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 processing[6], insulation of genomic domains[7] and facilitating DNA replication[8]. 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 microscopy[9]. 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

chromatin loops via interaction at S/MARs. Conversely, the matrix is sensitive to RNase digestion, indicating that ribonucleoprotein complexes are integral to the structure, and presumably functions, of the matrix[10]. One of the major challenges in understanding the nuclear matrix is defining the major protein constituents that underlie the observed filamentous structure; proteomic analyses indicate a highly complex composition[11], including many RNA binding proteins. The matrix-association of proteins known to be active in various nuclear processes argues for the functional importance of the matrix. Nevertheless it should be noted that the concept of a nuclear matrix is not universally accepted[12, 13].

Matrin3 was identified by analysis of the protein complement of the biochemically defined nuclear matrix fraction[1]. To characterize the key players of the nuclear matrix, Berezney and colleagues used two-dimensional polyacrylamide gels to separate and identify the major protein components of the extracted nuclear matrix from rat liver cells[1]. Together with the lamins A, B and C, other abundant proteins were identified, including Matrins 3, 12, 13, D/E, F/G (Prostaglandin transporter SLC21A2), PSF/SFPQ (at the time named Matrin4) and nucleophosmin (also known as B-23), as well as several hnRNPs. Subsequently, many other proteins have been found to localize to this structure and have been identified using quantitative proteomic tools[11]. Taking a complementary approach Sugano and colleagues identified a 130kDa protein, initially named P130 after its gel migration size, which binds to AT-rich sequences found in highly repetitive matrix-associated DNA sequences, and upon protein sequence analysis revealed to be Matrin3[14, 15].

Although a major component of the matrix that provides structure and organization to the nucleus, the majority of the 847 amino acids of Matrin3 comprise intrinsically disordered regions, with the exceptions of two zinc-finger domains and two tandem RNA binding domains[16]. The assumption that its matrix localization implies a passive structural role could not be further from the truth; Matrin3 has been associated with functions ranging from transcription regulation, pre-mRNA splicing, mRNA stability, DNA damage repair to cell proliferation (see below). The involvement of Matrin3 and other nuclear matrix proteins in these processes suggests that the nuclear matrix might have important roles not only in the spatial organization and compartmentalization, but also in the activity of the specialized 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 C_2H_2 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 regions[18]. 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 proteins[19].

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 blot[20]. Nevertheless, subsequent studies have shown that Matrin3 can bind to at least some DNA sequences[14-16]. 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, U1C[21], the ZF domains in Matrin3 mediate interaction with DNA[16]. 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 chromatin[16]. The ZF domains cannot compensate binding to RNA when the RRM domains are deleted[16]. 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 events[19].

The two RRM domains of Matrin3 are most similar to the RRM domains of PTBP1 and hnRNPL[16], 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 proteins[22]. 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

domain[23]. There is evidence that some RNA-dependent protein interactions require the second RRM but not the first, suggesting that RRM2 might play the dominant role in RNA binding[24]. The AUCUU motif is similar to the UUUCU motif bound by PTBP1, which directly interacts with Matrin3 and cooperates with it in alternative splicing regulation. The two tandem RRMs of Matrin3 bind to the regulated RNA targets and are required for its role in splicing regulation and RNA stabilization[19, 24]. RNA map constructed by mapping Matrin3 iCLIP tags onto the Matrin3 regulated splicing events suggests that *in vivo* RNA binding might involve initial binding to specific sites followed by oligomerization along the RNA[25] (see below).

Immunofluorescence microscopy found Matrin3 as a mainly nuclear protein in various mammalian cell lines, where it localizes throughout the nucleoplasm with the exception of the nucleolus[26, 27]. Nuclear matrix localization of proteins like SATB1, Runx and AML-1 is mediated through a nuclear matrix targeting sequence (NMTS) distinct from the NLS[28, 29], but a functionally equivalent motif could not be identified in Matrin3. Although Matrin3 was originally discovered by its presence in nuclear matrix fractions, biochemical fractionation and western blotting show it is also present in soluble nucleoplasmic fractions[16, 19], and to some extent also in cytoplasmic fractions, albeit at lower levels than in the nucleus[16]. The lack of an identified NMTS in Matrin3 means that it is not yet possible to determine which of its nuclear functions depend on its matrix localization. Shuttling between the nucleus and cytoplasm is mediated by the nuclear export signal (NES) located at the N-terminus, and the bi-partite nuclear localization signal (NLS) located between the second RRM and the second ZF domain[16, 17] (Fig.1A). Although no evidence for a cytoplasmic role has yet been described, the presence of a NES and some cytoplasmic localization suggest that such a role may exist.

Molecular interactions of Matrin3

Matrin3 can interact with multiple nuclear proteins, a high proportion of which are involved in RNA binding and/or processing (Table 1). Some of these are important for Matrin3 function, including the interactions with PTBP1, NONO (also known as p54nrb) and PSF (reviewed in[30]), but the function of other interactions remains unclear. The domains and motifs mediating most direct protein-protein

interactions remain unclear, with one exception being the PRI-mediated interaction with PTBP1[19]. In fact, some of the reported interactions might be indirectly bridged by RNA, since they were identified by techniques that do not distinguish between direct and RNA-bridged interactions, including yeast two hybrid (Y2H) and immunoprecipitation (IP) without nuclease treatment (Table 1). Moreover, interaction sites for binding partners may overlap, and their competition for Matrin3 binding might be relevant for the different Matrin3 functions. Some of these interactions may require presence of Matrin3 within the nuclear matrix, which could serve as a scaffold for specific functions. The interaction between Matrin3 and LaminA[26] is one such matrix associated interaction. Matrin3 binds to the tail of LaminA and immunofluorescence shows co-localization on the nuclear rim of differentiated C2C12 myotubules, although it is unclear if this is in the nuclear membrane or nuclear matrix. This interaction might be involved in the anchoring of the nuclear envelope bound lamins to the nuclear matrix, thereby ensuring structural integrity of the nucleus.

Matrin3-related proteins

Mammals possess two Matrin3-related proteins, RBM20 and ZNF638, which share its overall domain organization, including the related ZF and RRM domains (Fig. 1A). While Matrin3 and ZNF638 are widely expressed across different cell types, RBM20 expression is mostly restricted to the foetal and adult heart (Fig 1B). ZNF638 (originally known as NP220) has a pair of RRMs between the two C_2H_2 ZF domains[31]. Sequence identity is highest in the ZF and RRM domains, although RBM20 only has a single RRM domain. RBM20 shares 29% amino acid identity with Matrin3 over 43% of its length. Mapping of the RNA targets of RBM20 by CLIP revealed an optimal UCUU binding motif[32], which is remarkably similar to the optimal binding sites for both Matrin3 (AUCUU)[22], and PTBP1 (UUUCU/UCUU)[22, 33]. This is consistent with the fact that the first RRM of PTBP1 recognises YCU motif[34] and has a high degree of identity with the RRMs of Matrin3 (40% and 35%) and RBM20 (51%). RBM20 and ZNF638 lack the PRI motif found in Matrin3, and so presumably do not interact with the splicing regulator PTBP1. Nevertheless, both proteins were

found to regulate splicing[35, 36], and have regions enriched in RS dipeptides, a feature commonly associated with splicing factors and regulators

Matrin3 function in transcriptional regulation

A number of functions have been suggested for Matrin3 throughout the life cycle of mRNAs, from the regulation of transcription, alternative splicing, viral RNA export, mRNA stability, to the DNA damage response. The role of Matrin3 in transcriptional regulation originates from the finding that Matrin3 interacts with the regions of DNA that are present within the scaffold/matrix attachment regions (S/MARs)[3]. These Matrin3-bound DNA regions augmented transcription when placed upstream of a promoter in a reporter plasmid[37]. Matrin3 binding decreased upon methylation of this DNA region, with a corresponding decrease in transcription, thereby suggesting that Matrin3 can activate transcription[38]. Matrin3 might not be the only factor involved in transcriptional activation by these DNA regions, given that these regions were isolated from the nuclear matrix scaffold that contain many proteins involved in transcription[11, 37]. Matrin3 was also found to bind to Pit1, a transcription factor involved in the activation of multiple genes, most notably the pituary hormone gene. Enhancer-bound Pit1 has to interact with the nuclear matrix to recruit the transcription factors beta-catenin and SATB1 to specific enhancer loci. The interaction of Pit1 with the nuclear matrix and Matrin3, requires beta-catenin and SATB1, and in absence of these factors Pit1 is unable to activate transcription. A disease-causing dominant negative mutation of Pit1 (R271W) disrupts the interaction with beta-catenin and SATB1, and consequent loss of association of enhancer occupied Pit-1 with the nuclear matrix. This interaction loss can be rescued by fusing Pit1 R271W to the nuclear matrix targeting region from hnRNPU/SAF-A, with the remarkable rescue of enhancer activation and recruitment of transcription co-activators such as p300, and consequent transcription activation[39].

Transcription is thought to occur in a limited number of “transcription factories” at fixed locations in the nucleus[40], which implies anchoring to a structural component that limits diffusion. Besides Matrin3, other components of the nuclear matrix impact transcription, positively and negatively. While Lamin B is associated with insulator sequences[41], matrix-associated RNA-binding binding proteins seem to aid in

maintaining a transcriptional active environment. The nuclear matrix component hnRNPU is associated with sites of RNA pol II transcription[42], and expression of a dominant negative mutant of hnRNPU destabilises the matrix in a similar manner as does RNase treatment[43]. Matrin3 has been found at RNA pol II initiation sites together with hnRNPU, highlighting the involvement of Matrin3 in a network of transcriptional activators[44].

Matrin3 function in splicing regulation

Generation of alternative spliced mRNAs allows cells to expand their proteome[45]. 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 activator[19]. While Matrin3 required its RRMs 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 PTBP1[19]. This interaction is mediated through a specific small linear motif GILGPPP found between Matrin3's first ZF and the RRMs. 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 PTBP1[50] 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 events [19] (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).

Interestingly, Matrin3 interacts with broad sections of the pre-mRNA within the repressed exons and the flanking introns. In contrast to the splicing maps for many other splicing regulators[25], Matrin3 binding was relatively uniform over a region encompassing 500 nucleotides flanking the repressed exons, with no specific peaks of binding. Matrin3 RNA-compete motifs were also enriched in the flanking introns, but not within the repressed exons[19]. This suggests a "bind and spread" model in which Matrin3 is recruited initially to the intronic high affinity binding sites, with subsequent recruitment of other Matrin3 molecules that interact and spread across the exon and flanking introns (Fig. 2). In support of this hypothesis, Matrin3 was found to interact with itself in a yeast two hybrid screen[27]. Another striking feature is that the introns flanking the Matrin3 repressed exons are significantly longer than those flanking other cassette exons, including those regulated by PTBP1. Longer introns may be required to prevent the "bind and spread" mode of action from affecting the flanking constitutive splice sites.

Notably, both of the Matrin3-related proteins RBM20 and ZNF638 have also been reported to regulate alternative splicing[32, 35, 36], suggesting that it is a core function of this group of proteins. RBM20 has similar RNA binding preference to PTBP1 and Matrin3, consistent with the similarity between their RRMs (Fig 1). Strikingly, RBM20 appears to act primarily as a splicing repressor and like Matrin3 binds within both introns flanking repressed exons [32]. Despite these similarities, there are interesting contrasts. While Matrin3 interacts with a known splicing repressor PTBP1 via its PRI, RBM20 interacts with a number of core splicing factors, consistent with having an RS domain, as well as with hnRNPs[32].

Matrin3 function in RNA stability

Steady-state RNA levels are determined by the rates of RNA synthesis and degradation. In addition to its roles in transcription, Matrin3 has also been implicated the stability of a number of mRNAs[24]. Since Matrin3 interacts with several transcription associated factors, such as DHX9 and hnRNPK, and the noncoding small RNA 7SK, the authors examined alterations in gene expression upon Matrin3 depletion in U2OS cells. Matrin3 depletion decreases the mRNA levels of 77 genes, and Matrin3 binding to these mRNAs was indicated by RNA immunoprecipitation.

This interaction is dependent on the second RRM, which interestingly is also the one involved in the RNA-dependent interaction with DHX9 and hnRNPK. The reduced levels of these mRNAs were shown to be due to reduced RNA stability rather than transcriptional down-regulation, indicated by reduced mRNA half-life of three tested mRNAs[24]. The exact molecular mechanism of Matrin3-dependent mRNA stabilization is not entirely understood. However, a subset of stabilized mRNAs might be explained by nonsense mediated decay (NMD) linked to alternative splicing (AS-NMD)[19]. These are splicing events in which one of the splicing patterns introduces a premature termination codon (PTC) leading to NMD[51]. Analysis of mRNA level and splicing changes upon Matrin3 knockdown showed that 15 out of 22 genes with changes in expression greater than 1.5 fold had an associated AS-NMD event that might explain the changes in mRNA level[19].

Matrin3 function in viral biology

Viruses exploit their host systems to serve their own interests in several steps of their life cycle. Generation of infectious HIV-1 viral progeny requires the cytoplasmic export of several spliced and unspliced subgenomic HIV-1 mRNAs. The Rev protein is crucial in HIV-1 biogenesis and is produced by the fully spliced RNA, and in turn Rev facilitates the export of incompletely spliced RNAs that will express the other essential HIV-1 components, and ultimately genomic RNA for assembly of new HIV-1 particles. Rev binds to the Rev Response Elements (RRE) present in the RNA and together with host components it can escape the nucleus and lead the RNAs to the ribosome for expression of viral proteins (Reviewed in[52]). Two parallel studies using different approaches identified Matrin3 as a regulator of HIV-1 expression: one by RNA-affinity isolation of proteins interacting with the HIV-1 RNA and the other by yeast two hybrid screen using PTBP1 as a bait, as this splicing factor has been shown to affect HIV-1 gene expression[53, 54]. Manipulation of Matrin3 levels greatly affected the activity of Rev, indicative of a role of Matrin3 as a Rev co-regulator. Rev, Matrin3 and RRE-containing RNAs together form a complex suggesting that RNA bridges the Rev-Matrin3 interaction, and supporting this is the requirement for Matrin3's RRMs for this activity. Here, as in some of the other Matrin3 associated functions, PSF also plays a role[55]. However their RNA association is only partially

overlapping. PSF is recruited to the RRE-containing RNA along with Rev co-transcriptionally and is thought to be released before RNA accumulation at the nuclear matrix or its export, while Matrin3 is recruited post-transcriptionally and remains with the RNA in the nuclear matrix until its export. Viral use of Matrin3 is possibly not restricted to HIV-1, and accordingly a kinase encoded by alphaherpesvirus ORF66 targets predominantly Matrin3, modifying threonine 150, which is required to avoid cytoplasmic aggregates of Matrin3 in later stages of infection similar to what has been observed with the lamins[56, 57].

Along with PSF and NONO, Matrin3 was also identified as a component of a complex involved in nuclear retention of RNA with long double-stranded regions, possibly associated with adenosine to inosine editing[58]. This could be of interest as a possible cellular antiviral mechanism. However, subsequent analyses suggest that this resulted from nuclear paraspeckle localization mediated by PSF and NONO, with no evidence for involvement of Matrin3[59].

Matrin3 function in cell survival and DNA damage response

The importance of the multiple functions of Matrin3 is evident from the dramatic effect of its depletion on cell proliferation and on the ability of cells to respond to DNA damaging compounds[17, 60, 61]. Matrin3 is involved in the response to several signals, such as the activation of NMDA receptors[62] or the induction of DNA double strand breaks[60]. Both lead to post-translation modification of Matrin3 by phosphorylation at serine residues, with the former mediated by protein kinase A (PKA) and the latter by the kinase ataxia-telangiectasia mutated (ATM). PKA targets mainly a serine at position 188[57] while ATM targets serine 208[60]. The consequences of these modifications are quite distinct. Matrin3 is the main PKA target upon NMDA receptor activation in rat brain, which culminates in Matrin3 degradation and neuronal cell death, effects that can be blocked by PKA inhibitors[62]. Several caspases, typically activated/released during apoptosis, can cleave Matrin3, although it is not clear if this is the mechanism occurring during PKA-mediated degradation[63]. Down-regulation of Matrin3 by siRNA transfection in endothelial cells culminates in a decrease of cell proliferation followed by cell necrosis[61]. This

suggests that although Matrin3 down-regulation is involved in the regulated apoptotic death response to NMDA signalling, it is not sufficient and that in the absence of signalling Matrin3 depletion alone leads to necrotic cell death. The exact mechanism by which Matrin3 ensures proper cell proliferation is not clear, although it most likely will be related to its nuclear functions as it requires its nuclear localisation[17].

Persistent DNA damage can lead to cell death, and Matrin3 can function in several steps ensuring that only undamaged cells escape cell death, although no direct links between its role in DNA damage response and cell death have yet been reported. DNA double strand breaks (DDB) trigger ATM mediated phosphorylation of Matrin3 at serine 208, and this seems to be required for the DNA damage response[60]. This response involves activation of a cell cycle checkpoint that stalls cells to ensure sufficient time for DNA repair. Matrin3 can form a complex with proteins involved in DNA damage response, PSF and NONO, as well as the DNA binding proteins Ku80/Ku70, independent of any DNA damage. PSF and NONO are recruited to sites of DNA damage, and while Matrin3 itself is neither recruited nor affects the recruitment of other components, it does significantly alter the retention time of these proteins at the damage sites. This suggests that Matrin3 is involved in the early steps of the DNA damage response and is required for the dynamic recruitment and release of PSF/NONO from the damage site, although it is itself not recruited to the site[60].

Associations of Matrin3 with human diseases

In addition to its effect on viruses such as HIV1, inherited mutations of Matrin3 have also been shown to be associated with the neurodegenerative disease amyotrophic lateral sclerosis (ALS). ALS is a neurodegenerative disease affecting lower and upper motor neurons including the spinal cord, typically with adult onset. A Ser85Cys mutation in Matrin3 was found in 1998 as the autosomal dominant mutation in a family with multiple members diagnosed with myopathy[64]. Subsequently, a Phe115Cys mutation in Matrin3 was found to segregate with disease in a Sardinian family suffering from slow progressing ALS, and re-evaluation of the original family established that the Ser85Cys mutations also causes ALS[65]. Matrin3 mutations are rare in ALS, since in 96 patients with sporadic disease only

one missense mutation was found in Matrin3. Patients with Matrin3 Ser85Cys or Phe115Cys mutation suffer from brisk knee reflexes and a 'split-hand' pattern of weakness[65, 66], symptoms also observed in other ALS patients.

At the molecular level, Phe115Cys mutation leads to intra-nuclear inclusion, and a pronounced increase in Matrin3 protein abundance in the spinal cord neurons. Aggregation of Matrin3 is also seen in ALS patients with the more common C9orf72 tri-nucleotide expansion, suggesting that aggregate formation of Matrin3 (and other RBPs) is widespread in ALS patients[65]. This phenotype is highly similar to other RBPs in which mutations lead to aggregation and manifestation of ALS or related motor neuron diseases, including TDP43, FUS, hnRNPA1 and hnRNPA2[67]. The basis for pathology could be the loss of function of the protein(s), which may lead to deregulated alternative splicing. Alternatively, the aggregates may actively disrupt cellular functions via a toxic gain of function. The two mechanisms are not mutually exclusive and evidence has been presented for both in the cases of other RNA binding proteins such as TDP43 and FUS [68].

As noted above, expression of the Matrin3-related protein RBM20 is restricted to the heart and is especially high in early development. Consistent with this, mutations of RBM20 were identified in 8 independent families associated with dilated cardiomyopathy[69, 70]. The missense mutations were all located in a hotspot corresponding to the arginine-serine domain of RBM20. Moreover, a large deletion of the RBM20 gene was found to be responsible for altered splicing of titin and other mRNAs in a rat model that also showed symptoms of dilated cardiomyopathy[35]. This suggests that the cardiomyopathy results from missplicing associated with loss of RBM20 function. It remains to be determined whether the mechanisms of ALS pathology arising from Matrin3 mutations are similar to the cardiomyopathy arising from RBM20 mutations.

Conclusions and perspectives

Evidence of the multiple functions of Matrin3 has accumulated over the last 25 years since it was discovered as a component of nuclear matrix. The full extent of functional coupling between the different stages of gene expression has also come into focus during this period, and like many other proteins, Matrin3 has clear roles at

numerous levels of gene expression. While it seems obvious that Matrin3 function is linked to the nuclear matrix, the extent to which its involvement in processes from transcriptional activation and pre-mRNA splicing to export of viral RNAs, requires anchoring of RNPs to the nuclear matrix remains unresolved. A precise mutation to specifically disrupt matrix localization would be an invaluable tool in this regard. Matrin3 function might in part be attributed to limiting diffusion of several co-factors, as suggested by the change in retention time of PSF and NONO upon DNA damage[60]. Much remains to be learned about the molecular interactions and cellular roles of Matrin3 and its related proteins, RBM20 and ZNF638, as well as the molecular basis of pathologies arising from their malfunction. The pace of discoveries about Matrin3 has accelerated over the past few years, with new insights emerging from several directions, including investigations of viral mechanisms and of diseases of motor neurons and the heart. We anticipate that Matrin3 will become a prime exemplar of the multiple ways in which RNP functions are inter-linked with genome organisation and the nuclear matrix.

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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 Atlas[71].

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
RNA processing	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]
	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]

	PCBP2	Y2H		Q15366	[27]
	PTBP1	IP^{R} & GPD	✔[75, 78]	P26599	[19, 24]
	PTBP2	Y2H		Q9UKA9	[80]
	PTBP3	IP^{R}		O95758	[81]
	RBMX	Y2H		P38159	[27]
	PSF	IP^{R}	✔[78]	P23246	[60, 55]
	SFRS7	Y2H	✔[11]	Q16629	[27]
	SRPK2	Y2H		P78362	[27]
	TARDBP/TDP43	IP		Q13148	Freibaum et al, 2010
Transcription	DDX3X	IP^{R}	✔[11]	O00571	[76]
	DHX9	IP	✔[11]	Q08211	[24]
	ERG	IP & GPD		P11308	[82]
	FOXG1B	Y2H		P55316	[27]
	NFX1	IP	✔[11]	Q12986	[83]
	NR2F1	Y2H		P10589	[27]
	RGS6	Y2H		P49758	[27]
	POU1F1/Pit1	IP	✔[39]	P28069	[39]
	POLR2A	IP	✔[6]	P24928	[84]
	RPAP1	Y2H		Q9BWH6	[27]
	SAFB	Y2H	✔[85]	Q15424	[27]
	SLTM	Y2H	✔[11]	Q9NWH9	[27]

	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]
	SMARCAD1	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]
Signal Transduction	BRE	Y2H		Q9NXR7	[27]
	PLCG1	Y2H		P19174	[27]

	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

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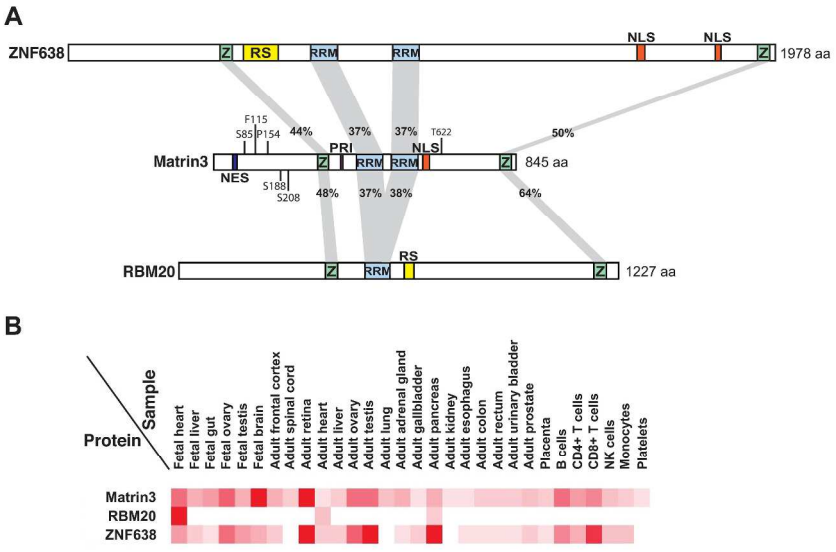

Figure 1 - Matrin3 and related protein structure and expression
297x420mm (300 x 300 DPI)

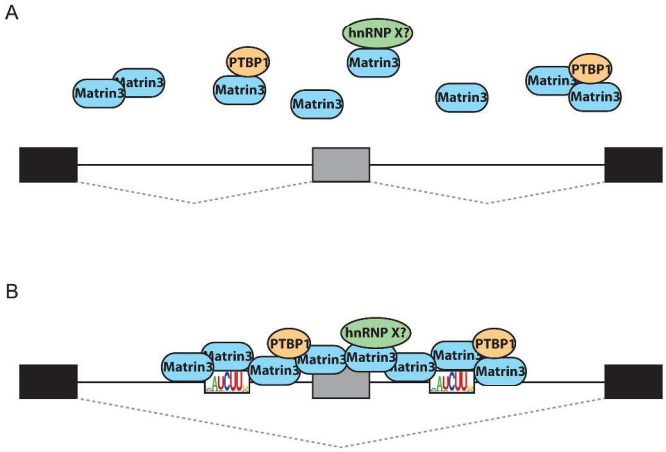

Figure 2 - Splicing Regulation by Matrin3
297x420mm (300 x 300 DPI)