

Architectural roles of long noncoding RNAs in the intranuclear formation of functional paraspeckles

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1. ABSTRACT

The eukaryotic nucleus is highly compartmentalized, and this structural complexity allows the regulation of complex gene expression pathways. Some of the subnuclear structures called nuclear bodies are known to contain RNAs. Recently multiple noncoding RNAs (ncRNAs) have been identified as products from regions covering large portions of mammalian genomes. Several abundant ncRNAs were found to localize in nuclear bodies, suggesting new roles for ncRNAs in these nuclear bodies. Paraspeckle, one of these nuclear bodies, contains specific ncRNAs, termed MEN (Multiple Endocrine Neoplasia) epsilon/beta ncRNAs, and characteristic RNA-binding proteins. Paraspeckle is ubiquitously observed in cultured cell lines but is cell type-specific in mouse tissues. Paraspeckle reportedly plays an important role in the nuclear retention of inosine-containing mRNAs and is regulated under stress conditions. Intriguingly, MEN epsilon/beta ncRNAs are prerequisite for the formation of paraspeckles, indicating architectural roles for these ncRNAs and, presumably, significant roles in the nuclear retention of mRNAs as well. This review focuses on known aspects of the paraspeckle structure and its components, and we attempt here to construct a model of the ncRNA-dependent formation of nuclear body structures.

2. INTRODUCTION

Postgenomic transcriptomic analyses, including full-length cDNA sequencing and tiling array analyses, have revealed that large numbers of transcripts that are unlikely to code for polypeptides are produced from regions covering large portions of the human and mouse genomes (1-6). These transcripts are commonly termed noncoding RNAs (ncRNAs). Extensive analyses of the subcellular localization of human transcripts have revealed an obvious enrichment of ncRNAs in the cell nucleus, suggesting diverse roles for ncRNAs in nuclear events (7-13). Recently, several nuclear abundant long ncRNAs have been shown to localize in specific subnuclear structures, raising the intriguing possibility that these subnuclear structures are sites where ncRNAs play important regulatory roles in nuclear events, including gene expression or the modulation of protein function. For instance, Gomafu ncRNA (also known as MIAT) is highly expressed in a subset of neurons in the central nervous system and localizes in novel nuclear structures (14). Malat-1 ncRNA (also known as MEN alpha and NEAT2) is a highly expressed nuclear ncRNA of 8 kb in size that is significantly associated with cancer metastasis (15, 16). It was recently reported that Malat-1 ncRNA is localized to nuclear speckles; that it regulates alternative splicing

Table 1. Nuclear bodies

Name	Number/cell	Diameter(micrometer)	Marker protein	Associated genomic locus	References
Paraspeckle	2-20	0.36(average)	PSP1	MENepsilon/beta	37, 90
Nucleolus	1-5	0.5-5.0	Nucleolin	rDNA	22, 23
Cajal body	0-6	0.2-2.0	Coilin	U-snRNA	20, 24, 26
Histone locus body	n/d	n/d	NPAT	Histone	26
Gem	1-4	0.3-1.0	SMN	n/d	20, 28
Nuclear speckle	25-50	0.8-1.8	SC35	n/d	20, 30
Sam68 body	2-5	0.6-1.0	Sam68	n/d	32, 33
Nuclear stress body	4-6	2.0-2.5	HSF1	pericentromeric	34, 35
Cleavage body	1-4	0.2-1.0	CstF64	n/d	20, 33
Polycomb body	12-16	0.3-1.0	Bmi	n/d	20, 33
PML body	10-30	0.3-1.0	PML	MHC class I	20, 36

patterns through the modulation of the phosphorylation status of SR (Serine/Arginine) splicing factor (15,17); and that it controls synaptogenesis by modulating gene expression (18).

The eukaryotic nucleus is highly compartmentalized, and this structural complexity is thought to allow the regulation of complex gene expression pathways, especially in mammalian cells. In such cells, more than ten membraneless suborganelles (Table 1) (19) have been discovered and are thought to serve as specialized hubs for various nuclear events (Figure 1A, B) (20,21). These subnuclear organelles, called nuclear bodies, exist in the interchromosomal space, and multiple nuclear regulatory factors, such as transcription factors and RNA processing factors, are enriched in these nuclear bodies. The nucleolus, the most classical nuclear body, is known to be a “factory for ribosome biogenesis” in which RNA polymerase I transcription, pre-rRNA processing and modification, and subsequent ribosomal protein assembly are thought to occur sequentially in the hierarchical structure of the nucleolus (22, 23). Cajal bodies were originally discovered at the beginning of the 20th century and are spherical sub-organelles of 0.2-2.0 micrometer in diameter in size that occur in the nucleus of various cell types (24). Cajal bodies are involved in the biogenesis of multiple small nuclear ribonucleoprotein particles (snRNPs) (25, 26). Cajal bodies often overlap with distinct nuclear structures called histone locus bodies, in which histone gene loci along with histone mRNA processing factors, such as U7 snRNP, are localized (26, 27). Another nuclear body called Gems that contain a protein complex that includes the survival motor neuron (SMN) protein often overlaps with Cajal bodies (26, 28). Nuclear speckles are 25-50 irregularly shaped nuclear structures of 0.8-1.8 micrometer in diameter in size that are found in the nucleus of various cell types. Nuclear speckles are relatively well-characterized nuclear bodies in which various SR splicing factors are localized, along with several other RNA-binding proteins, Malat-1 ncRNA as well as uncharacterized polyadenylated RNAs (29, 30). Although still controversial, nuclear speckles are widely considered to be the reservoir of various splicing factors that are translocated to transcriptionally active sites of the chromosome (31). Among the splicing regulatory factors, Sam68 (Src-associated in mitosis, 68 kDa) is specifically localized to distinct nuclear foci on the periphery of the nucleolus. Electron microscopic analysis has revealed that Sam68-containing foci, called Sam68 bodies, are electron dense,

suggesting that RNA is a major constituent of these structures (32, 33). Nuclear stress bodies (nSBs) are formed on specific pericentromeric regions in response to thermal and chemical stress (34). Under such stress conditions, polyadenylated ncRNAs are transcribed from the satellite III (SatIII) DNA regions of the pericentromeric heterochromatin of human 9q11-12. RNA-binding proteins, including splicing factors, are captured with SatIII ncRNAs in nuclear stress bodies (35). Although some nuclear bodies still remain to be functionally characterized, others are recognized as sites for the localization of specific regulatory factors of gene expression; cleavage body, polycomb body (PcG body) and PML body contain the protein factors involved in mRNA 3' end processing, epigenetic regulation and transcriptional control, respectively (20, 33, 36). Many nuclear bodies contain specific RNA molecules, together with RNA-binding proteins, and serve as platforms for ribonucleoprotein biogenesis in addition to their functions as reservoirs. This review will focus on the structure and function of the specific nuclear body called the paraspeckle. The paraspeckle structure includes nuclear-retained long ncRNAs and RNA-binding proteins. Current understanding regarding the components, building process, and function of these nuclear bodies will be introduced, and the significance of ncRNAs employed for cellular structural formation will be considered.

3. THE PARASPECKLE: AN RNA-CONTAINING NUCLEAR BODY

The paraspeckle is a nuclear body that was discovered in 2002 (37). Lamond and his colleagues performed large-scale proteomics analysis of isolated HeLa cell nucleoli treated with the transcription inhibitor, actinomycin D. Among 271 nucleolar protein candidates identified, more than 30% of these candidates were functionally uncharacterized proteins. Subsequent analysis of these uncharacterized proteins revealed that two of the proteins exhibited nuclear punctate localization. They were not enriched in nucleoli but were enriched in other unknown nuclear bodies. These newly discovered nuclear bodies were localized in close proximity to nuclear speckles (37-40). Therefore, they were named “paraspeckles” and the colocalized proteins were named paraspeckle protein 1, (PSP1) and paraspeckle protein 2 (PSP2 or CoAA/RBM14). Non-POU domain-containing octamer-binding protein (NONO or p54nrb) was simultaneously identified as a paraspeckle component.

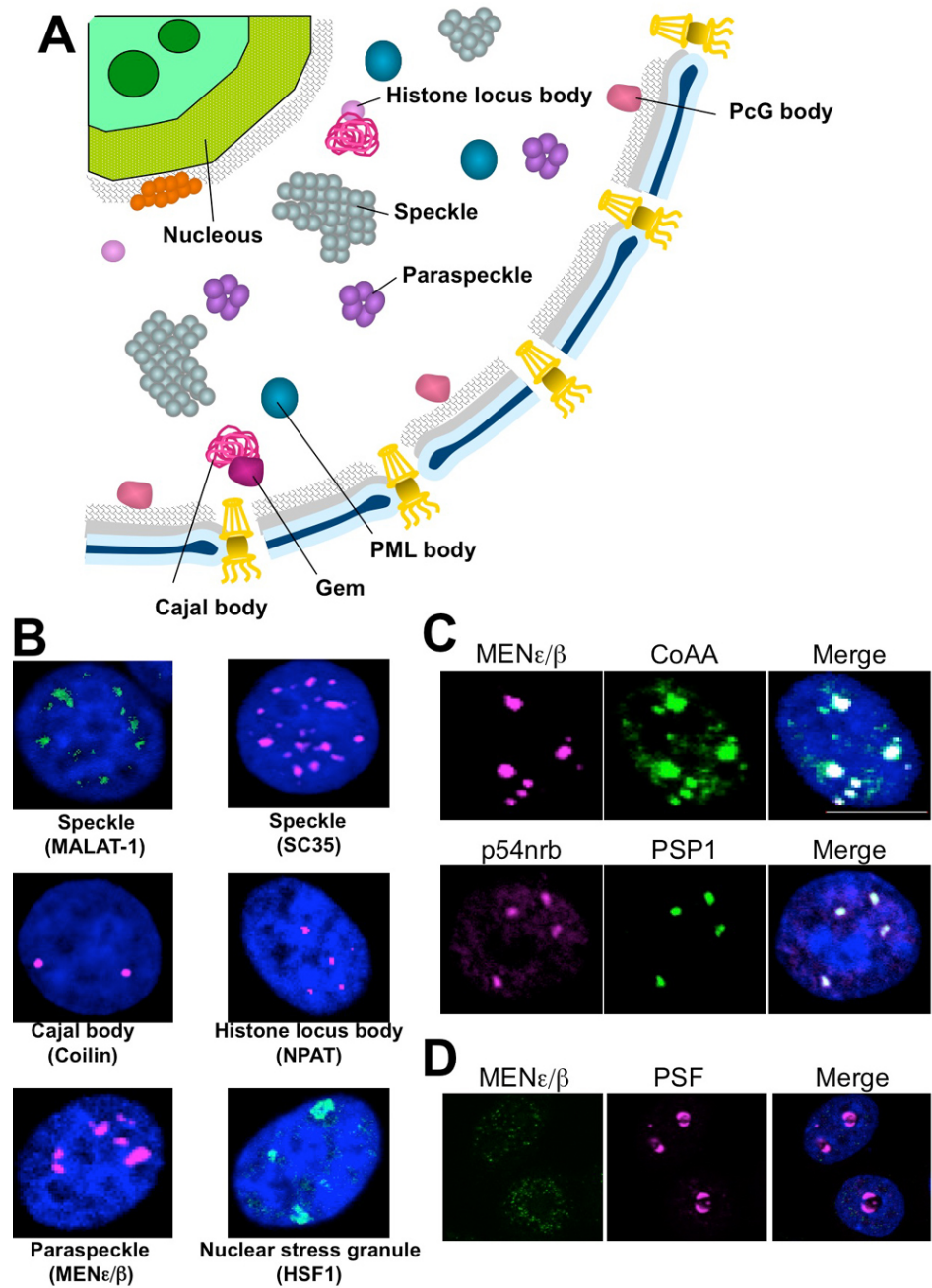


Figure 1. A. Schematic of nuclear structure and major nuclear bodies. B. Visualization of nuclear bodies by immunostaining or RNA-FISH to detect molecular markers corresponding to each nuclear body. Malat-1 ncRNA and SC35 are markers of nuclear speckle-localized RNA and protein, respectively. Coilin and NPAT are markers of partially overlapped Cajal bodies and histone locus bodies, respectively. PSP1 and HSF1 are markers of paraspeckles and nuclear stress bodies, respectively. C. Paraspeckles visualized by RNA-FISH of MEN epsilon/beta ncRNAs and the immunostaining of paraspeckle marker proteins including CoAA, p54nrb, and PSP1. D. Relocation of paraspeckle proteins to the perinucleolar cap structure upon actinomycin D treatment. It should be noted that MEN epsilon/beta ncRNAs never relocates to perinucleolar caps.

Subsequently, Splicing factor, proline- and glutamine-rich (SFPQ or PSF), and Cleavage and polyadenylation specificity factor subunit 6 (CPSF6) were identified as additional paraspeckle proteins. Normally, paraspeckles occur as 2–20 punctate nuclear structures with an average

size of 0.36 micrometer in diameter (Figure 1C, 37-40). Paraspeckles have been found in mammalian cells from humans and mice; however, the presence of phylogenetically orthologous nuclear bodies remains to be investigated. Orthologues of paraspeckle proteins (p54nrb,

PSF and PSP1) with >60% amino acid sequence identities to mammal paraspeckle proteins are present in other vertebrate species such as chicken and tetradon, suggesting that similar nuclear bodies may exist in these vertebrate species. Paraspeckles exist in various mammalian cultured cells, including primary and transformed cell lines. The number of paraspeckles per interphase nucleus is variable and depends on the cell line (37). Paraspeckle is reminiscent of the interchromatin granule-associated zones (IGAZs) that were previously detected as electron-dense interchromatin structures by high resolution *in situ* hybridization with electron microscopic observation (38, 39, 41). IGAZs are electron-dense fibrillar structures located in close proximity to interchromatin granules that correspond to nuclear speckles (41). In contrast to cultured cell lines, observation in adult mouse tissues revealed that the intact paraspeckles were observed in the specific cell types (42). For example, in digestive organs, such as stomach and intestine, the paraspeckle-possessing cells are typically observed only in the most distal regions of the surface but not in the deep layers of the gastric epithelium. Even in cultured cell lines, embryonic stem cells and a few transformed cell lines lack intact paraspeckles (43, Kawaguchi *et al.*, unpublished). Therefore, this nuclear body is likely a highly dynamic structure. Paraspeckle formation is tightly regulated during the cell cycle. Time-lapse fluorescent imaging analysis using a fluorescent protein tagged paraspeckle protein, YFP-PSP1, expressed in a stable cell line revealed that paraspeckles persist throughout interphase and almost throughout mitosis, including prophase as well as metaphase, anaphase, and telophase, and that they exist in the cytoplasm during mitosis because of the elimination of the nuclear envelope (44). Subsequently, the cytoplasmic paraspeckles disappear between telophase and early G1 phase and reappear during the G1 phase (44).

Blocking RNA polymerase II transcription with transcription inhibitors such as actinomycin D or alpha-amanitin disassembles paraspeckles, resulting in the relocation of paraspeckle proteins to the nucleolar cap structure formed on the nucleolar periphery (Figure 1D). Treatment of the cells with the reversible transcription inhibitor DRB (5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole) (45) also disassembles paraspeckles; however, they recommence assembly quickly after the removal of DRB, suggesting that paraspeckle formation requires active transcription by RNA polymerase II (37, 44). Paraspeckle structure is resistant to DNase I treatment but disappears after RNase A treatment, suggesting that the paraspeckle structure contains RNA component(s) required to maintain the structural integrity of the paraspeckle. Therefore, paraspeckles are considered to be RNA-containing nuclear bodies or tremendously large ribonucleoprotein complexes, the maintenance of which requires certain RNA molecule(s) and active transcription by RNA polymerase II. In 2009, four groups using different approaches (43, 46-48) simultaneously found that two ncRNAs (MEN epsilon/beta ncRNAs) transcribed from the Multiple Endocrine Neoplasia type 1 (MEN1) locus are paraspeckle architectural components and are essential for paraspeckle formation in human and mouse cultured cell

lines. Our group identified a paraspeckle enriched fraction in HeLa cell nuclei by biochemical purification (47). Spector and colleagues identified an ncRNA that was upregulated 3-fold during the differentiation of a C2C12 mouse myoblast cell line into myotubes (48). Lawrence and colleagues identified an ncRNA by searching for nuclear abundant ncRNA (46). Carmichael and colleagues reached MEN epsilon/beta ncRNAs during their research on nuclear retention of mRNAs (43).

4. RNA COMPONENTS INVOLVED IN STRUCTURAL FORMATION AND FUNCTIONS OF PARASPECKLES

Discovery of MEN epsilon/beta ncRNAs involved in paraspeckle structural formation opened a new window in the investigation of the roles of ncRNAs in the construction of intracellular structures. The paraspeckle could be a model system to dissect the mechanism of ncRNA-dependent cellular structure formation. Paraspeckles are composed of not only ncRNAs but also multiple protein factors. In this chapter, the known components of paraspeckles are introduced.

4.1. MEN epsilon/beta ncRNAs as the structural core of paraspeckles

4.1.1. Unique structure of MEN epsilon/beta ncRNAs

MEN epsilon/beta ncRNAs were originally identified as transcripts produced from the MEN 1 (Multiple Endocrine Neoplasia type 1) locus on human chromosome 11 (11q13) (49). Their counterparts are found on mouse chromosome 19 (19qA). MEN epsilon/beta ncRNAs (also known as NEAT1(Nuclear Enrichment Abundant Transcript 1), Vinc(Virus-inducible non-coding RNA), TSU (Trophoblast Stat Utron), or TNC (Trophoblast noncoding RNA) (15, 50-52) are comprised of two isoforms, MEN epsilon ncRNA, the shorter isoform (3.7 kb in human and 3.2 kb in mouse), and MEN beta ncRNA, the longer isoform (23 kb in human and 20 kb in mouse). These two isoforms share a common promoter recognized by RNA polymerase II and overlap across 3.7 kb of their sequence, at the 5' terminal region, in humans (Figure 2A, 38-40, 47, 48). Although MEN epsilon/beta ncRNAs are products of RNA polymerase II, they have several unique features distinct from those of protein-coding mRNAs. First, both MEN epsilon/beta ncRNAs are transcribed as intronless single-exon transcripts. Second, MEN epsilon/beta ncRNAs are never exported to the cytoplasm, but are exclusively retained in the nucleus (15, 43, 46-48). Third, the MEN beta ncRNA is not canonically polyadenylated; instead it is matured via a unique 3' end processing pathway (Figure 2B) (48). In mammalian cells, more than 90% of protein-coding genes are divided by at least one intron, and the primary mRNA transcripts are matured via pre-mRNA splicing. Pre-mRNA splicing is known to induce dynamic remodeling of ribonucleoprotein on the mRNAs, resulting in the recruitment of factors involved in nuclear export (53). The unique structure of MEN epsilon/beta ncRNAs may be related to the pathway responsible for producing RNAs that do not need to be exported. It would be intriguing to pursue the process of cotranscriptional assembly of ribonucleoproteins onto

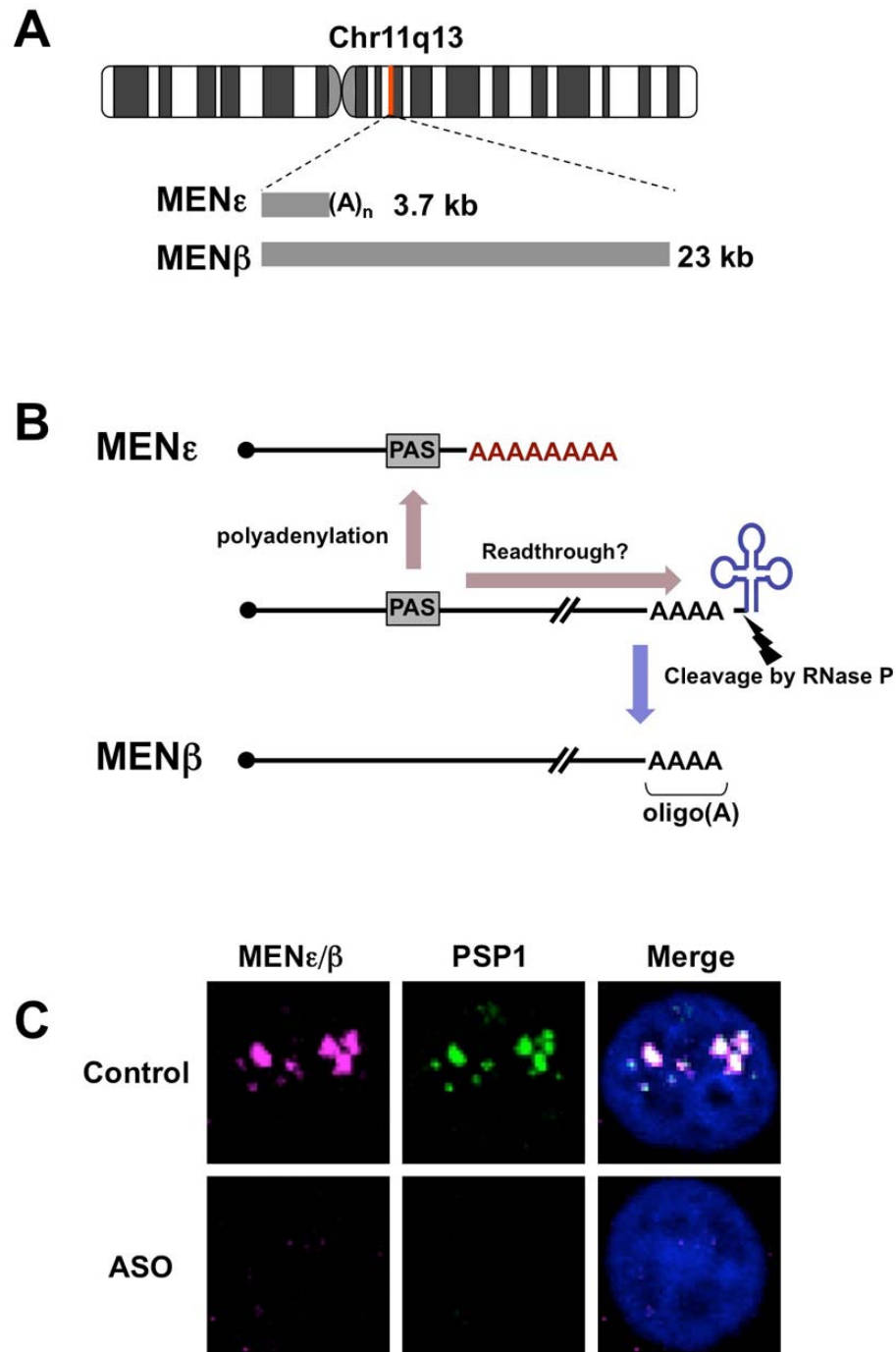


Figure 2. A. Schematic of MEN epsilon/beta ncRNAs and their genomic locus. The length of each isoform is shown on the right. The MEN epsilon ncRNA is polyadenylated as shown by (A)_n, but the MEN beta ncRNA does not possess the canonical poly(A) tail. B. RNA processing of MEN epsilon/beta ncRNAs. At the 3' end of MEN epsilon ncRNA, canonical polyadenylation that depends on a polyadenylation signal (PAS) (AAUAAA) takes place to produce MEN epsilon ncRNA. Alternatively, transcription readthrough occurs to produce the MEN beta ncRNA. The mature 3' end of MEN beta ncRNA is processed by RNase P cleavage without the addition of a poly (A) tail. The tRNA-like structure located adjacent to the MEN beta ncRNA 3' end is recognized by RNase P. The genomically-encoded oligoadenylates (oligo(A)) are located at the processed MEN beta ncRNA 3' end. C. MEN epsilon/beta ncRNAs are essential components of paraspeckles. MEN epsilon/beta ncRNAs, PSP1, and merged images are shown at the top (control). The images obtained upon MEN epsilon/beta ncRNAs knockdown using an antisense oligonucleotides are shown at the bottom (ASO).

nascent MEN epsilon/beta ncRNAs, which avoid the canonical mRNA biogenesis pathway. MEN epsilon ncRNA is polyadenylated at its 3' end through its canonical polyadenylation signal (PAS) (AAUAAA or AUUAAA), while MEN beta ncRNA lacks the canonical poly(A) tail and a PAS. Instead, the 3' terminus of MEN beta ncRNA is reported to be processed by the tRNA-processing endonuclease RNase P, which recognizes a tRNA-like structure located downstream of the 3' end of MEN beta ncRNA (Figure 2B, 48). A similar mechanism is also employed for 3' end processing of Malat-1 ncRNAs, which are transcribed from a region in MEN I locus that is 55 kb apart from MEN epsilon/beta ncRNA gene (54). The processed 3' termini of MEN beta ncRNA and Malat-1 ncRNA include short A-rich sequences (oligo (A)) that may be important for the stable accumulation of these ncRNAs in the nucleus. The mechanism of RNA stability control in the nucleus largely remains to be investigated. PAN RNA (polyadenylated nuclear RNA), viral ncRNA that is highly enriched in the nucleus and is associated with Kaposi's sarcoma, possesses a specific 79-nucleotides RNA motif called PAN-ENE (PAN RNA expression and nuclear retention element) at the 3' terminus. This motif plays a crucial role in both the nuclear enrichment and stability of PAN RNA (55). Recently, X-ray crystallography of this RNA motif revealed that it formed a non-canonical triple helix structure that contributes to the stabilization of PAN RNA (56). Although the significance of the non-canonical 3' end processing of MEN beta ncRNA remains to be elucidated, such 3' end processing would be critical for the stabilization or selective degradation of MEN beta ncRNA and Malat-1 ncRNA under certain conditions.

4.1.2. Architectural function of MEN epsilon/beta ncRNAs

MEN epsilon/beta ncRNAs and Malat-1 ncRNA are exceptionally highly abundant long ncRNAs, in comparison to thousands of other ncRNAs transcribed from the intergenic genomic regions (15). The apparent evidence of specific paraspeckle localization of MEN epsilon/beta ncRNAs together with a previous report showing RNase-sensitive paraspeckle structure raised the intriguing possibility that MEN epsilon/beta ncRNAs serve as structural components of paraspeckle. This hypothesis was confirmed by specific elimination of MEN epsilon/beta ncRNAs by knockdown with antisense chimeric oligonucleotides or siRNAs (43, 46-48). Knockdown of MEN epsilon/beta ncRNAs causes the disintegration of paraspeckle, resulting in the dispersal of paraspeckle protein components in the nucleoplasm (Figure 2C). Using the reversible transcriptional inhibitor DRB, the paraspeckle reassembly process was monitored after depletion of MEN epsilon/beta ncRNAs. The disassembled paraspeckle proteins were unable to reassemble in the absence of MEN epsilon/beta ncRNAs (47, 48). Taken together, it was concluded that MEN epsilon/beta ncRNAs are prerequisite for both the maintenance and formation of the intact paraspeckle structure (43, 46-48). This is consistent with evidence that paraspeckle is absent in human embryonic stem cells in which MEN epsilon/beta ncRNAs are poorly expressed (43). To understand the architectural roles of MEN epsilon/beta ncRNAs, it is

critical to determine the RNA regions required for their architectural function. In mouse NIH3T3 cells in which mouse MEN epsilon/beta ncRNAs were eliminated by antisense oligonucleotides, neither human MEN epsilon ncRNA nor a truncated 13 kb-MEN beta ncRNA lacking the 10 kb 3' terminal region were able to rescue paraspeckle formation (47). Future experiments using full-length MEN beta ncRNA and its deletion constructs should identify the RNA region responsible for architectural function. Another important issue is the respective roles of MEN epsilon ncRNA and MEN beta ncRNA. This is described below, with respect to the roles of paraspeckle protein components in paraspeckle formation.

4.2. Paraspeckle-localized nuclear-retained mRNAs

The function of paraspeckle and the significance of the ncRNA core of this nuclear body remain largely enigmatic. A number of reports have indicated that paraspeckles are involved in unique regulatory mechanisms of gene expression through the nuclear retention of specific mRNA transcripts. The starting point of this line of work is to attempt to identify the factors that recognize inosine-containing RNAs. Inosine nucleotides in RNAs are produced by deamination of specific adenosine nucleotides with an enzyme called ADAR (adenosine deaminase acting on RNA) (57). ADARs recognize double stranded RNAs (dsRNAs) and convert adenosine to inosine (so called A-to I RNA editing) within the dsRNA region (57, 58). Some virus-produced dsRNAs are edited at multiple sites and retained in the nucleus (59). Carmichael and his colleagues identified the protein complex containing p54nrb, known as a major paraspeckle protein component (described below) that specifically binds to inosine-containing RNAs (60). A search of potential dsRNA structures in genomically encoded mRNAs revealed that more than 333 mRNAs were found to contain the inverted repeat Alu element (IR_{Alu}) in their 3' untranslated regions (UTRs) (61). The double stranded inverted repeats (IRs) are potentially recognized by ADARs; therefore, they may possibly be retained in the nucleus. The insertion of IRs into the 3' UTR of green fluorescent protein (GFP) reporter constructs revealed that GFP mRNA was localized to the paraspeckles, suggesting that paraspeckles serve as nuclear retention sites of unique subsets of mRNA (61).

CTN-RNA (cationic amino acid transporter 2 transcribed nuclear RNA) is a natural paraspeckle-localized RNA with several unique features. It was originally identified as an RNA component enriched in the purified nuclear speckle fraction (62). By fluorescent *in situ* hybridization (FISH), CTN-RNA was found to be specifically localized in paraspeckles. CTN-RNA is a relatively long isoform of mRNA encoding mouse cationic amino acid transporter 2 (mCat2). The full-length 3' UTR containing CTN-RNA is retained in the nucleus; however, the 3' UTR is endonucleolytically cleaved upon external stimulation (e.g., Interferon gamma or lipopolysaccharide), producing the processed mCat2 mRNA with a shorter 3' UTR that is exported to the cytoplasm for translation (Figure 3) (62). CTN-RNA contains a long inverted repeat (IR) sequence in its 3' UTR that forms a long stem-loop structure that is subjected to A-to-I RNA editing. This

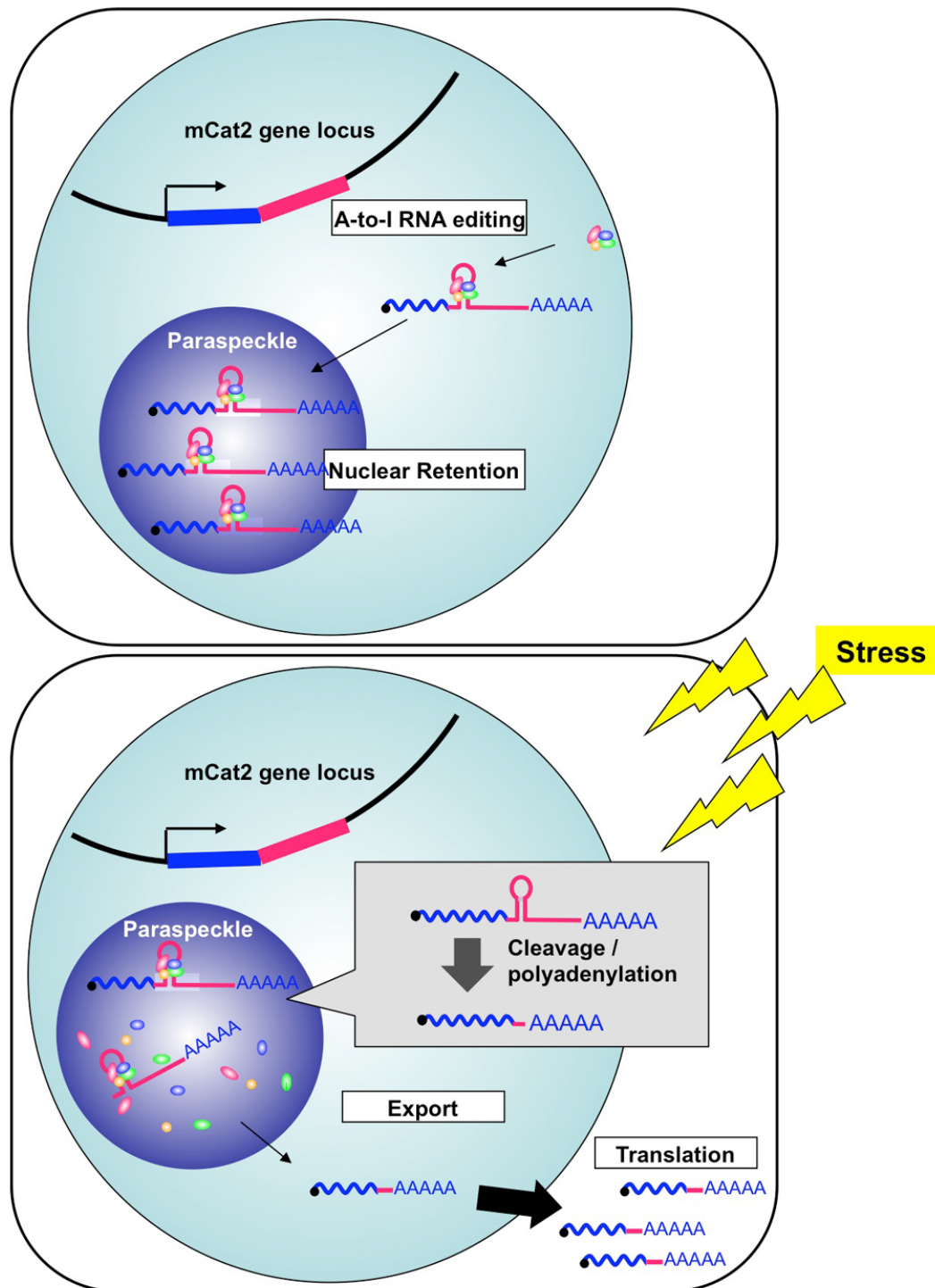


Figure 3. A. Paraspeckle-mediated mechanism of the nuclear retention of CTN-RNA. The status of CTN-RNA in normal cells is shown at the top. CTN-RNA is transcribed as an isoform transcript from the mCat2 gene locus, which is not attached to the paraspeckles. The stable stem-loop structure in the 3' UTR (red line) is shown. The putative nuclear retention factors may recognize this stem-loop structure. B. Release of the nuclear-retained CTN-RNA under stress conditions. The long 3' UTR of CTN-RNA is cleaved by an unidentified endonuclease, resulting in its release from nuclear retention status. The cleaved CTN-RNA (corresponding to mCat2 mRNA) may be subsequently polyadenylated and exported to the cytoplasm, where it is translated.

Table 2. Paraspeckle components

RNAs	Synonyms	Known function	References
RNA components			
MEN epsilon/beta	NEAT1	Essential for paraspeckle integrity	43, 46-48
CTN-RNA	mCAT2	Nuclear retained mRNA	62
U1 snRNA		Unknown in paraspeckles	37, 38, 84, 90
Protein components			
Proteins	Synonyms	Known function	References
p54nrb	NONO	DBHS, Essential for paraspeckle integrity	44,47
PSF	SFPQ	DBHS, Essential for paraspeckle integrity	47, 60
PSP1	PSPC1	DBHS	37,44
CoAA	PSP2, RBM14	Transcription/RNA processing coregulator	37
CPSF6	CFIm68	RNA 3'end processing factor	81
HnRNP M	NA	Pre-mRNA binding protein	85
Sox9	SRA1	Transcription factor	87
WTX	NA	Transcription factor	88
RNA polymerase II	NA	Transcription	86

situation is consistent with the above scenario, in which the p54nrb-containing complex recognizes A-to-I edited RNA and retains it in the nucleus. Indeed, p54nrb and another paraspeckle protein, PSP1, were shown to bind to CTN-RNA. Furthermore, artificial reporter mRNA containing an inverted repeat sequence of CTN-RNA has been shown to be retained in the nucleus. It is endonucleolytically cleaved at its 3' UTR in response to external stimuli, and then processed reporter mRNA is likely to be polyadenylated and exported to the cytoplasm where it is translated. CTN-RNA is the first identified natural paraspeckle-localized RNA; however, CTN-RNA is not capable of paraspeckle formation, in contrast to MEN epsilon/beta ncRNAs.

The mechanism underlying the nuclear retention of mRNA through the paraspeckle remains to be understood. Carmichael and his colleagues have reported that intact paraspeckle is required for the nuclear retention of several mRNAs that contain IRs in their 3' UTRs (43). One of these, Lin28 mRNA, was shown to be exported to the cytoplasm and translated in human embryonic stem cells (ES cells). Here, the expression of MEN epsilon/beta ncRNAs are silenced and paraspeckles are not formed. On the other hand, when the ES cells are induced to differentiate into the trophoblast, where MEN epsilon/beta ncRNAs are substantially expressed, intact paraspeckles are formed, and Lin28 mRNA is retained in the nucleus (43). These data provide the insight that MEN epsilon/beta ncRNA expression and concomitant paraspeckle formation is regulated under various physiological conditions, and, consistently, the nuclear retention of IR-containing mRNAs is controlled by the status of paraspeckle formation. Our recent report indicated that the expression of MEN epsilon/beta ncRNAs exhibited tissue-specific patterns in adult mouse tissues (42). Furthermore, a number of other reports also indicated dynamic regulation of MEN epsilon/beta ncRNA expression under various conditions, including myoblast differentiation, virus infection, and carcinogenesis (48, 50, 63), suggesting that nuclear retention of IR-containing mRNAs is regulated under such conditions.

5. MULTIPLE FUNCTIONS OF PARASPECKLE PROTEIN COMPONENTS

The paraspeckle structure is organized by cooperative interactions between MEN epsilon/beta

ncRNAs and multiple protein components. In other words, the paraspeckle proteins are able to localize to the paraspeckles through interaction with MEN epsilon/beta ncRNAs or other protein components. Intriguingly, one of the common features of paraspeckle proteins is that they rapidly dissociate from MEN epsilon/beta ncRNAs and relocate to the nucleolar periphery when RNA polymerase II transcription is arrested. In this condition, MEN epsilon/beta ncRNAs never relocate to the nucleolar periphery and instead diffuse in the nucleoplasm, suggesting that a common mechanism controls paraspeckle protein interactions with MEN epsilon ncRNA and MEN beta ncRNAs (47, 48). The paraspeckle protein components work not only for paraspeckle structure formation but also for paraspeckle function. The known protein components of paraspeckles (summarized in Table 2) and the events in which these proteins are involved are introduced.

5.1. DBHS protein family and RNA-binding proteins

The DBHS (*Drosophila melanogaster* behavior, human splicing) protein family includes PSP1, p54nrb, and PSF. These proteins are characteristically localized to paraspeckles (37-40, 44, 62). They are composed of similar conserved domains, including two RNA recognition motifs (RRMs) and a coiled coil domain (Figure 4A). The three proteins are capable of forming each combination of heterodimer (PSP1/p54nrb, p54nrb/PSF, and PSP1/PSF) that likely serves as part of the paraspeckle core (47). DBHS proteins are multi-functional nuclear proteins that are involved in a wide variety of gene expression events (64). The p54nrb/PSF complex plays roles in multiple steps of transcription, from initiation to termination, as well as in cotranscriptional RNA processing events. P54nrb and PSF are known to bind to the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (65). P54nrb/PSF acts as a coactivator or corepressor of transcription, and modulates the activity of transcription factors in response to various signals such as steroid hormones and cAMP (66-68). The p54nrb/PSF heterodimer interacts with Brm, the catalytic subunit of the SWI/SNF chromatin remodeling complex, and is involved in the transactivation of telomerase reverse transcriptase (TERT) gene transcription as well as the modulation of its pre-mRNA splicing pattern (69). P54nrb is a component of a huge ribonucleoprotein complex formed on the 5' splice site during the splicing

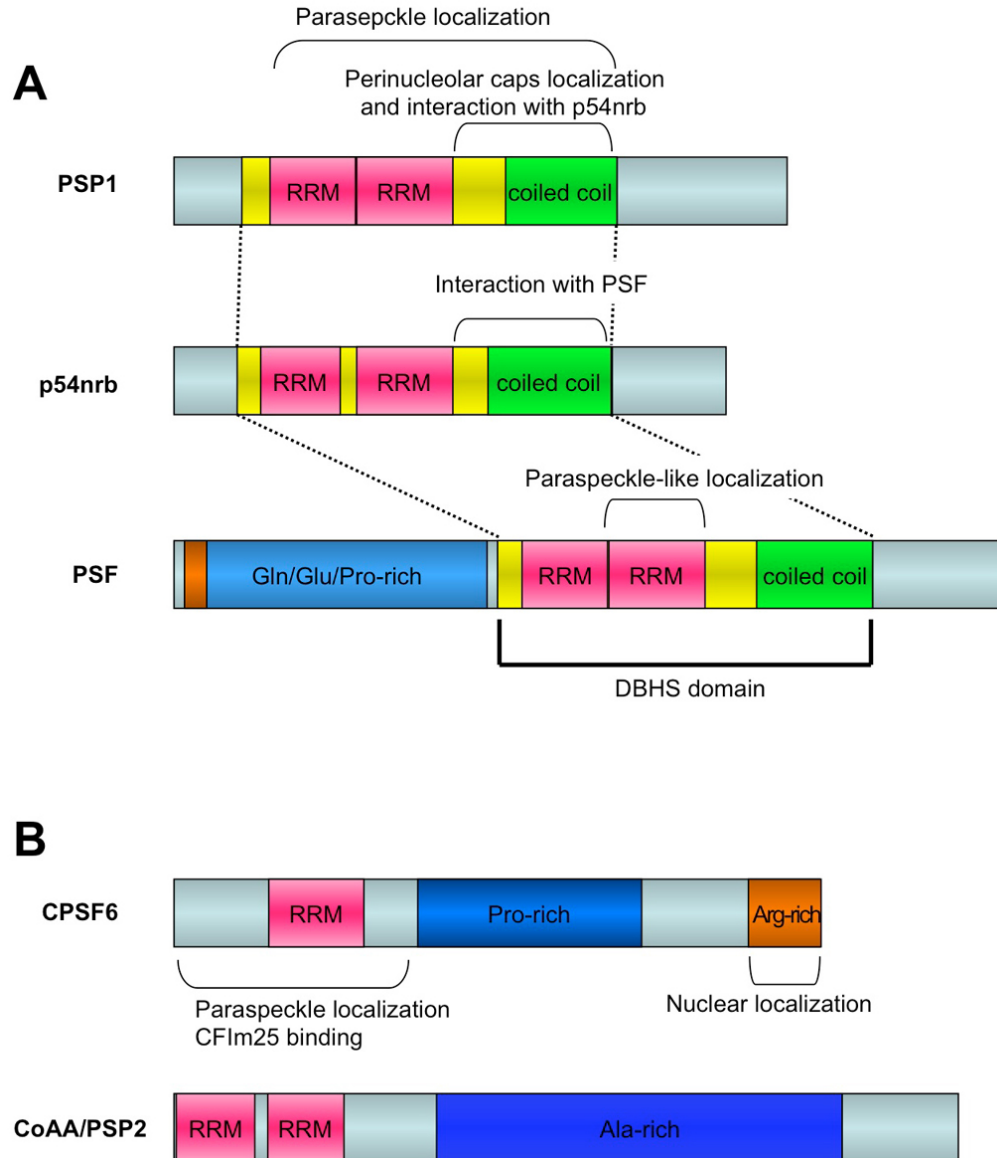


Figure 4. The domain structures of paraspeckle-localized DBHS proteins (A) and other RNA-binding proteins (B). The characteristic domains of each paraspeckle protein are shown. The functional regions that are involved in protein-protein interactions or intracellular localization are shown.

reaction (70). P54nrb and PSF are required for the coupling of the processes of transcriptional termination and mRNA 3' end processing, in which PSF recruits the Xrn2 exoribonuclease (71). Meanwhile, PSP1 exhibits the most prominent paraspeckle localization pattern, and PSP1 protein has been used as a major paraspeckle protein marker. However, the elimination of PSP1 using RNAi failed to change the integrity of paraspeckles (47). The functional domains of the DBHS proteins required for interaction and localization have been identified (Figure 4A): p54nrb binds PSF through its C-terminal region, which contains a coiled-coil domain (69, 72), while PSP1 binds p54nrb through its coiled-coil domain (44). Although the responsible domain remains unidentified, PSF also

binds PSP1 (73). The region containing RRM as well as the coiled-coil domain is required for the paraspeckle localization of PSP1 (44), while the second RRM is required for the localization of PSF in paraspeckle-like foci (74).

CoAA/PSP2 is identified as a paraspeckle component together with PSP1 and p54nrb (37). CoAA is known as a “coactivator activator” that coactivates transcription by association with the thyroid hormone receptor binding protein (TRBP) (75). CoAA contains two RRM that are sufficient for its regulatory function in transcriptional control. In contrast, the role of CoAA in paraspeckle formation and the domain required for

paraspeckle localization remain to be determined. Additional CoAA functions include pre-mRNA splicing regulation as well as transcription repression through the prevention of the function of Runt domain transcription factors (76, 77). CoAA is also known as SIP (SYT-interacting protein) and interacts with proto-oncoprotein SYT (synovial sarcoma translocation, also known as SS18), the DNA-dependent protein kinase subunit, 86 kDa subunit of Ku antigen (Ku86), and Poly-(ADP-ribose) polymerase (PARP) (75, 78). Intriguingly, CoAA pre-mRNA is regulated by the DBHS paraspeckle protein heterodimer p54nrb/PSF; transient expression of p54nrb/PSF switches the splicing pattern of CoAA mRNAs, resulting in the production of the dominant negative isoforms (79). Overexpression of CoAA promotes cell proliferation in the NIH3T3 stable cell line, while knockdown of CoAA downregulates cell proliferation in the NCI-H69 human lung cancer cell line (80). These aspects of the CoAA structure may also be related to the functions of the homologous domains of the oncoproteins EWS (Ewing's sarcoma) and TLS/FUS (translocation/fusion in liposarcoma), which are also known as regulators of RNA processing (78).

CPSF6 (cleavage and polyadenylation specificity factor 6, also known as CFIm68) is a paraspeckle protein component (81). CPSF6 is a subunit of the Pre-messenger RNA cleavage factor I (CFIm) complex, which is involved in mRNA 3' end processing. CFIm targets UGUAN sequences (N: arbitrary nucleotide) clustered upstream of the cleavage/polyadenylation site, which is followed by the recruitment of the catalytic CPSF complex (82). Recently, it was reported that the CFIm complex is involved in the regulation of alternative polyadenylation of various mRNA species (83). Deletion analysis of CPSF6 revealed that an RNA recognition motif is required for localization in paraspeckles and interaction with CPSF5/CFIm25, suggesting that CPSF6 associates with RNA component(s) in paraspeckles (Fig 4B, 81, 84). This raises the interesting possibility that CPSF6 is involved in the regulation of the 3' end processing of paraspeckle-localized RNAs. MEN epsilon/beta ncRNAs are comprised of two isoforms that are likely synthesized by alternative 3' end processing (Figure 2B); therefore, CFIm, including CPSF6, may be responsible for isoform synthesis of MEN epsilon/beta ncRNAs. Another possibility is that CPSF6 is involved in the processing of nuclear-retained mRNA. As described above, the long 3' UTR of CTN-RNA is processed in response to certain stresses (Figure 3). A CFIm-dependent RNA processing mechanism is a candidate for the processing of the CTN-RNA 3' UTR as well as other nuclear-retained mRNAs.

Finally, it was recently reported that heterogeneous nuclear ribonucleoprotein M (hnRNP M) interacts with p54nrb and PSF and colocalizes within defined nuclear structures that are probably paraspeckles (85). hnRNP M and PSF showed counteracting activity in alternative splicing for the inclusion of exon 4 of the preprotachykinin minigene transcript. Thus, several RNA-binding proteins that play multiple regulatory functions in transcriptional and posttranscriptional events are related to paraspeckles. These functions, in which paraspeckle proteins are involved, will be important in

the characterization of the physiological functions of paraspeckles.

5.2. Other protein components suggest paraspeckle functions

Several proteins other than RNA binding proteins have been reported to localize in paraspeckles. First, immunofluorescent observations have revealed that paraspeckles contain RNA polymerase II. However, paraspeckles are not considered to be sites of active transcription, since neither the elongating form of RNA polymerase II with a phosphorylated second serine (Ser 2P) in the heptad repeat (YSPTSPS) of CTD nor newly synthesized RNAs labeled by bromo-uridine incorporation has been detected in paraspeckles (86). It is noteworthy that active transcription may occur in the periphery of paraspeckles, suggesting the transient involvement of paraspeckles in transcriptional regulation (86). Indeed, several transcription factors have been detected to localize in paraspeckles. The transcription factor SRY (sex determining region Y)-box 9 (Sox9) interacts with p54nrb and colocalizes in paraspeckles in a murine chondrogenic cell line. Also, various functional analyses have revealed a link between p54nrb- and Sox9-mediated transcription and pre-mRNA splicing during chondrogenesis in mice (87). The nucleo-cytoplasmic shuttling protein WTX (an X chromosome gene commonly inactivated in Wilms tumor), which interacts with and modulates the transcription factor WT1 (Wilms tumor 1), is localized to paraspeckles (88). Although the significance remains to be determined, BCL11A-XL, the longest isoform of BCL11A acting as a DNA-sequence specific transcriptional repressor, and the interacting BCL6 proto-oncogene product colocalize in paraspeckles (89). Although paraspeckles are not the major sites of active transcription as described above, the presence of several transcription factors in paraspeckles suggests that either the transcription of limited gene loci occurs in paraspeckles or the repression of transcription of specific gene loci is localized on paraspeckles. It is also intriguing that several transcription factors are preassembled with RNA polymerase II before recruitment onto the chromosome.

Paraspeckles correspond to interchromatin granule-associated zones (IGAZs), which are observed by electron microscopy (41, 90). High resolution *in situ* hybridization analysis has shown that IGAZs contain U1 snRNA but not U2 snRNA, suggesting that IGAZs are the sites of U1 snRNP maturation rather than the sites of pre-mRNA splicing (41). Recently, it was reported that U1 snRNA has a distinct function from that involved in splicing, and that U1 snRNP prevents aberrant polyadenylation within introns (91). It is possible that U1 snRNA acts in the synthesis of non-truncated MEN epsilon/beta ncRNAs in paraspeckles.

6. HOW ARE PARASPECKLES BUILT ON NCRNAS?

The present review has described RNA and protein components of paraspeckles and also their known functions. These aspects may be useful in the exploration of

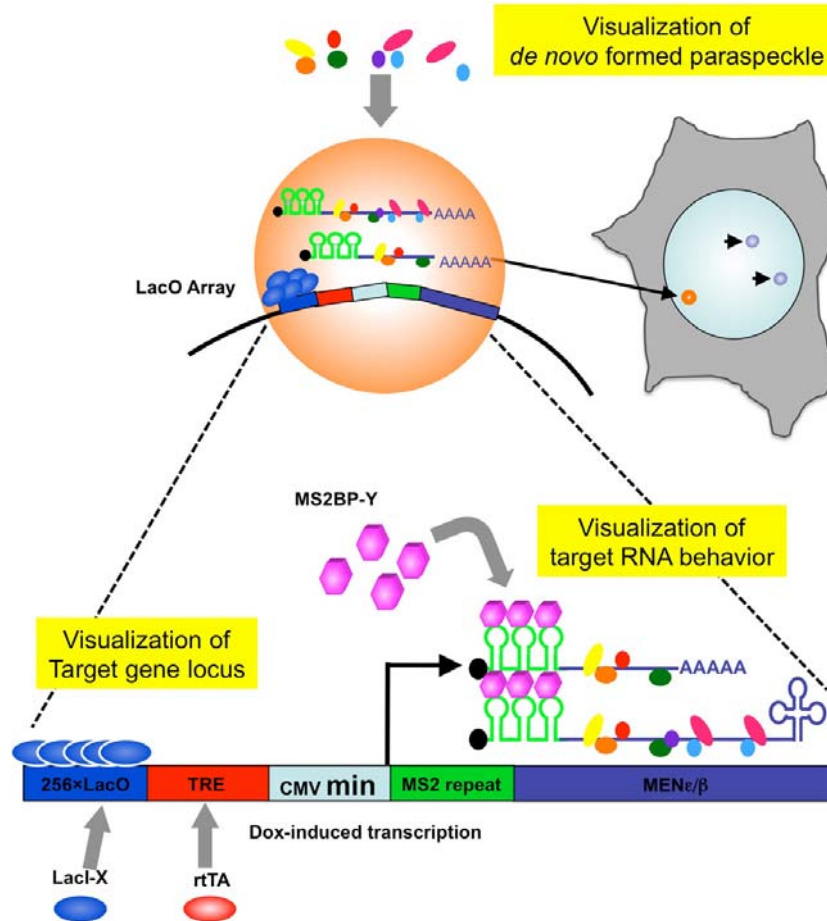


Figure 5. Detection of *de novo* synthesis of paraspeckle at the MEN epsilon/beta ncRNAs transcribing locus. The position of the artificial MEN epsilon/beta gene locus is marked by the binding of a fluorescent protein fused with the Lac repressor (Lac I-X). Lac I-X binds to the integrated 256x tandem repeats of Lac operator (Lac O) sequences, which are referred to as the Lac O array. Transcription of MEN epsilon/beta ncRNAs is driven by a tetracycline-induced promoter (TRE) which is transactivated by the tetracycline-dependent transactivator, rtTA. Doxycycline (dox) is a tetracycline derivative. The synthesized MEN epsilon/beta ncRNAs are tagged with MS2 phage coat protein-binding sequences (MS2-binding sequence) that can be visualized by the binding of fluorescent protein fused to MS2 coat protein (MS2BP-Y) to these sequences. The *de novo* paraspeckle signal is superimposed onto the Lac I signal, which can be recognized as the integrated genomic site of the artificial MEN epsilon/beta gene.

the biological roles of paraspeckles. We would now like to integrate the knowledge described above and consider the prerequisite steps of paraspeckle construction from the multiple components. The paraspeckle is an RNase-sensitive nuclear body that is built on MEN epsilon/beta ncRNAs. It can therefore be considered to be a huge ribonucleoprotein complex. Electron microscopic observation-based estimates indicate that the average size of paraspeckles is ~0.36 micrometer in diameter (90), more than 1,500 times larger than the ribosome, a classical ribonucleoprotein complex. The biogenesis process underlying the construction of intact ribosomes is extremely complicated, with many of the details not yet understood. Briefly, however, ribosome biogenesis begins with the transcription of pre-rRNA by RNA polymerase I and is followed by the processing and modification of pre-rRNA (92). Multiple ribosomal proteins may assemble onto the pre-rRNA molecule cotranscriptionally and/or

posttranscriptionally in a specific order (92). How does paraspeckle assembly proceed? First, paraspeckle formation is initiated by the transcription of MEN epsilon/beta ncRNAs by RNA polymerase II from the corresponding locus on human chromosome 11. Combined DNA-FISH and RNA-FISH analyses show that subpopulations of MEN epsilon/beta ncRNAs localize to the MEN epsilon/beta genomic locus in interphase HeLa cell nuclei, indicating that the initial stage of paraspeckle assembly occurs in close proximity to the MEN epsilon/beta chromosomal locus (46). Spector and his colleagues recently demonstrated that the ongoing transcription of MEN epsilon/beta ncRNAs is required for paraspeckle formation (93). In their analyses, an artificial MEN epsilon/beta gene conjugated with a LacO array was integrated onto a specific chromosomal locus (Figure 5). Paraspeckle formation was observed on the visualized chromosomal locus, marked by the LacO array, where

transcription of the designed MEN epsilon/beta ncRNAs was induced. Importantly, no paraspeckle formation was detectable if the MS2-tagged MEN epsilon/beta ncRNAs were tethered onto the specific chromosomal locus (Figure 5). Taken together, it was concluded that ongoing transcription of MEN epsilon/beta ncRNAs is required for initial paraspeckle formation on the corresponding chromosomal locus. It should be noted that the majority of paraspeckle foci are detected outside of MEN epsilon/beta chromosomal loci, indicating that the assembled paraspeckles either dissociate from the chromosomal locus of origin and then diffuse randomly or are transported to a programmed specific location in the nucleoplasm. The identification of the detailed locations of released paraspeckles as well as the investigation of their association with specific chromosomal loci would represent an intriguing avenue of inquiry.

The next important point regarding paraspeckle formation is the role of each isoform of MEN epsilon/beta ncRNAs. Two paraspeckle proteins, p54nrb and PSF, are essential for paraspeckle formation, since the elimination of either of them leads to the disintegration of the paraspeckles. Importantly, under the same conditions, it has been clearly shown that MEN beta ncRNA can become destabilized and disappear while MEN epsilon ncRNA remains stably accumulated (47). Furthermore, immunoprecipitation experiments have shown that PSF and p54nrb selectively bind the MEN beta ncRNA. These data strongly suggest that MEN beta is an essential ncRNA isoform for paraspeckle formation, and that the interaction of p54nrb and PSF with the MEN beta ncRNA serves to sustain the paraspeckle structure (Figure 6)(40, 47). Meanwhile, it has been reported that MEN epsilon ncRNA interacts with p54nrb and PSP1 *in vivo* and *in vitro* (46, 94) and that RRM of PSP1 are required for the colocalization with MEN epsilon ncRNA in paraspeckles (46), although MEN epsilon and MEN beta isoforms were not distinguished from each other in these reports. Spector and his colleagues have reported that p54nrb coimmunoprecipitates with both MEN epsilon ncRNA and MEN beta ncRNA (48). In addition, it has been reported that a MEN epsilon ncRNA-overexpressing stable cell line increases the number of paraspeckle foci in accordance with MEN epsilon ncRNA expression level (46). These data suggest facilitative and decorative functions for MEN epsilon ncRNA in concert with DBHS proteins in paraspeckle organization. Furthermore, they are consistent with the electron microscopic observation of MEN epsilon ncRNA localization at the paraspeckle periphery (90), which raises the possibility that the MEN epsilon ncRNA may be required for functional execution occurring at the paraspeckle periphery rather than paraspeckle structural organization. Electron microscopic observations also indicate that the 3' terminal region of MEN beta ncRNA, as well as the MEN epsilon/beta ncRNA overlapping region, localizes in the paraspeckle periphery, while the middle region of MEN beta ncRNA is localized in the interior of the paraspeckle (Figure 6, 90). This observation is consistent with our model postulating that the MEN beta ncRNA is critical to the maintenance of paraspeckle structural integrity. This model is also supported by our

recent observation that paraspeckles are formed only in limited adult mouse tissues in which the MEN beta ncRNA is highly expressed (42). To clarify the significance of each isoform, it is obviously important to attempt a rescue experiment of either the MEN epsilon ncRNA or the MEN beta ncRNA in cells in which MEN epsilon/beta ncRNAs has been eliminated.

The assignment of the functions of the MEN epsilon/beta ncRNA isoforms in paraspeckle formation raises the importance of alternative 3' end processing, which leads to the creation of the two MEN epsilon/beta ncRNA isoforms. As described above, paraspeckle-localized CPSF6 is presumably involved in MEN epsilon ncRNA production though facilitating 3' end processing. Another factor, which would serve to counteract the effect of CPSF6, would arrest MEN epsilon ncRNA 3' end processing, and this would eventually lead to the synthesis of the MEN beta ncRNA. A search for additional paraspeckle protein components will identify such an additional essential factor(s) for paraspeckle formation.

Are MEN beta ncRNA and its stabilizing protein factors, such as p54nrb and PSF, sufficient for paraspeckle formation? Careful observation of paraspeckles and the related accumulation levels of MEN beta ncRNA suggest that certain mouse tissues and a few cultured cell lines exhibit an absence of intact paraspeckles, in spite of the substantial accumulation of the MEN beta ncRNA. This suggests that MEN beta ncRNA accumulation is required but not sufficient for paraspeckle formation (42, Kawaguchi *et al.*, unpublished). Additional factors may be required to mediate the assembly of individual ribonucleoprotein subcomplexes containing either MEN beta ncRNA or MEN epsilon ncRNA. Another intriguing possibility is that posttranslational modifications, such as phosphorylation, acetylation, and methylation could modulate characteristics of paraspeckle proteins, such as properties related to interaction with MEN epsilon/beta ncRNAs or other paraspeckle proteins. Indeed, various posttranslational modifications have been reported in p54nrb, PSF, PSP1, and CoAA paraspeckle proteins (95-99). This mechanism may also account for the drastic disintegration of paraspeckles upon actinomycin D treatment through the dissociation of paraspeckle proteins from MEN epsilon/beta ncRNAs (Figure 1D).

7. INTRACELLULAR STRUCTURES BUILT WITH RNA MOLECULES

Several cellular bodies are known to contain RNAs, and it is intriguing to explore additional RNA molecules that may play architectural roles. Thermal and various chemical stresses induce the formation of transient nuclear structures called nuclear stress bodies (nSBs) (35). These nuclear bodies contain the transcription factor Heat shock factor protein 1 (HSF1) and a specific subset of pre-mRNA splicing factors. nSBs are assembled on specific pericentromeric heterochromatic domains containing satellite III (SatIII) DNA. In response to stress, these domains change their epigenetic status from heterochromatin to euchromatin, and subsequently

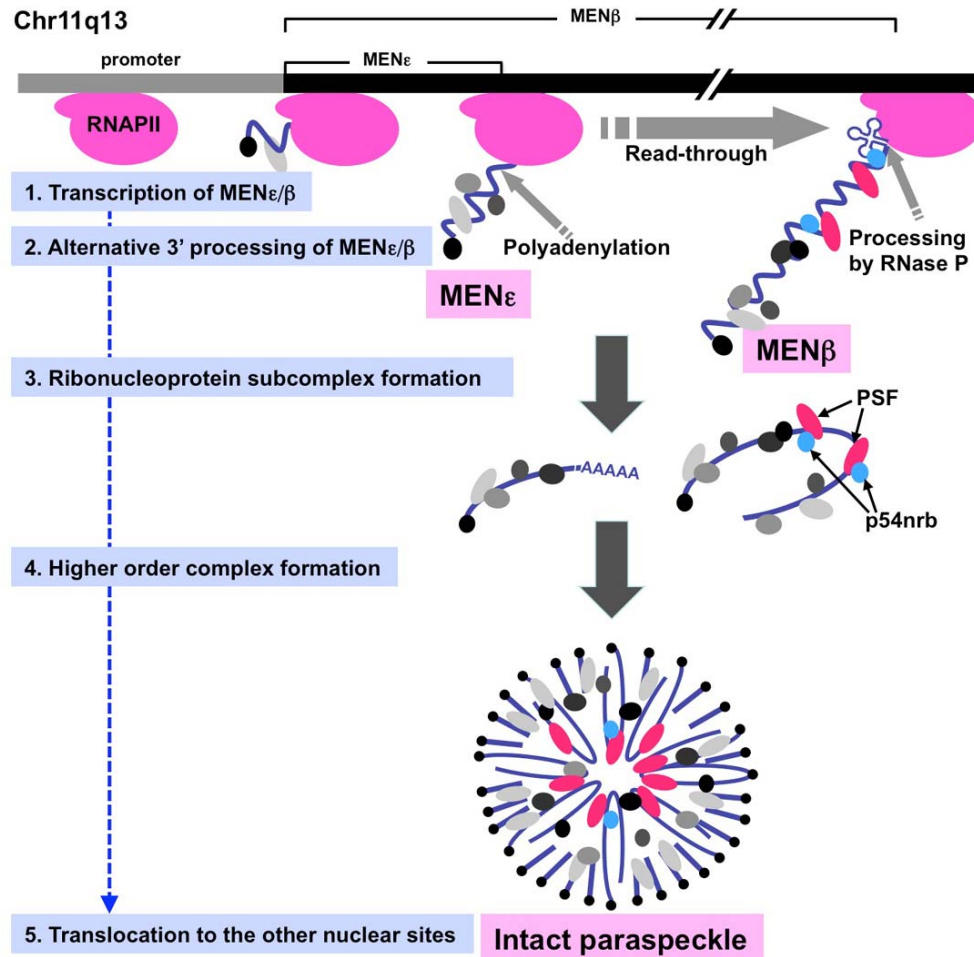


Figure 6. The current model of paraspeckle structural formation. The required steps for intact paraspeckle formation are summarized on the left. First, MEN epsilon/beta ncRNA transcription needs to be initiated by RNA polymerase II (RNAPII). Some of the paraspeckle proteins are thought to associate with nascent MEN epsilon/beta ncRNAs cotranscriptionally. Canonical 3' end processing and polyadenylation takes place 3.7 kb from the 5' end to produce the MEN epsilon ncRNA; however, this processing is relatively inefficient, resulting in the occurrence of transcriptional readthrough to produce the MEN beta ncRNA. At the end of MEN beta ncRNA, a tRNA-like structure acts as a signal for processing with RNase P. The MEN beta ncRNA preferentially binds with DBHS proteins (p54nrb and PSF), resulting in the formation of a putative MEN beta ribonucleoprotein subcomplex, while MEN epsilon ncRNA is considered to form its own ribonucleoprotein subcomplex. These two subcomplexes are bundled to form a higher order complex in which the 5' terminal and 3' terminal regions of MEN beta ncRNA as well as MEN epsilon ncRNA are located at the paraspeckle periphery.

transcribe polyadenylated SatIII RNAs that remain associated with the nSBs. The elimination of SatIII RNAs significantly affects the recruitment of RNA splicing factors to nSBs without altering the association of HSF1 with these structures. Recently, the tethering of MS2-tagged RNA revealed that SatIII RNA could initiate *de novo* formation of nSBs (100), indicating that SatIII RNAs play a major role in the formation of functional nSBs.

RNA has a structural role in the organization of the cytoskeleton and the mitotic spindle. In *Xenopus* oocytes, short interspersed repeat transcripts (Xlirts ncRNA) and mRNA for T-box factor from the vegetally localized transcript (VegT) are integrated within the cytoskeleton and are required for the proper organization of

the cytoskeleton (101, 102). The elimination of either transcript disrupts the cytoskeleton, but not the actin cytoskeleton. Interestingly, VegT mRNA plays a role as a nontranslated RNA, since blocking its translation had no effect on the cytoskeleton network (102, 103). Mitotic spindles have been found to associate with various RNA species, including ribosomal RNAs and a number of uncharacterized transcripts (104). RNase treatment, but not translation inhibitors, disrupts spindle assembly and causes the spindle to collapse, indicating that the associated RNAs play an important role in spindle assembly during M phase (104). Further research will reveal additional ncRNAs as well as mRNAs that play architectural roles in organizing intracellular structures. The functional analysis of these RNA-containing structures and their assembly process will

expand our understanding of the biological significance of RNA as a structural molecule.

7. SUMMARY AND PERSPECTIVES

The paraspeckle is a unique nuclear body that is constructed on MEN epsilon/beta ncRNAs. This nuclear body contains multiple characteristic RNA-binding proteins that play various transcriptional and posttranscriptional regulatory events. These protein factors are likely integrated by MEN epsilon/beta ncRNAs to form the functional paraspeckle. Paraspeckles play important roles in the retention of mRNA subsets in the nucleus, in processes which may be regulated by certain signals. It is important to investigate whether natural nuclear-retained mRNAs are localized to paraspeckles or not, since CTN-RNA is the only natural mRNA whose localization to paraspeckles has been confirmed. For the formation of the paraspeckle core, the ongoing transcription of MEN epsilon/beta ncRNAs, alternative 3' RNA processing of MEN epsilon/beta ncRNAs, and subsequent association of DBHS RNA binding proteins, including PSF and p54nrb, with the longer MEN beta ncRNA must occur in a programmed order. Other paraspeckle proteins, including PSP1 and the shorter MEN epsilon ncRNA, may join the core ribonucleoprotein complex to form intact paraspeckles. Future research on paraspeckles has the important potential to elucidate the following biological concepts: [1] new roles for long ncRNA; [2] new construction processes for intracellular structures; and [3] new regulatory mechanisms through intracellular structures. Further dissection of RNA-protein interactions and their modulation under various physiological conditions would be advantageous for the exploration of the concepts listed above. The identification of additional paraspeckle components and the investigation of each component would also be required to achieve a solid understanding of the process of paraspeckle formation. Animal models in which paraspeckles have been removed by blocking the expression of MEN epsilon/beta ncRNA by gene targeting would serve as a useful tool for the investigation of the physiological roles of this nuclear body.

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9. REFERENCES

1. P Bertone, V Stolc, TE Royce, JS Rozowsky, AE Urban, X Zhu, JL Rinn, W Tongprasit, M Samanta, S Weissman, M Gerstein, M Snyder: Global identification of human transcribed sequences with genome tiling arrays.. *Science* 306:2242-2246(2004)
2. P Carninci, T Kasukawa, S Katayama, J Gough, MC Frith, N Maeda, R Oyama, T Ravasi, B Lenhard, C Wells, R Kodzius, K Shimokawa, VB Bajic, SE Brenner, S

Batalov, AR Forrest, M Zavolan, MJ Davis, LG Wilming, V Aidinis, JE Allen, A Ambesi-Impiombato, R Apweiler, RN Aturaliya, TL Bailey, M Bansal, L Baxter, KW Beisel, T Bersano, H Bono, AM Chalk, KP Chiu, V Choudhary, A Christoffels, DR Clutterbuck, ML Crowe, E Dalla, BP Dalrymple, B de Bono, G Della Gatta, D di Bernardo, T Down, P Engstrom, M Fagiolini, G Faulkner, CF Fletcher, T Fukushima, M Furuno, S Futaki, M Gariboldi, P Georgii-Hemming, TR Gingeras, T Gojobori, RE Green, S Gustincich, M Harbers, Y Hayashi, TK Hensch, N Hirokawa, D Hill, L Huminiecki, M Iacono, K Ikeo, A Iwama, T Ishikawa, M Jakt, A Kanapin, M Katoh, Y Kawasaki, J Kelso, H Kitamura, H Kitano, G Kollias, SP Krishnan, A Kruger, SK Kummerfeld, IV Kurochkin, LF Lareau, D Lazarevic, L Lipovich, J Liu, S Liuni, S McWilliam, M Madan Babu, M Madera, L Marchionni, H Matsuda: The transcriptional landscape of the mammalian genome.. *Science* 309:1559-1563(2005)

3. Z Cheng, M Ventura, X She, P Khaitovich, T Graves, K Osogawa, D Church, P DeJong, RK Wilson, S Paabo, M Rocchi, EE Eichler: A genome-wide comparison of recent chimpanzee and human segmental duplications.. *Nature* 437:88-93(2005)

4. S Katayama, Y Tomaru, T Kasukawa, K Waki, M Nakanishi, M Nakamura, H Nishida, CC Yap, M Suzuki, J Kawai, H Suzuki, P Carninci, Y Hayashizaki, C Wells, M Frith, T Ravasi, KC Pang, J Hallinan, J Mattick, DA Hume, L Lipovich, S Batalov, PG Engstrom, Y Mizuno, MA Faghihi, A Sandelin, AM Chalk, S Mottagui-Tabar, Z Liang, B Lenhard, C Wahlestedt: Antisense transcription in the mammalian transcriptome.. *Science* 309:1564-1566(2005)

5. The ENCODE Project Consortium: Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project.. *Nature* 447:799-816(2007)

6. P Kapranov, J Cheng, S Dike, DA Nix, R Duttagupta, AT Willingham, PF Stadler, J Hertel, J Hackermuller, IL Hofacker, I Bell, E Cheung, J Drenkow, E Dumais, S Patel, G Helt, M Ganesh, S Ghosh, A Piccolboni, V Sementchenko, H Tammana, TR Gingeras: RNA maps reveal new RNA classes and a possible function for pervasive transcription.. *Science* 316:1484-1488(2007)

7. KV Prasanth, DL Spector: Eukaryotic regulatory RNAs: an answer to the 'genome complexity' conundrum.. *Genes Dev* 21:11-42(2007)

8. O Yazgan, JE Krebs: Noncoding but nonexpendable: transcriptional regulation by large noncoding RNA in eukaryotes.. *Biochem Cell Biol* 85:484-496(2007)

9. R Kurokawa, MG Rosenfeld, CK Glass: Transcriptional regulation through noncoding RNAs and epigenetic modifications.. *RNA Biol* 6:233-236(2009)

10. JE Wilusz, H Sunwoo, DL Spector: Long noncoding RNAs: functional surprises from the RNA world.. *Genes Dev* 23:1494-1504(2009)

Noncoding RNA-dependent formation of nuclear body structure

11. LL Chen, GG Carmichael: Decoding the function of nuclear long non-coding RNAs.. *Curr Opin Cell Biol* 22:357-364(2010)
12. X Wang, X Song, CK Glass, MG Rosenfeld: The Long Arm of Long Noncoding RNAs: Roles as Sensors Regulating Gene Transcriptional Programs.. *Cold Spring Harb Perspect Biol.* 2010 Jun 23.(2010)
13. MB Clark, JS Mattick: Long noncoding RNAs in cell biology.. *Semin Cell Dev Biol.* 2011 Jan 20.(2011)
14. M Sone, T Hayashi, H Tarui, K Agata, M Takeichi, S Nakagawa: The mRNA-like noncoding RNA Gomafu constitutes a novel nuclear domain in a subset of neurons.. *J Cell Sci* 120:2498-2506(2007)
15. JN Hutchinson, AW Ensminger, CM Clemson, CR Lynch, JB Lawrence, A Chess: A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains.. *BMC Genomics* 8:39(2007)
16. P Ji, S Diederichs, W Wang, S Boing, R Metzger, PM Schneider, N Tidow, B Brandt, H Buerger, E Bulk, M Thomas, WE Berdel, H Serve, C Muller-Tidow: MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer.. *Oncogene* 22:8031-8041(2003)
17. V Tripathi, JD Ellis, Z Shen, DY Song, Q Pan, AT Watt, SM Freier, CF Bennett, A Sharma, PA Bubulya, BJ Blencowe, SG Prasanth, KV Prasanth: The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation.. *Mol Cell* 39:925-938(2010)
18. D Bernard, KV Prasanth, V Tripathi, S Colasse, T Nakamura, Z Xuan, MQ Zhang, F Sedel, L Jourden, F Culpier, A Triller, DL Spector, A Bessis: A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression.. *EMBO J* 29:3082-3093(2010)
19. DL Spector: SnapShot: Cellular bodies.. *Cell* 127:1071(2006)
20. DL Spector: Nuclear domains.. *J Cell Sci* 114:2891-2893(2001)
21. RI Kumaran, R Thakar, DL Spector: Chromatin dynamics and gene positioning.. *Cell* 132:929-934(2008)
22. S Boulon, BJ Westman, S Hutten, FM Boisvert, AI Lamond: The nucleolus under stress.. *Mol Cell* 40:216-227(2010)
23. T Pederson: The Nucleolus.. *Cold Spring Harb Perspect Biol.* 2010 Nov 24.(2010)
24. Ramon y Cajal, SUn sencillo metodo de coloracion selectiva del reticulo protoplasmico y sus efectos en los diversos organos nerviosos de vertebrados e invertebrados. *Trab. Lab. Invest. Biol* 2:129-221. (1903)
25. M Cioce, AI Lamond: Cajal bodies: a long history of discovery.. *Annu Rev Cell Dev Biol* 21:105-131(2005)
26. Z Nizami, S Deryusheva, JG Gall: The Cajal body and histone locus body.. *Cold Spring Harb Perspect Biol* 2:a000653(2010)
27. WF Marzluff, EJ Wagner, RJ Duronio: Metabolism and regulation of canonical histone mRNAs: life without a poly(A) tail.. *Nat Rev Genet* 9:843-854(2008)
28. Q Liu, G Dreyfuss: A novel nuclear structure containing the survival of motor neurons protein.. *EMBO J* 15:3555-3565(1996)
29. AI Lamond, DL Spector: Nuclear speckles: a model for nuclear organelles.. *Nat Rev Mol Cell Biol* 4:605-612(2003)
30. DL Spector, AI Lamond: Nuclear Speckles.. *Cold Spring Harb Perspect Biol.* 2010 Oct 6.(2010)
31. Q Hu, YS Kwon, E Nunez, MD Cardamone, KR Hutt, KA Ohgi, I Garcia-Bassets, DW Rose, CK Glass, MG Rosenfeld, XD Fu: Enhancing nuclear receptor-induced transcription requires nuclear motor and LSD1-dependent gene networking in interchromatin granules.. *Proc Natl Acad Sci U S A* 105:19199-19204(2008)
32. T Chen, FM Boisvert, DP Bazett-Jones, S Richard: A role for the GSG domain in localizing Sam68 to novel nuclear structures in cancer cell lines.. *Mol Biol Cell* 10:3015-3033(1999)
33. M Carmo-Fonseca, MT Berciano, M Lafarga: Orphan nuclear bodies.. *Cold Spring Harb Perspect Biol* 2:a000703(2010)
34. G Biamonti, C Vourc'h: Nuclear stress bodies.. *Cold Spring Harb Perspect Biol* 2:a000695(2010)
35. I Chiodi, M Corioni, M Giordano, R Valgardsdottir, C Ghigna, F Cobianni, RM Xu, S Riva, G Biamonti: RNA recognition motif 2 directs the recruitment of SF2/ASF to nuclear stress bodies.. *Nucleic Acids Res* 32:4127-4136,
36. V Lallemand-Breitenbach, H de The: PML nuclear bodies.. *Cold Spring Harb Perspect Biol* 2:a000661(2010)
37. AH Fox, YW Lam, AK Leung, CE Lyon, J Andersen, M Mann, AI Lamond: Paraspeckles: a novel nuclear domain.. *Curr Biol* 12:13-25(2002)
38. CS Bond, AH Fox: Paraspeckles: nuclear bodies built on long noncoding RNA.. *J Cell Biol* 186:637-644(2009)
39. AH Fox, AI Lamond: Paraspeckles.. *Cold Spring Harb Perspect Biol* 2:a000687(2010)
40. YT Sasaki, T Hirose: How to build a paraspeckle.. *Genome Biol* 10:227(2009)

Noncoding RNA-dependent formation of nuclear body structure

41. N Visa, F Puvion-Dutilleul, JP Bachellerie, E Puvion: Intracellular distribution of U1 and U2 snRNAs visualized by high resolution *in situ* hybridization: revelation of a novel compartment containing U1 but not U2 snRNA in HeLa cells.. *Eur J Cell Biol* 60:308-321(1993)
42. S Nakagawa, T Naganuma, G Shioi, T Hirose: Paraspeckles are subpopulation-specific nuclear bodies that are not essential in mice.. *J Cell Biol* 193:31-39(2011)
43. LL Chen, GG Carmichael: Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: functional role of a nuclear noncoding RNA.. *Mol Cell* 35:467-478(2009)
44. AH Fox, CS Bond, AI Lamond: P54nrb forms a heterodimer with PSP1 that localizes to paraspeckles in an RNA-dependent manner.. *Mol Biol Cell* 16:5304-5315(2005)
45. Y Yamaguchi, T Takagi, T Wada, K Yano, A Furuya, S Sugimoto, J Hasegawa, H Handa: NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation.. *Cell* 97:41-51(1999)
46. CM Clemson, JN Hutchinson, SA Sara, AW Ensminger, AH Fox, A Chess, JB Lawrence: An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles.. *Mol Cell* 33:717-726(2009)
47. YT Sasaki, T Ideue, M Sano, T Mituyama, T Hirose: MENepsilon/beta noncoding RNAs are essential for structural integrity of nuclear paraspeckles.. *Proc Natl Acad Sci U S A* 106:2525-2530(2009)
48. H Sunwoo, ME Dinger, JE Wilusz, PP Amaral, JS Mattick, DL Spector: MEN epsilon/beta nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles.. *Genome Res* 19:347-359(2009)
49. SC Guru, SK Agarwal, P Manickam, SE Olufemi, JS Crabtree, JM Weisemann, MB Kester, YS Kim, Y Wang, MR Emmert-Buck, LA Liotta, AM Spiegel, MS Boguski, BA Roe, FS Collins, SJ Marx, L Burns, SC Chandrasekharappa: A transcript map for the 2.8-Mb region containing the multiple endocrine neoplasia type 1 locus.. *Genome Res* 7:725-735(1997)
50. S Saha, S Murthy, PN Rangarajan: Identification and characterization of a virus-inducible non-coding RNA in mouse brain.. *J Gen Virol* 87:1991-1995(2006)
51. JA Peyman: Repression of major histocompatibility complex genes by a human trophoblast ribonucleic acid.. *Biol Reprod* 60:23-31(1999)
52. A Geirsson, RJ Lynch, I Paliwal, AL Bothwell, GL Hammond: Human trophoblast noncoding RNA suppresses CIITA promoter III activity in murine B-lymphocytes.. *Biochem Biophys Res Commun* 301:718-724(2003)
53. G Dreyfuss, VN Kim, N Kataoka: Messenger-RNA-binding proteins and the messages they carry.. *Nat Rev Mol Cell Biol* 3:195-205(2002)
54. JE Wilusz, SM Freier, DL Spector: 3' end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA.. *Cell* 135:919-932(2008)
55. NK Conrad, JA Steitz: A Kaposi's sarcoma virus RNA element that increases the nuclear abundance of intronless transcripts.. *EMBO J* 24:1831-1841(2005)
56. RM Mitton-Fry, SJ DeGregorio, J Wang, TA Steitz, JA Steitz: Poly(A) tail recognition by a viral RNA element through assembly of a triple helix.. *Science* 330:1244-1247(2010)
57. K Nishikura: Functions and regulation of RNA editing by ADAR deaminases.. *Annu Rev Biochem* 79:321-349(2010)
58. K Nishikura: Editor meets silencer: crosstalk between RNA editing and RNA interference.. *Nat Rev Mol Cell Biol* 7:919-931(2006)
59. M Kumar, GG Carmichael: Nuclear antisense RNA induces extensive adenosine modifications and nuclear retention of target transcripts.. *Proc Natl Acad Sci U S A* 94:3542-3547(1997)
60. Z Zhang, GG Carmichael: The fate of dsRNA in the nucleus: a p54(nrb)-containing complex mediates the nuclear retention of promiscuously A-to-I edited RNAs.. *Cell* 106:465-475(2001)
61. LL Chen, JN DeCervo, GG Carmichael: Alu element-mediated gene silencing.. *EMBO J* 27:1694-1705(2008)
62. KV Prasanth, SG Prasanth, Z Xuan, S Hearn, SM Freier, CF Bennett, MQ Zhang, DL Spector: Regulating gene expression through RNA nuclear retention.. *Cell* 123:249-263(2005)
63. YS Kim, JD Hwan, S Bae, DH Bae, WA Shick: Identification of differentially expressed genes using an annealing control primer system in stage III serous ovarian carcinoma.. *BMC Cancer* 10:576(2010)
64. Y Shav-Tal, D Zipori: PSF and p54(nrb)/NonO--multi-functional nuclear proteins.. *FEBS Lett* 531:109-114(2002)
65. A Emili, M Shales, S McCracken, W Xie, PW Tucker, R Kobayashi, BJ Blencowe, CJ Ingles: Splicing and transcription-associated proteins PSF and p54nrb/nonO bind to the RNA polymerase II CTD.. *RNA* 8:1102-1111(2002)
66. K Ishitani, T Yoshida, H Kitagawa, H Ohta, S Nozawa, S Kato: p54nrb acts as a transcriptional coactivator for activation function 1 of the human androgen receptor.. *Biochem Biophys Res Commun* 306:660-665(2003)

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67. AL Amelio, LJ Miraglia, JJ Conkright, BA Mercer, S Batalov, V Cavett, AP Orth, J Busby, JB Hogenesch, MD Conkright: A coactivator trap identifies NONO (p54nrb) as a component of the cAMP-signaling pathway.. *Proc Natl Acad Sci U S A* 104:20314-20319(2007)
68. X Dong, J Sweet, JR Challis, T Brown, SJ Lye: Transcriptional activity of androgen receptor is modulated by two RNA splicing factors, PSF and p54nrb.. *Mol Cell Biol* 27:4863-4875(2007)
69. T Ito, H Watanabe, N Yamamichi, S Kondo, T Tando, T Haraguchi, T Mizutani, K Sakurai, S Fujita, T Izumi, T Isobe, H Iba: Brm transactivates the telomerase reverse transcriptase (TERT) gene and modulates the splicing patterns of its transcripts in concert with p54(nrb).. *Biochem J* 411:201-209(2008)
70. S Kameoka, P Duque, MM Konarska: p54(nrb) associates with the 5' splice site within large transcription/splicing complexes.. *EMBO J* 23:1782-1791(2004)
71. S Kaneko, O Rozenblatt-Rosen, M Meyerson, JL Manley: The multifunctional protein p54nrb/PSF recruits the exonuclease XRN2 to facilitate pre-mRNA 3' processing and transcription termination.. *Genes Dev* 21:1779-1789(2007)
72. R Peng, BT Dye, I Perez, DC Barnard, AB Thompson, JG Patton: PSF and p54nrb bind a conserved stem in U5 snRNA.. *RNA* 8:1334-1347(2002)
73. R Myojin, S Kuwahara, T Yasaki, T Matsunaga, T Sakurai, M Kimura, S Uesugi, Y Kurihara: Expression and functional significance of mouse paraspeckle protein 1 on spermatogenesis.. *Biol Reprod* 71:926-932(2004)
74. BT Dye, JG Patton: An RNA recognition motif (RRM) is required for the localization of PTB-associated splicing factor (PSF) to subnuclear speckles.. *Exp Cell Res* 263:131-144(2001)
75. T Iwasaki, WW Chin, L Ko: Identification and characterization of RRM-containing coactivator activator (CoAA) as TRBP-interacting protein, and its splice variant as a coactivator modulator (CoAM).. *J Biol Chem* 276:33375-33383(2001)
76. X Li, LH Hoepfner, ED Jensen, R Gopalakrishnan, JJ Westendorf: Co-activator activator (CoAA) prevents the transcriptional activity of Runt domain transcription factors.. *J Cell Biochem* 108:378-387(2009)
77. D Auboeuf, A Honig, SM Berget, BW O'Malley: Coordinate regulation of transcription and splicing by steroid receptor coregulators.. *Science* 298:416-419(2002)
78. M Perani, P Antonson, R Hamoudi, CJ Ingram, CS Cooper, MD Garrett, GH Goodwin: The proto-oncoprotein SYT interacts with SYT-interacting protein/co-activator activator (SIP/CoAA), a human nuclear receptor co-activator with similarity to EWS and TLS/FUS family of proteins.. *J Biol Chem* 280:42863-42876(2005)
79. Z Yang, Y Sui, S Xiong, SS Liour, AC Phillips, L Ko: Switched alternative splicing of oncogene CoAA during embryonal carcinoma stem cell differentiation.. *Nucleic Acids Res* 35:1919-1932(2007)
80. Y Sui, Z Yang, S Xiong, L Zhang, KL Blanchard, SC Peiper, WS Dynan, D Tuan, L Ko: Gene amplification and associated loss of 5' regulatory sequences of CoAA in human cancers.. *Oncogene* 26:822-835(2007)
81. S Dettwiler, C Aringhieri, S Cardinale, W Keller, SM Barabino: Distinct sequence motifs within the 68-kDa subunit of cleavage factor Im mediate RNA binding, protein-protein interactions, and subcellular localization.. *J Biol Chem* 279:35788-35797(2004)
82. KM Brown, GM Gilmartin: A mechanism for the regulation of pre-mRNA 3' processing by human cleavage factor Im.. *Mol Cell* 12:1467-1476(2003)
83. T Kubo, T Wada, Y Yamaguchi, A Shimizu, H Handa: Knock-down of 25 kDa subunit of cleavage factor Im in HeLa cells alters alternative polyadenylation within 3'-UTRs.. *Nucleic Acids Res* 34:6264-6271(2006)
84. S Cardinale, B Cisterna, P Bonetti, C Aringhieri, M Biggiogera, SM Barabino: Subnuclear localization and dynamics of the Pre-mRNA 3' end processing factor mammalian cleavage factor I 68-kDa subunit.. *Mol Biol Cell* 18:1282-1292(2007)
85. M Marko, M Leichter, M Patrino-Georgoula, A Guialis: hnRNP M interacts with PSF and p54(nrb) and co-localizes within defined nuclear structures.. *Exp Cell Res* 316:390-400(2010)
86. SQ Xie, S Martin, PV Guillot, DL Bentley, A Pombo: Splicing speckles are not reservoirs of RNA polymerase II, but contain an inactive form, phosphorylated on serine2 residues of the C-terminal domain.. *Mol Biol Cell* 17:1723-1733(2006)
87. K Hata, R Nishimura, S Muramatsu, A Matsuda, T Matsubara, K Amano, F Ikeda, VR Harley, T Yoneda: Paraspeckle protein p54nrb links Sox9-mediated transcription with RNA processing during chondrogenesis in mice.. *J Clin Invest* 118:3098-3108(2008)
88. MN Rivera, WJ Kim, J Wells, A Stone, A Burger, EJ Coffman, J Zhang, DA Haber: The tumor suppressor WTX shuttles to the nucleus and modulates WT1 activity.. *Proc Natl Acad Sci U S A* 106:8338-8343(2009)
89. H Liu, GC Ippolito, JK Wall, T Niu, L Probst, BS Lee, K Pulford, AH Banham, L Stockwin, AL Shaffer, LM Staudt, C Das, MJ Dyer, PW Tucker: Functional studies of BCL11A: characterization of the conserved BCL11A-XL splice variant and its interaction with BCL6 in nuclear paraspeckles of germinal center B cells.. *Mol Cancer* 5:18(2006)
90. S Souquere, G Beauclair, F Harper, A Fox, G Pierron: Highly ordered spatial organization of the structural long

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noncoding NEAT1 RNAs within paraspeckle nuclear bodies.. *Mol Biol Cell* 21:4020-4027(2010)

91. D Kaida, MG Berg, I Younis, M Kasim, LN Singh, L Wan, G Dreyfuss: U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation.. *Nature* 468:664-668(2010)

92. S Granneman, SJ Baserga: Crosstalk in gene expression: coupling and co-regulation of rDNA transcription, pre-ribosome assembly and pre-rRNA processing.. *Curr Opin Cell Biol* 17:281-286(2005)

93. YS Mao, H Sunwoo, B Zhang, DL Spector: Direct visualization of the co-transcriptional assembly of a nuclear body by noncoding RNAs.. *Nat Cell Biol* 13:95-101(2011)

94. UM Murthy, PN Rangarajan: Identification of protein interaction regions of VINC/NEAT1/Men epsilon RNA.. *FEBS Lett* 584:1531-1535(2010)

95. A Proteau, S Blier, AL Albert, SB Lavoie, AM Traish, M Vincent: The multifunctional nuclear protein p54nrb is multiphosphorylated in mitosis and interacts with the mitotic regulator Pin1.. *J Mol Biol* 346:1163-1172(2005)

96. M Buxade, N Morrice, DL Krebs, CG Proud: The PSF.p54nrb complex is a novel Mnk substrate that binds the mRNA for tumor necrosis factor alpha.. *J Biol Chem* 283:57-65(2008)

97. KE Lukong, ME Huot, S Richard: BRK phosphorylates PSF promoting its cytoplasmic localization and cell cycle arrest.. *Cell Signal* 21:1415-1422(2009)

98. S Matsuoka, BA Ballif, A Smogorzewska, ER 3rd McDonald, KE Hurov, J Luo, CE Bakalarski, Z Zhao, N Solimini, Y Lerenthal, Y Shiloh, SP Gygi, SJ Elledge: ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage.. *Science* 316:1160-1166(2007)

99. C Choudhary, C Kumar, F Gnad, ML Nielsen, M Rehman, TC Walther, JV Olsen, M Mann: Lysine acetylation targets protein complexes and co-regulates major cellular functions.. *Science* 325:834-840(2009)

100. SP Shevtsov, M Dundr: Nucleation of nuclear bodies by RNA.. *Nat Cell Biol* 13:167-173(2011)

101. M Kloc, S Bilinski, MT Dougherty: Organization of cytokeatin cytoskeleton and germ plasm in the vegetal cortex of *Xenopus laevis* oocytes depends on coding and non-coding RNAs: three-dimensional and ultrastructural analysis.. *Exp Cell Res* 313:1639-1651(2007)

102. M Kloc, K Wilk, D Vargas, Y Shirato, S Bilinski, LD Etkin: Potential structural role of non-coding and coding RNAs in the organization of the cytoskeleton at the vegetal cortex of *Xenopus* oocytes.. *Development* 132:3445-3457(2005)

103. J Heasman, O Wessely, R Langland, EJ Craig, DS Kessler: Vegetal localization of maternal mRNAs is disrupted by VegT depletion.. *Dev Biol* 240:377-386(2001)

104. MD Blower, M Nachury, R Heald, K Weis: A Rae1-containing ribonucleoprotein complex is required for mitotic spindle assembly.. *Cell* 121:223-234(2005)

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