the dorsal surface of PTBP1 RRM2^{46, 47}, a domain important for PTBP1 repressor activity^{48, 49}. Despite this route of identification, comparison between splicing events regulated by Matrin3 or PTBP1⁵⁰ revealed that only a subset of events are co-regulated by both proteins. This indicates that Matrin3 regulates most of its targets independently of PTBP1. Nevertheless, the activity of Matrin3 as a splicing regulator requires its PRI motif, for both PTBP1-dependent and -independent events¹⁹ (and unpublished observations). This raises the hypothesis that Matrin3 might not only interact with PTBP1, but also with other co-regulatory proteins through its PRI motif, depending on the regulated splicing event. In fact, Matrin3 can interact with multiple RNA binding proteins (see Table 1) although it is not yet known which are involved in splicing co-regulation.

The mechanism of splicing repression was examined by mapping Matrin3 binding sites with nucleotide-resolution crosslinking immunoprecipitation (iCLIP).

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3 Interestingly, Matrin3 interacts with broad sections of the pre-mRNA within the
4 repressed exons and the flanking introns. In contrast to the splicing maps for many
5 other splicing regulators²⁵, Matrin3 binding was relatively uniform over a region
6 encompassing 500 nucleotides flanking the repressed exons, with no specific peaks
7 of binding. Matrin3 RNA-compete motifs were also enriched in the flanking introns,
8 but not within the repressed exons¹⁹. This suggests a “bind and spread” model in
9 which Matrin3 is recruited initially to the intronic high affinity binding sites, with
10 subsequent recruitment of other Matrin3 molecules that interact and spread across
11 the exon and flanking introns (Fig. 2). In support of this hypothesis, Matrin3 was
12 found to interact with itself in a yeast two hybrid screen²⁷. Another striking feature is
13 that the introns flanking the Matrin3 repressed exons are significantly longer than
14 those flanking other cassette exons, including those regulated by PTBP1. Longer
15 introns may be required to prevent the “bind and spread” mode of action from
16 affecting the flanking constitutive splice sites.
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19 Notably, both of the Matrin3-related proteins RBM20 and ZNF638 have also
20 been reported to regulate alternative splicing^{32, 35, 36}, suggesting that it is a core
21 function of this group of proteins. RBM20 has similar RNA binding preference to
22 PTBP1 and Matrin3, consistent with the similarity between their RRM s (Fig 1).
23 Strikingly, RBM20 appears to act primarily as a splicing repressor and like Matrin3
24 binds within both introns flanking repressed exons³². Despite these similarities, there
25 are interesting contrasts. While Matrin3 interacts with a known splicing repressor
26 PTBP1 via its PRI, RBM20 interacts with a number of core splicing factors,
27 consistent with having an RS domain, as well as with hnRNPs³².
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44 **Matrin3 function in RNA stability**

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46 Steady-state RNA levels are determined by the rates of RNA synthesis and
47 degradation. In addition to its roles in transcription, Matrin3 has also been implicated
48 the stability of a number of mRNAs²⁴. Since Matrin3 interacts with several
49 transcription associated factors, such as DHX9 and hnRNPK, and the noncoding
50 small RNA 7SK, the authors examined alterations in gene expression upon Matrin3
51 depletion in U2OS cells. Matrin3 depletion decreases the mRNA levels of 77 genes,
52 and Matrin3 binding to these mRNAs was indicated by RNA immunoprecipitation.
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3 This interaction is dependent on the second RRM, which interestingly is also the one
4 involved in the RNA-dependent interaction with DHX9 and hnRNPk. The reduced
5 levels of these mRNAs were shown to be due to reduced RNA stability rather than
6 transcriptional down-regulation, indicated by reduced mRNA half-life of three tested
7 mRNAs²⁴. The exact molecular mechanism of Matrin3-dependent mRNA
8 stabilization is not entirely understood. However, a subset of stabilized mRNAs might
9 be explained by nonsense mediated decay (NMD) linked to alternative splicing (AS-
10 NMD)¹⁹. These are splicing events in which one of the splicing patterns introduces a
11 premature termination codon (PTC) leading to NMD⁵¹. Analysis of mRNA level and
12 splicing changes upon Matrin3 knockdown showed that 15 out of 22 genes with
13 changes in expression greater than 1.5 fold had an associated AS-NMD event that
14 might explain the changes in mRNA level¹⁹.
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26 **Matrin3 function in viral biology**

27 Viruses exploit their host systems to serve their own interests in several steps of
28 their life cycle. Generation of infectious HIV-1 viral progeny requires the cytoplasmic
29 export of several spliced and unspliced subgenomic HIV-1 mRNAs. The Rev protein
30 is crucial in HIV-1 biogenesis and is produced by the fully spliced RNA, and in turn
31 Rev facilitates the export of incompletely spliced RNAs that will express the other
32 essential HIV-1 components, and ultimately genomic RNA for assembly of new HIV-
33 1 particles. Rev binds to the Rev Response Elements (RRE) present in the RNA
34 and together with host components it can escape the nucleus and lead the RNAs to
35 the ribosome for expression of viral proteins (Reviewed in⁵²). Two parallel studies
36 using different approaches identified Matrin3 as a regulator of HIV-1 expression: one
37 by RNA-affinity isolation of proteins interacting with the HIV-1 RNA and the other by
38 yeast two hybrid screen using PTBP1 as a bait, as this splicing factor has been
39 shown to affect HIV-1 gene expression^{53, 54}. Manipulation of Matrin3 levels greatly
40 affected the activity of Rev, indicative of a role of Matrin3 as a Rev co-regulator. Rev,
41 Matrin3 and RRE-containing RNAs together form a complex suggesting that RNA
42 bridges the Rev-Matrin3 interaction, and supporting this is the requirement for
43 Matrin3's RRMs for this activity. Here, as in some of the other Matrin3 associated
44 functions, PSF also plays a role⁵⁵. However their RNA association is only partially
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3 overlapping. PSF is recruited to the RRE-containing RNA along with Rev co-
4 transcriptionally and is thought to be released before RNA accumulation at the
5 nuclear matrix or its export, while MatrIn3 is recruited post-transcriptionally and
6 remains with the RNA in the nuclear matrix until its export. Viral use of MatrIn3 is
7 possibly not restricted to HIV-1, and accordingly a kinase encoded by
8 alphaherpesvirus ORF66 targets predominantly MatrIn3, modifying threonine 150,
9 which is required to avoid cytoplasmic aggregates of MatrIn3 in later stages of
10 infection similar to what has been observed with the lamins^{56, 57}.

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17 Along with PSF and NONO, MatrIn3 was also identified as a component of a
18 complex involved in nuclear retention of RNA with long double-stranded regions,
19 possibly associated with adenosine to inosine editing⁵⁸. This could be of interest as a
20 possible cellular antiviral mechanism. However, subsequent analyses suggest that
21 this resulted from nuclear paraspeckle localization mediated by PSF and NONO,
22 with no evidence for involvement of MatrIn3⁵⁹.

31 **MatrIn3 function in cell survival and DNA damage response**

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33 The importance of the multiple functions of MatrIn3 is evident from the
34 dramatic effect of its depletion on cell proliferation and on the ability of cells to
35 respond to DNA damaging compounds^{17, 60, 61}. MatrIn3 is involved in the response to
36 several signals, such as the activation of NMDA receptors⁶² or the induction of DNA
37 double strand breaks⁶⁰. Both lead to post-translation modification of MatrIn3 by
38 phosphorylation at serine residues, with the former mediated by protein kinase
39 A (PKA) and the latter by the kinase ataxia-telangiectasia mutated (ATM). PKA
40 targets mainly a serine at position 188⁵⁷ while ATM targets serine 208⁶⁰. The
41 consequences of these modifications are quite distinct. MatrIn3 is the main PKA
42 target upon NMDA receptor activation in rat brain, which culminates in MatrIn3
43 degradation and neuronal cell death, effects that can be blocked by PKA inhibitors⁶².
44 Several caspases, typically activated/released during apoptosis, can cleave MatrIn3,
45 although it is not clear if this is the mechanism occurring during PKA-mediated
46 degradation⁶³. Down-regulation of MatrIn3 by siRNA transfection in endothelial cells
47 culminates in a decrease of cell proliferation followed by cell necrosis⁶¹. This

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3 suggests that although Matrin3 down-regulation is involved in the regulated apoptotic
4 death response to NMDA signalling, it is not sufficient and that in the absence of
5 signalling Matrin3 depletion alone leads to necrotic cell death. The exact mechanism
6 by which Matrin3 ensures proper cell proliferation is not clear, although it most likely
7 will be related to its nuclear functions as it requires its nuclear localisation¹⁷.
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11 Persistent DNA damage can lead to cell death, and Matrin3 can function in
12 several steps ensuring that only undamaged cells escape cell death, although no
13 direct links between its role in DNA damage response and cell death have yet been
14 reported. DNA double strand breaks (DSBs) trigger ATM mediated phosphorylation of
15 Matrin3 at serine 208, and this seems to be required for the DNA damage
16 response⁶⁰. This response involves activation of a cell cycle checkpoint that stalls
17 cells to ensure sufficient time for DNA repair. Matrin3 can form a complex with
18 proteins involved in DNA damage response, PSF and NONO, as well as the DNA
19 binding proteins Ku80/Ku70, independent of any DNA damage. PSF and NONO are
20 recruited to sites of DNA damage, and while Matrin3 itself is neither recruited nor
21 affects the recruitment of other components, it does significantly alter the retention
22 time of these proteins at the damage sites. This suggests that Matrin3 is involved in
23 the early steps of the DNA damage response and is required for the dynamic
24 recruitment and release of PSF/NONO from the damage site, although it is itself not
25 recruited to the site⁶⁰.
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39 **Associations of Matrin3 with human diseases**

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41 In addition to its effect on viruses such as HIV1, inherited mutations of Matrin3
42 have also been shown to be associated with the neurodegenerative disease
43 amyotrophic lateral sclerosis (ALS). ALS is a neurodegenerative disease affecting
44 lower and upper motor neurons including the spinal cord, typically with adult onset. A
45 Ser85Cys mutation in Matrin3 was found in 1998 as the autosomal dominant
46 mutation in a family with multiple members diagnosed with myopathy⁶⁴.
47 Subsequently, a Phe115Cys mutation in Matrin3 was found to segregate with
48 disease in a Sardinian family suffering from slow progressing ALS, and re-evaluation
49 of the original family established that the Ser85Cys mutations also causes ALS⁶⁵.
50 Matrin3 mutations are rare in ALS, since in 96 patients with sporadic disease only
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3 one missense mutation was found in Matrin3. Patients with Matrin3 Ser85Cys or
4 Phe115Cys mutation suffer from brisk knee reflexes and a 'split-hand' pattern of
5 weakness^{65,66}, symptoms also observed in other ALS patients.
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8 At the molecular level, Phe115Cys mutation leads to intra-nuclear inclusion,
9 and a pronounced increase in Matrin3 protein abundance in the spinal cord neurons.
10 Aggregation of Matrin3 is also seen in ALS patients with the more common C9orf72
11 tri-nucleotide expansion, suggesting that aggregate formation of Matrin3 (and other
12 RBPs) is widespread in ALS patients⁶⁵. This phenotype is highly similar to other
13 RBPs in which mutations lead to aggregation and manifestation of ALS or related
14 motor neuron diseases, including TDP43, FUS, hnRNPA1 and hnRNPA2⁶⁷. The
15 basis for pathology could be the loss of function of the protein(s), which may lead to
16 deregulated alternative splicing. Alternatively, the aggregates may actively disrupt
17 cellular functions via a toxic gain of function. The two mechanisms are not mutually
18 exclusive and evidence has been presented for both in the cases of other RNA
19 binding proteins such as TDP43 and FUS⁶⁸.
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28 As noted above, expression of the Matrin3-related protein RBM20 is restricted
29 to the heart and is especially high in early development. Consistent with this,
30 mutations of RBM20 were identified in 8 independent families associated with dilated
31 cardiomyopathy^{69, 70}. The missense mutations were all located in a hotspot
32 corresponding to the arginine-serine domain of RBM20. Moreover, a large deletion of
33 the RBM20 gene was found to be responsible for altered splicing of titin and other
34 mRNAs in a rat model that also showed symptoms of dilated cardiomyopathy³⁵. This
35 suggests that the cardiomyopathy results from missplicing associated with loss of
36 RBM20 function. It remains to be determined whether the mechanisms of ALS
37 pathology arising from Matrin3 mutations are similar to the cardiomyopathy arising
38 from RBM20 mutations.
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49 **Conclusions and perspectives**

50 Evidence of the multiple functions of Matrin3 has accumulated over the last 25 years
51 since it was discovered as a component of nuclear matrix. The full extent of
52 functional coupling between the different stages of gene expression has also come
53 into focus during this period, and like many other proteins, Matrin3 has clear roles at
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3 numerous levels of gene expression. While it seems obvious that MatrIn3 function is
4 linked to the nuclear matrix, the extent to which its involvement in processes from
5 transcriptional activation and pre-mRNA splicing to export of viral RNAs, requires
6 anchoring of RNPs to the nuclear matrix remains unresolved. A precise mutation to
7 specifically disrupt matrix localization would be an invaluable tool in this regard.
8 MatrIn3 function might in part be attributed to limiting diffusion of several co-factors,
9 as suggested by the change in retention time of PSF and NONO upon DNA
10 damage⁶⁰. Much remains to be learned about the molecular interactions and cellular
11 roles of MatrIn3 and its related proteins, RBM20 and ZNF638, as well as the
12 molecular basis of pathologies arising from their malfunction. The pace of
13 discoveries about MatrIn3 has accelerated over the past few years, with new insights
14 emerging from several directions, including investigations of viral mechanisms and of
15 diseases of motor neurons and the heart. We anticipate that MatrIn3 will become a
16 prime exemplar of the multiple ways in which RNP functions are inter-linked with
17 genome organisation and the nuclear matrix.
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31 **Acknowledgments**

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33 We thank Clare Gooding and Dipen Rajgor for critical comments on the manuscript.
34 Work in the CWJS lab on MatrIn3 is funded by a grant from the Wellcome Trust
35 (092900). JA was funded by a Boehringer Ingelheim Fonds studentship.
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Figure Legends

Figure 1. Domain organization of Matrin3 and related proteins

A) Schematic diagram of structural organization of Matrin3 (middle), ZNF638 (top) and RBM20 (bottom). The percent sequence identity between the RRM and ZF domains of Matrin3 compared to ZNF638 and RBM20 is indicated. Disease – associated aminoacid residues S85, F115, P154 and T622, and the phosphorylation sites at S188 and S208, are indicated.

B) Relative expression of Matrin3, RBM20 and ZNF638 across human tissues. Darker red correlates with high expression levels and light red with low expression. Data taken from The Human Protein Atlas⁷¹.

Figure 2. Proposed model for splicing regulation by Matrin3

Cartoon depicting an exon not regulated by Matrin3 (A) and one regulated by Matrin3 (B). The presence of Matrin3 binding site (B – boxed sequence) on the introns flanking the exon recruits the Matrin3 molecules, which can be bound or not to other splicing regulators like PTBP1.

Table 1 – MatrIn3 interacting proteins

	Protein Name	Detection method	Detected in Nuclear Matrix	Uniprot Accession #	Reference
	CDC5L	IP		Q99459	72
	CLK1	Y2H		P49759	27
	DDX17	IP		Q92841	24
	DDX39B	Y2H		Q13838	27
	DDX5	IP ^R , Y2H & GPD	✓ ⁷³	P17844	24, 27, 74
	HNRNPA1	IP ^R	✓ ⁷⁵	P09651	76
	HNRNPA2/B1	IP ^R	✓ ⁷⁵	P22626	76
	HNRNPC1/C2	IP ^R	✓ ⁷⁵	P07910	76
RNA processing	HNRNPL	IP & Y2H	✓ ^{75, 11}	P14866	24, 27
	HNRNPK	IP	✓ ^{75, 77}	P61978	24
	HNRNPM	IP ^R	✓ ^{11, 75}	P52272	76
	HNRNPU	IP ^R	✓ ^{11, 75}	Q00839	76
	ILF2	IP		Q12905	24
	NONO	IP ^R	✓ ⁷⁸	Q15233	60
	NOVA1	Y2H		P51513	79
	NOVA2	Y2H		Q9UNW9	79
	PABPC1	IP	✓ ⁷⁵	P11940	24

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3		PCBP2	Y2H	Q15366	27	
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5						
6						
7		PTBP1	IP ^R & GPD	✓ ^{75 78}	P26599	19, 24
8						
9						
10		PTBP2	Y2H	Q9UKA9	80	
11						
12		PTBP3	IP ^R	O95758	81	
13						
14		RBMX	Y2H	P38159	27	
15						
16						
17						
18		PSF	IP ^R	✓ ⁷⁸	P23246	60 55
19						
20		SFRS7	Y2H	✓ ¹¹	Q16629	27
21						
22		SRPK2	Y2H		P78362	27
23						
24		TARDBP/TDP4	IP	Q13148	Freibaum et al,	
25		3			2010	
26						
27						
28		DDX3X	IP ^R	✓ ¹¹	O00571	76
29						
30		DHX9	IP	✓ ¹¹	Q08211	24
31						
32		ERG	IP & GPD		P11308	82
33						
34		FOXG1B	Y2H		P55316	27
35						
36						
37		NFX1	IP	✓ ¹¹	Q12986	83
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39						
40		NR2F1	Y2H		P10589	27
41						
42	Transcription	RGS6	Y2H		P49758	27
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44						
45		POU1F1/Pit1	IP	✓ ³⁹	P28069	39
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47						
48		POLR2A	IP	✓ ⁶	P24928	84
49						
50		RPAP1	Y2H		Q9BWH6	27
51						
52		SAFB	Y2H	✓ ⁸⁵	Q15424	27
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55		SLTM	Y2H	✓ ¹¹	Q9NWH9	27
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	YBX1	Y2H		P67809	27
	ZHX1	Y2H		Q9UKY1	27
Chromatin / Chromatin Remodeling	BAZ1A	Y2H		Q9NRL2	27
	CBX4	Y2H		O00257	27
	CHD3	Y2H		Q12873	27
	CTCF	GPD		P49711	74
	H2AFX	IP		P16104	86
	SIRT6	IP		Q8N6T7	87
	SMARCA4	Y2H	✓ ⁸⁸	P51532	27
	SMARCA4	IP ^R		Q9H4L7	89
Translation	RPL5	Y2H		P46777	27
	RPL18A	Y2H	✓ ¹¹	Q02543	27
	RPL10	Y2H		P27635	27
	RPS15A	Y2H		P62244	27
DNA replication / Repair	GADD45GIP1	Y2H		Q8TAE8	27
	XRCC6	IP	✓ ⁹⁰	P12956	60
	XRCC5	IP	✓ ⁹⁰	P13010	60
	MCM7	IP & Y2H		P33993	27, 91
	TDG	Y2H		Q13569	27
Apoptosis	DAXX	Y2H		Q9UER7	27
	HIPK1	Y2H		Q86Z02	27
	BRE	Y2H		Q9NXR7	27
Signal Transduction	PLCG1	Y2H		P19174	27

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	CIB1	Y2H		Q99828	27
Nuclear Organization	LMNA	IP ^R & GPD	✓ ^{1, 11}	P02545	26

Y2H – Yeast two hybrid; IP – Immunoprecipitation; GPD – GST pulldown, ^R – Ribonuclease resistant interaction

For Peer Review

References

1. Nakayasu H, Berezney R. Nuclear matrins: identification of the major nuclear matrix proteins. *Proc Natl Acad Sci U S A* 1991, 88:10312-10316.
2. Belgrader P, Dey R, Berezney R. Molecular cloning of matrin 3. A 125-kilodalton protein of the nuclear matrix contains an extensive acidic domain. *J Biol Chem* 1991, 266:9893-9899.
3. Bode J, Goetze S, Heng H, Krawetz SA, Benham C. From DNA structure to gene expression: mediators of nuclear compartmentalization and dynamics. *Chromosome Res* 2003, 11:435-445.
4. Martins RP, Ostermeier GC, Krawetz SA. Nuclear matrix interactions at the human protamine domain: a working model of potentiation. *J Biol Chem* 2004, 279:51862-51868.
5. Linnemann AK, Platts AE, Krawetz SA. Differential nuclear scaffold/matrix attachment marks expressed genes. *Hum Mol Genet* 2009, 18:645-654.
6. Mortillaro MJ, Blencowe BJ, Wei X, Nakayasu H, Du L, Warren SL, Sharp PA, Berezney R. A hyperphosphorylated form of the large subunit of RNA polymerase II is associated with splicing complexes and the nuclear matrix. *Proc Natl Acad Sci U S A* 1996, 93:8253-8257.
7. Yusufzai TM, Felsenfeld G. The 5'-HS4 chicken beta-globin insulator is a CTCF-dependent nuclear matrix-associated element. *Proc Natl Acad Sci U S A* 2004, 101:8620-8624.
8. Mesner LD, Hamlin JL, Dijkwel PA. The matrix attachment region in the Chinese hamster dihydrofolate reductase origin of replication may be required for local chromatid separation. *Proc Natl Acad Sci U S A* 2003, 100:3281-3286.
9. Nickerson J. Experimental observations of a nuclear matrix. *J Cell Sci* 2001, 114:463-474.
10. Ma H, Siegel AJ, Berezney R. Association of chromosome territories with the nuclear matrix. Disruption of human chromosome territories correlates with the release of a subset of nuclear matrix proteins. *J Cell Biol* 1999, 146:531-542.
11. Engelke R, Riede J, Hegermann J, Wuerch A, Eimer S, Dengjel J, Mittler G. The quantitative nuclear matrix proteome as a biochemical snapshot of nuclear organization. *J Proteome Res* 2014, 13:3940-3956.
12. Pederson T. Half a century of "the nuclear matrix". *Mol Biol Cell* 2000, 11:799-805.
13. Razin SV, Iarovaia OV, Vassetzky YS. A requiem to the nuclear matrix: from a controversial concept to 3D organization of the nucleus. *Chromosoma* 2014, 123:217-224.
14. Hibino Y, Nakamura K, Tsukada S, Sugano N. Purification and characterization of nuclear scaffold proteins which bind to a highly repetitive bent DNA from rat liver. *Biochim Biophys Acta* 1993, 1174:162-170.
15. Hibino Y, Tsukada S, Sugano N. Properties of a DNA-binding protein from rat nuclear scaffold fraction. *Biochem Biophys Res Commun* 1993, 197:336-342.
16. Hibino Y, Usui T, Morita Y, Hirose N, Okazaki M, Sugano N, Hiraga K. Molecular properties and intracellular localization of rat liver nuclear scaffold protein P130. *Biochim Biophys Acta* 2006, 1759:195-207.
17. Hisada-Ishii S, Ebihara M, Kobayashi N, Kitagawa Y. Bipartite nuclear localization signal of matrin 3 is essential for vertebrate cells. *Biochem Biophys Res Commun* 2007, 354:72-76.
18. Dinkel H, Van Roey K, Michael S, Davey NE, Weatheritt RJ, Born D, Speck T, Kruger D, Grebnev G, Kuban M, et al. The eukaryotic linear motif resource ELM: 10 years and counting. *Nucleic Acids Res* 2014, 42:D259-266.

19. Coelho MB, Attig J, Bellora N, Konig J, Hallegger M, Kayikci M, Eyraş E, Ule J, Smith CW. Nuclear matrix protein Matrin3 regulates alternative splicing and forms overlapping regulatory networks with PTB. *EMBO J* 2015, 34:653-668.
20. Hakes DJ, Berezney R. DNA binding properties of the nuclear matrix and individual nuclear matrix proteins. Evidence for salt-resistant DNA binding sites. *J Biol Chem* 1991, 266:11131-11140.
21. Muto Y, Pomeranz Krummel D, Oubridge C, Hernandez H, Robinson CV, Neuhaus D, Nagai K. The structure and biochemical properties of the human spliceosomal protein U1C. *J Mol Biol* 2004, 341:185-198.
22. Ray D, Kazan H, Cook KB, Weirauch MT, Najafabadi HS, Li X, Gueroussov S, Albu M, Zheng H, Yang A, et al. A compendium of RNA-binding motifs for decoding gene regulation. *Nature* 2013, 499:172-177.
23. Clery A, Blatter M, Allain FH. RNA recognition motifs: boring? Not quite. *Curr Opin Struct Biol* 2008, 18:290-298.
24. Salton M, Elkon R, Borodina T, Davydov A, Yaspo ML, Halperin E, Shiloh Y. Matrin 3 binds and stabilizes mRNA. *PLoS One* 2011, 6:e23882.
25. Witten JT, Ule J. Understanding splicing regulation through RNA splicing maps. *Trends Genet* 2011, 27:89-97.
26. Depreux FF, Puckelwartz MJ, Augustynowicz A, Wolfgeher D, Labno CM, Pierre-Louis D, Cicka D, Kron SJ, Holaska J, McNally EM. Disruption of the lamin A and matrin-3 interaction by myopathic LMNA mutations. *Hum Mol Genet* 2015, 24:4284-4295.
27. Zeitz MJ, Malyavantham KS, Seifert B, Berezney R. Matrin 3: chromosomal distribution and protein interactions. *J Cell Biochem* 2009, 108:125-133.
28. Zeng C, van Wijnen AJ, Stein JL, Meyers S, Sun W, Shopland L, Lawrence JB, Penman S, Lian JB, Stein GS, et al. Identification of a nuclear matrix targeting signal in the leukemia and bone-related AML/CBF-alpha transcription factors. *Proc Natl Acad Sci U S A* 1997, 94:6746-6751.
29. Seo J, Lozano MM, Dudley JP. Nuclear matrix binding regulates SATB1-mediated transcriptional repression. *J Biol Chem* 2005, 280:24600-24609.
30. Yarosh CA, Iacona JR, Lutz CS, Lynch KW. PSF: nuclear busy-body or nuclear facilitator? *Wiley Interdiscip Rev RNA* 2015, 6:351-367.
31. Inagaki H, Matsushima Y, Nakamura K, Ohshima M, Kadowaki T, Kitagawa Y. A large DNA-binding nuclear protein with RNA recognition motif and serine/arginine-rich domain. *J Biol Chem* 1996, 271:12525-12531.
32. Maatz H, Jens M, Liss M, Schafer S, Heinig M, Kirchner M, Adami E, Rintisch C, Dauksaite V, Radke MH, et al. RNA-binding protein RBM20 represses splicing to orchestrate cardiac pre-mRNA processing. *J Clin Invest* 2014, 124:3419-3430.
33. Perez I, Lin CH, McAfee JG, Patton JG. Mutation of PTB binding sites causes misregulation of alternative 3' splice site selection in vivo. *RNA* 1997, 3:764-778.
34. Oberstrass FC, Auweter SD, Erat M, Hargous Y, Henning A, Wenter P, Reymond L, Amir-Ahmady B, Pitsch S, Black DL, et al. Structure of PTB bound to RNA: specific binding and implications for splicing regulation. *Science* 2005, 309:2054-2057.
35. Guo W, Schafer S, Greaser ML, Radke MH, Liss M, Govindarajan T, Maatz H, Schulz H, Li S, Parrish AM, et al. RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing. *Nat Med* 2012, 18:766-773.
36. Du C, Ma X, Meruvu S, Hugendubler L, Mueller E. The adipogenic transcriptional cofactor ZNF638 interacts with splicing regulators and influences alternative splicing. *J Lipid Res* 2014, 55:1886-1896.
37. Hibino Y, Ohzeki H, Sugano N, Hiraga K. Transcription modulation by a rat nuclear scaffold protein, P130, and a rat highly repetitive DNA component or various types of animal and

- 1
2
3 plant matrix or scaffold attachment regions. *Biochem Biophys Res Commun* 2000, 279:282-
4 287.
- 5 38. Hibino Y, Ohzeki H, Hirose N, Morita Y, Sugano N. Involvement of DNA methylation in
6 binding of a highly repetitive DNA component to nuclear scaffold proteins from rat liver.
7 *Biochem Biophys Res Commun* 1998, 252:296-301.
- 8 39. Skowronska-Krawczyk D, Ma Q, Schwartz M, Scully K, Li W, Liu Z, Taylor H, Tollkuhn J, Ohgi
9 KA, Notani D, et al. Required enhancer-matrin-3 network interactions for a homeodomain
10 transcription program. *Nature* 2014, 514:257-261.
- 11 40. Cook PR. The organization of replication and transcription. *Science* 1999, 284:1790-1795.
- 12 41. Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, Eussen BH, de Klein A,
13 Wessels L, de Laat W, et al. Domain organization of human chromosomes revealed by
14 mapping of nuclear lamina interactions. *Nature* 2008, 453:948-951.
- 15 42. Martens JH, Verlaan M, Kalkhoven E, Dorsman JC, Zantema A. Scaffold/matrix attachment
16 region elements interact with a p300-scaffold attachment factor A complex and are bound
17 by acetylated nucleosomes. *Mol Cell Biol* 2002, 22:2598-2606.
- 18 43. Hall LL, Carone DM, Gomez AV, Kolpa HJ, Byron M, Mehta N, Fackelmayer FO, Lawrence JB.
19 Stable COT-1 repeat RNA is abundant and is associated with euchromatic interphase
20 chromosomes. *Cell* 2014, 156:907-919.
- 21 44. Malyavantham KS, Bhattacharya S, Barbeitos M, Mukherjee L, Xu J, Fackelmayer FO,
22 Berezney R. Identifying functional neighborhoods within the cell nucleus: proximity analysis
23 of early S-phase replicating chromatin domains to sites of transcription, RNA polymerase II,
24 HP1gamma, matrin 3 and SAF-A. *J Cell Biochem* 2008, 105:391-403.
- 25 45. Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing.
26 *Nature* 2010, 463:457-463.
- 27 46. Rideau AP, Gooding C, Simpson PJ, Monie TP, Lorenz M, Huttelmaier S, Singer RH, Matthews
28 S, Curry S, Smith CW. A peptide motif in Raver1 mediates splicing repression by interaction
29 with the PTB RRM2 domain. *Nat Struct Mol Biol* 2006, 13:839-848.
- 30 47. Joshi A, Coelho MB, Kotik-Kogan O, Simpson PJ, Matthews SJ, Smith CW, Curry S.
31 Crystallographic analysis of polypyrimidine tract-binding protein-Raver1 interactions
32 involved in regulation of alternative splicing. *Structure* 2011, 19:1816-1825.
- 33 48. Gueroussov S, Gonatopoulos-Pournatzis T, Irimia M, Raj B, Lin ZY, Gingras AC, Blencowe BJ.
34 RNA SPLICING. An alternative splicing event amplifies evolutionary differences between
35 vertebrates. *Science* 2015, 349:868-873.
- 36 49. Robinson F, Smith CW. A splicing repressor domain in polypyrimidine tract-binding protein. *J*
37 *Biol Chem* 2006, 281:800-806.
- 38 50. Llorian M, Schwartz S, Clark TA, Hollander D, Tan LY, Spellman R, Gordon A, Schweitzer AC,
39 de la Grange P, Ast G, et al. Position-dependent alternative splicing activity revealed by
40 global profiling of alternative splicing events regulated by PTB. *Nat Struct Mol Biol* 2010,
41 17:1114-1123.
- 42 51. McGlincy NJ, Smith CW. Alternative splicing resulting in nonsense-mediated mRNA decay:
43 what is the meaning of nonsense? *Trends Biochem Sci* 2008, 33:385-393.
- 44 52. Cullen BR. Viral RNAs: lessons from the enemy. *Cell* 2009, 136:592-597.
- 45 53. Kula A, Guerra J, Knezevich A, Kleva D, Myers MP, Marcello A. Characterization of the HIV-1
46 RNA associated proteome identifies Matrin 3 as a nuclear cofactor of Rev function.
47 *Retrovirology* 2011, 8:60.
- 48 54. Yedavalli VS, Jeang KT. Matrin 3 is a co-factor for HIV-1 Rev in regulating post-transcriptional
49 viral gene expression. *Retrovirology* 2011, 8:61.
- 50 55. Kula A, Gharu L, Marcello A. HIV-1 pre-mRNA commitment to Rev mediated export through
51 PSF and Matrin 3. *Virology* 2013, 435:329-340.
- 52
53
54
55
56
57
58
59
60

- 1
2
3 56. Mou F, Forest T, Baines JD. US3 of herpes simplex virus type 1 encodes a promiscuous
4 protein kinase that phosphorylates and alters localization of lamin A/C in infected cells. *J*
5 *Virology* 2007, 81:6459-6470.
- 6 57. Erazo A, Yee MB, Banfield BW, Kinchington PR. The alphaherpesvirus US3/ORF66 protein
7 kinases direct phosphorylation of the nuclear matrix protein matrin 3. *J Virol* 2011, 85:568-
8 581.
- 9 58. Zhang Z, Carmichael GG. The fate of dsRNA in the nucleus: a p54(nrb)-containing complex
10 mediates the nuclear retention of promiscuously A-to-I edited RNAs. *Cell* 2001, 106:465-475.
- 11 59. Chen LL, Carmichael GG. Altered nuclear retention of mRNAs containing inverted repeats in
12 human embryonic stem cells: functional role of a nuclear noncoding RNA. *Mol Cell* 2009,
13 35:467-478.
- 14 60. Salton M, Lerenthal Y, Wang SY, Chen DJ, Shiloh Y. Involvement of Matrin 3 and SFPQ/NONO
15 in the DNA damage response. *Cell Cycle* 2010, 9:1568-1576.
- 16 61. Przygodzka P, Boncela J, Cierniewski CS. Matrin 3 as a key regulator of endothelial cell
17 survival. *Exp Cell Res* 2011, 317:802-811.
- 18 62. Giordano G, Sanchez-Perez AM, Montoliu C, Berezney R, Malyavantham K, Costa LG, Calvete
19 JJ, Felipe V. Activation of NMDA receptors induces protein kinase A-mediated
20 phosphorylation and degradation of matrin 3. Blocking these effects prevents NMDA-
21 induced neuronal death. *J Neurochem* 2005, 94:808-818.
- 22 63. Valencia CA, Ju W, Liu R. Matrin 3 is a Ca²⁺/calmodulin-binding protein cleaved by caspases.
23 *Biochem Biophys Res Commun* 2007, 361:281-286.
- 24 64. Feit H, Silbergleit A, Schneider LB, Gutierrez JA, Fitoussi RP, Reyes C, Rouleau GA, Brais B,
25 Jackson CE, Beckmann JS, et al. Vocal cord and pharyngeal weakness with autosomal
26 dominant distal myopathy: clinical description and gene localization to 5q31. *Am J Hum*
27 *Genet* 1998, 63:1732-1742.
- 28 65. Johnson JO, Pioro EP, Boehringer A, Chia R, Feit H, Renton AE, Pliner HA, Abramzon Y,
29 Marangi G, Winborn BJ, et al. Mutations in the Matrin 3 gene cause familial amyotrophic
30 lateral sclerosis. *Nat Neurosci* 2014, 17:664-666.
- 31 66. Yamashita S, Ando Y. Genotype-phenotype relationship in hereditary amyotrophic lateral
32 sclerosis. *Transl Neurodegener* 2015, 4:13.
- 33 67. Kim HJ, Kim NC, Wang YD, Scarborough EA, Moore J, Diaz Z, MacLea KS, Freibaum B, Li S,
34 Mollieux A, et al. Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause
35 multisystem proteinopathy and ALS. *Nature* 2013, 495:467-473.
- 36 68. Mackenzie IR, Rademakers R, Neumann M. TDP-43 and FUS in amyotrophic lateral sclerosis
37 and frontotemporal dementia. *Lancet Neurol* 2010, 9:995-1007.
- 38 69. Brauch KM, Karst ML, Herron KJ, de Andrade M, Pellikka PA, Rodeheffer RJ, Michels VV,
39 Olson TM. Mutations in ribonucleic acid binding protein gene cause familial dilated
40 cardiomyopathy. *J Am Coll Cardiol* 2009, 54:930-941.
- 41 70. Linke WA, Bucker S. King of hearts: a splicing factor rules cardiac proteins. *Nat Med* 2012,
42 18:660-661.
- 43 71. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A,
44 Kampf C, Sjostedt E, Asplund A, et al. Proteomics. Tissue-based map of the human
45 proteome. *Science* 2015, 347:1260419.
- 46 72. Lleres D, Denegri M, Biggiogera M, Ajuh P, Lamond AI. Direct interaction between hnRNP-M
47 and CDC5L/PLRG1 proteins affects alternative splice site choice. *EMBO Rep* 2010, 11:445-
48 451.
- 49 73. Akileswaran L, Taraska JW, Sayer JA, Gettemy JM, Coghlan VM. A-kinase-anchoring protein
50 AKAP95 is targeted to the nuclear matrix and associates with p68 RNA helicase. *J Biol Chem*
51 2001, 276:17448-17454.
- 52 74. Fujita T, Fujii H. Direct identification of insulator components by insertional chromatin
53 immunoprecipitation. *PLoS One* 2011, 6:e26109.
- 54
55
56
57
58
59
60

- 1
2
3 75. Mattern KA, Humbel BM, Muijsers AO, de Jong L, van Driel R. hnRNP proteins and B23 are
4 the major proteins of the internal nuclear matrix of HeLa S3 cells. *J Cell Biochem* 1996,
5 62:275-289.
- 6 76. Erazo A, Goff SP. Nuclear matrix protein Matrin 3 is a regulator of ZAP-mediated retroviral
7 restriction. *Retrovirology* 2015, 12:57.
- 8 77. Samuel SK, Spencer VA, Bajno L, Sun JM, Holth LT, Oesterreich S, Davie JR. In situ cross-
9 linking by cisplatin of nuclear matrix-bound transcription factors to nuclear DNA of human
10 breast cancer cells. *Cancer Res* 1998, 58:3004-3008.
- 11 78. Meissner M, Dechat T, Gerner C, Grimm R, Foisner R, Sauermaann G. Differential nuclear
12 localization and nuclear matrix association of the splicing factors PSF and PTB. *J Cell Biochem*
13 2000, 76:559-566.
- 14 79. Polydorides AD, Okano HJ, Yang YY, Stefani G, Darnell RB. A brain-enriched polypyrimidine
15 tract-binding protein antagonizes the ability of Nova to regulate neuron-specific alternative
16 splicing. *Proc Natl Acad Sci U S A* 2000, 97:6350-6355.
- 17 80. Hegele A, Kamburov A, Grossmann A, Sourlis C, Wowro S, Weimann M, Will CL, Pena V,
18 Luhrmann R, Stelzl U. Dynamic protein-protein interaction wiring of the human spliceosome.
19 *Mol Cell* 2012, 45:567-580.
- 20 81. Brazao TF, Demmers J, van IW, Strouboulis J, Fornerod M, Romao L, Grosveld FG. A new
21 function of ROD1 in nonsense-mediated mRNA decay. *FEBS Lett* 2012, 586:1101-1110.
- 22 82. Wang S, Kollipara RK, Srivastava N, Li R, Ravindranathan P, Hernandez E, Freeman E,
23 Humphries CG, Kapur P, Lotan Y, et al. Ablation of the oncogenic transcription factor ERG by
24 deubiquitinase inhibition in prostate cancer. *Proc Natl Acad Sci U S A* 2014, 111:4251-4256.
- 25 83. Katzenellenbogen RA, Egelkrout EM, Vliet-Gregg P, Gewin LC, Gafken PR, Galloway DA.
26 NFX1-123 and poly(A) binding proteins synergistically augment activation of telomerase in
27 human papillomavirus type 16 E6-expressing cells. *J Virol* 2007, 81:3786-3796.
- 28 84. Das R, Yu J, Zhang Z, Gygi MP, Krainer AR, Gygi SP, Reed R. SR proteins function in coupling
29 RNAP II transcription to pre-mRNA splicing. *Mol Cell* 2007, 26:867-881.
- 30 85. Oesterreich S, Lee AV, Sullivan TM, Samuel SK, Davie JR, Fuqua SA. Novel nuclear matrix
31 protein HET binds to and influences activity of the HSP27 promoter in human breast cancer
32 cells. *J Cell Biochem* 1997, 67:275-286.
- 33 86. Yang X, Zou P, Yao J, Yun D, Bao H, Du R, Long J, Chen X. Proteomic dissection of cell type-
34 specific H2AX-interacting protein complex associated with hepatocellular carcinoma. *J*
35 *Proteome Res* 2010, 9:1402-1415.
- 36 87. Simeoni F, Tasselli L, Tanaka S, Villanova L, Hayashi M, Kubota K, Isono F, Garcia BA,
37 Michishita-Kioi E, Chua KF. Proteomic analysis of the SIRT6 interactome: novel links to
38 genome maintenance and cellular stress signaling. *Sci Rep* 2013, 3:3085.
- 39 88. Reyes JC, Muchardt C, Yaniv M. Components of the human SWI/SNF complex are enriched in
40 active chromatin and are associated with the nuclear matrix. *J Cell Biol* 1997, 137:263-274.
- 41 89. Rowbotham SP, Barki L, Neves-Costa A, Santos F, Dean W, Hawkes N, Choudhary P, Will WR,
42 Webster J, Oxley D, et al. Maintenance of silent chromatin through replication requires
43 SWI/SNF-like chromatin remodeler SMARCD1. *Mol Cell* 2011, 42:285-296.
- 44 90. Yu E, Song K, Moon H, Maul GG, Lee I. Characteristic immunolocalization of Ku protein as
45 nuclear matrix. *Hybridoma* 1998, 17:413-420.
- 46 91. Huang TH, Huo L, Wang YN, Xia W, Wei Y, Chang SS, Chang WC, Fang YF, Chen CT, Lang JY, et
47 al. Epidermal growth factor receptor potentiates MCM7-mediated DNA replication through
48 tyrosine phosphorylation of Lyn kinase in human cancers. *Cancer Cell* 2013, 23:796-810.
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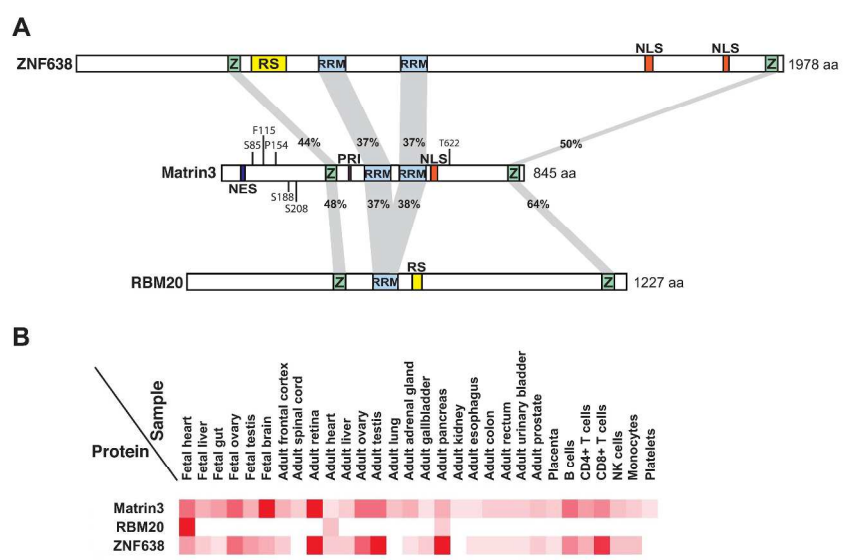


Figure 1 - Matrin3 and related protein structure and expression
297x420mm (300 x 300 DPI)

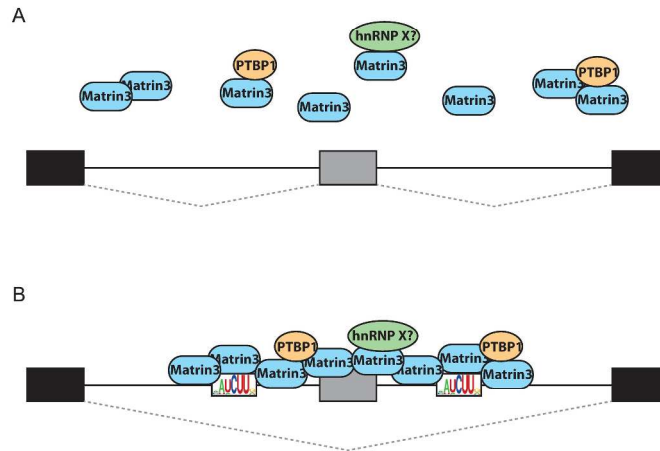


Figure 2 - Splicing Regulation by Matrin3
297x420mm (300 x 300 DPI)