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Review

Paraspeckles: possible nuclear hubs by the RNA for the RNA

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Abstract

The mammalian cell nucleus is a highly compartmentalized system in which multiple subnuclear structures, called nuclear bodies, exist in the nucleoplasmic spaces. Some of the nuclear bodies contain specific long non-coding RNAs (ncRNAs) as their components, and may serve as sites for long ncRNA functions that remain largely enigmatic. A paraspeckle is a nuclear body that is almost ubiquitously observed in mammalian cultured cells but is cell population-specific in adult mouse tissue. The paraspeckle structure is RNase-sensitive. Long ncRNAs, termed MENε/β ncRNAs (also referred to as NEAT1 ncRNAs), have been identified as the RNA components of the paraspeckles. Specific elimination has revealed that MENε/β ncRNAs are essential components for the formation of the intact paraspeckle structure. Paraspeckle formation requires the continual MENε/β ncRNA biogenesis process, including ongoing transcription, alternative 3′-end processing, and stabilization. Some paraspeckle-localized RNA-binding proteins (p54/nrb and PSF) direct paraspeckle formation through the selective stabilization of MENβ ncRNA. Both MENε/β ncRNA expression and their subsequent interactions with paraspeckle proteins can be regulated under environmental and developmental conditions, which are reflected in the size and number of the paraspeckles. However, how paraspeckles function remains largely unsolved. Paraspeckles appear to serve as the site of nuclear retention of specific mRNAs that are selectively transported to the cytoplasm upon certain signals. Alternatively, MENε/β ncRNAs may sequester paraspeckle proteins that function outside the paraspeckles. This review focuses on known aspects of paraspeckles and provides a model of their structure and function.

Keywords: long non-coding RNA; nuclear body; paraspeckle; RNA-binding protein; RNA-protein interaction.

Introduction

The eukaryotic cell nucleus is highly compartmentalized. More than 10 membraneless subnuclear organelles have been identified (1, 2). These so-called nuclear bodies exist in the interchromosomal space, where they are enriched in multiple nuclear regulatory factors, such as transcription and RNA-processing factors. These factors are thought to serve as specialized hubs for various nuclear events, including transcriptional regulation and RNA processing (3, 4). Some nuclear bodies serve as sites for the biogenesis of macromolecular machineries, such as ribosomes and spliceosomes. Multiple cancer cell types show striking alterations in their nuclear body organization, including changes in the numbers, shapes and sizes of certain nuclear bodies (5). The structural complexity and dynamics of nuclear bodies have been implicated in the regulation of complex gene expression pathways in mammalian cells.

Also known as the ‘ribosome factory’, the nucleolus is the classic nuclear body and the site at which RNA polymerase I transcription, pre-rRNA processing and modification and subsequent ribosomal protein assembly occur sequentially within the hierarchical structure of the nucleolus (6, 7).

Cajal bodies (CBs) were originally discovered in the nucleus of various cell types at the beginning of the 20th century (8). CBs are spherical suborganelles of 0.2–2.0 μm in diameter that can be specifically marked by autoantibodies against p80/Coilin. They are involved in the biogenesis of multiple small nuclear ribonucleoprotein particles (snRNPs) and small nucleolar ribonucleoprotein particles (snoRNPs) (9, 10). CBs often overlap with distinct subnuclear structures called histone locus bodies, in which histone gene clusters, histone gene transcription factors and histone mRNA processing factors, such as NPAT and U7 snRNP, are localized (10, 11).

Gems are nuclear bodies that contain a large protein complex, including the survival motor neuron (SMN) protein, and often overlap with CBs (10, 12). Although the SMN complex is involved in the biogenesis of snRNPs in the cytoplasm, its roles in nuclear gems are poorly understood.

The nuclei of various cell types each contain 25–50 irregularly shaped nuclear structures, called nuclear speckles, of 0.8–1.8 μm in diameter. These structures are identical to the interchromatin granule clusters (IGS) seen by electron microscopy. The pre-mRNA splicing machinery, including small nuclear ribonucleoprotein particles (snRNPs), spliceosome subunits, and other non-snRNP protein splicing

factors, shows a punctate nuclear localization pattern that is usually termed 'a speckled pattern' (13, 14). In spite of the accumulation of splicing factors in nuclear speckles, subsequent studies indicated that splicing generally does not occur in nuclear speckles. Currently, nuclear speckles are widely considered to be the reservoir of various splicing factors that are translocated to the transcriptionally active sites of the chromosome (14).

Many nuclear bodies contain specific RNA molecules, including those that are unlikely to code for polypeptides, so-called non-coding RNAs (ncRNAs). Genome-wide transcriptomic analyses have revealed that numerous ncRNAs are produced from multiple intergenic regions and various portions of the human and mouse genomes (15–20). Recently, several abundant long ncRNAs were shown to localize to specific nuclear bodies. This finding raised the intriguing possibility that these nuclear bodies are sites where the ncRNAs play certain regulatory roles in nuclear events, such as gene expression, the modulation of protein function or the biogenesis of macromolecular machineries (21–25).

For instance, nuclear speckles contain the highly abundant long ncRNA Malat-1 (also known as nuclear enrichment abundant transcript 2 [NEAT2]) and unidentified polyadenylated RNAs. Malat-1 is a nuclear ncRNA of ~8 kb in size that is significantly associated with cancer metastasis (26, 27). Malat-1 ncRNA reportedly regulates alternative splicing patterns by modulating the phosphorylation status of SR-rich splicing factors (28) and controls synaptogenesis by modulating the expression of genes involved in this process (29). Rosenfeld and colleagues reported that Malat-1 associates with unmethylated Polycomb 2 (Pc2) in nuclear speckles, which leads to relocation of the target gene locus. This event triggers the SUMOylation of Pc2, which leads to activation of the growth-control gene program (30). By contrast, methylated Pc2 associates with TUG1 ncRNA, which specifically localizes to Polycomb bodies (PcGs). This process leads to the relocation and suppression of the above genes under quiescent conditions (30). Thus, it has been suggested that long ncRNAs in the nuclear bodies integrate the various regulatory events by sequestering and dissociating the key regulatory factors.

Although the detailed functions remained uncharacterized, several nuclear bodies contain specific ncRNAs or uncharacterized RNAs that may play important regulatory functions. Nuclear stress bodies (nSBs) are formed on specific pericentromeric regions in response to thermal and chemical stresses (31). Under such stress conditions, polyadenylated ncRNAs are transcribed from the satellite III (SatIII) DNA regions of the pericentromeric heterochromatin of human 9q11-12, which is silent under unstressed conditions. RNA-binding proteins, including SR-splicing factors, are captured with SatIII ncRNAs in nSBs (31, 32).

Gomafu ncRNA (also known as MIAT) is highly expressed in a subset of neurons in the central nervous system and localizes to unidentified subnuclear structures (33). Gomafu associates with the splicing factor SF1 and is thought to control splicing events (34). The splicing regulatory factor Sam68 (Src-associated in mitosis, 68 kDa) is specifically localized to distinct nuclear foci (Sam68 bodies) at the periphery of the nucleolus. Electron microscopic analysis has revealed that Sam68 bodies contain nucleic acids, presumably RNA. The targeting of Sam68 to these nuclear bodies involves its highly conserved RNA-binding domain (GSG domain) (35). Another splicing regulatory factor, polypyrimidine tract binding protein (PTB), colocalizes to a distinct perinucleolar compartment (PNC) with several RNA polymerase III transcripts, including ribonuclease P RNA and RNase mitochondrial processing (MRP) RNA (36). Thus, many nuclear bodies contain specific ncRNA molecules and RNA-binding proteins and may serve as platforms for the regulation of gene expression and 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, which is comprised of specific nuclear-retained long ncRNAs and multiple RNA-binding proteins. Recent research has uncovered the unique architecture of the paraspeckle and the possible regulatory functions that are encoded by its abundant long ncRNAs. The significance of the ncRNAs in paraspeckle structural formation and its functional relevance are discussed, with consideration of other recently reported nuclear RNA granules involved in stress response and diseases.

The paraspeckle: its discovery and unusual features

The paraspeckle was discovered in 2002 by the Angus Lamond Laboratory at the University of Dundee, Scotland (37). Because these new nuclear bodies were localized in close proximity to nuclear speckles, they were named 'paraspeckles'. Initially, Lamond and his colleagues performed large-scale proteomics analyses of isolated HeLa cell nucleoli treated with the transcription inhibitor actinomycin D, identifying 279 nucleolar protein candidates. Among the functionally uncharacterized proteins in the identified nucleolar protein candidates, two proteins, named paraspeckle proteins 1 and 2 (PSP1 and PSP2, respectively), exhibited nuclear punctate localization (37). Interestingly, PSP1 and PSP2 were not enriched in nucleoli but were enriched in other uncharacterized nuclear bodies (37) (Figure 1A). Non-POU domain-containing octamer-binding protein (NONO or p54nrb) was simultaneously identified as a paraspeckle component (37).

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. Normally, paraspeckles occur as two to 20 punctate nuclear bodies with an average diameter of 0.36 μm [Figure 1A, (37–43)]. Paraspeckles are reminiscent of the interchromatin granule-associated zones (IGAZs) that were previously detected as electron-dense interchromatin structures by high resolution *in situ* hybridization under electron microscopic observation (38–43). The IGAZs are electron-dense fibrillar structures found in close proximity to interchromatin granules corresponding to nuclear speckles (42). In 2010, Pierron and colleagues analyzed the molecular

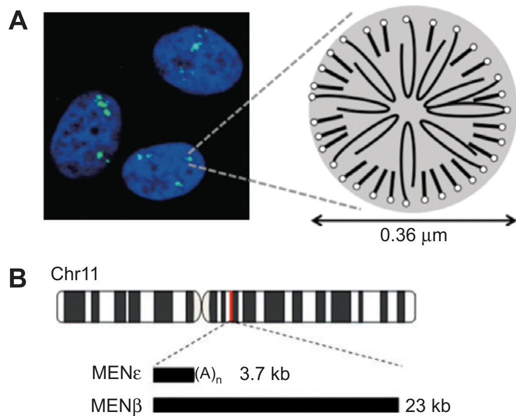

Figure 1 Paraspeckles are built on specific long ncRNAs.
(A) Paraspeckles detected by RNA-FISH of MENε/β ncRNAs (green signal). Nuclei are visualized by staining with DAPI (blue). Right: Cartoon of a paraspeckle, schematically showing the respective locations of MENε and MENβ, according to the electron microscopic observations by Souquere et al. (43). The average diameter of a paraspeckle is shown below. The cap structure at the 5′-terminus is represented by a small open circle. (B) Structure of MENε/β ncRNAs. The gene for the MENε/β ncRNAs is located at 13q of human chromosome 11. The size of each isoform is shown. MENε possesses a canonical poly(A) tail, but MENβ lacks it.

organization of paraspeckles by immuno- and *in situ* hybridization electron microscopy and confirmed that paraspeckles and IGAZs are the same structure (43).

Paraspeckles have been found in mammalian cells from humans and mice; however, the presence of phylogenetically orthologous nuclear bodies remains to be investigated. In contrast to cultured cell lines, observation in adult mouse tissues revealed intact paraspeckles in specific cell populations (44). Therefore, this nuclear body is likely a highly dynamic structure. Indeed, Fox et al. observed that paraspeckle formation is regulated during the cell cycle (37). Even in cultured cells, paraspeckle sizes dynamically change during cell differentiation (45). Time-lapse fluorescent imaging analysis of YFP-PSP1 revealed that paraspeckles persist throughout interphase and almost throughout mitosis. They exist in the cytoplasm during mitosis due to elimination of the nuclear envelope. Subsequently, the cytoplasmic paraspeckles disappear between telophase and early G1 phase and reappear during the G1 phase (37).

The inhibition of RNA polymerase II transcription with transcriptional inhibitors, such as actinomycin D or α-amanitin disassembles paraspeckles, results in the relocation of paraspeckle proteins to the perinucleolar cap structure formed on the nucleolar periphery. These dynamic alterations are not observed when RNA polymerase I transcription is inhibited with lower concentrations of actinomycin D (37–41, 46, 47). Therefore, this inhibition is not thought to be caused by nucleolar dysfunction. Treatment of the cells with the reversible transcription inhibitor DRB (5, 6-dichloro-1-β-D-ribofuranosylbenzimadizole) (48) also disassembles paraspeckles; however, they are quickly reassembled after DRB removal, suggesting that paraspeckle formation requires active transcription by RNA polymerase II (38, 49). Consistently, the paraspeckle structure is resistant to DNase I treatment but disappears after RNase A treatment, suggesting that the paraspeckle structure contains RNA component(s) that are required for maintenance of the structural integrity (37).

MENε/β ncRNAs are required for formation of the intact paraspeckle structure

The RNase-sensitive nature of the paraspeckle structure suggests that it contains RNA molecule(s) that sustain its structural integrity. The identified paraspeckle proteins commonly possess the canonical RNA-binding domains. Taken together, these findings suggest that the paraspeckle is a massive ribonucleoprotein particle.

In 2009, four research groups almost simultaneously discovered that two long ncRNAs (MENε/β or NEAT1 ncRNAs) are paraspeckle architectural components that are essential for paraspeckle formation in human and mouse cultured cell lines. Interestingly, each group obtained this evidence with a different approach (45, 49–51). Using cell fractionation, our group identified specific ncRNAs that were highly enriched in a high-density subnuclear fraction from a HeLa cell nuclei, which we later identified as the paraspeckle-enriched fraction (49). Spector and colleagues identified an ncRNA that was upregulated during the differentiation of a C2C12 mouse myoblast cell line into myotubes (45). Lawrence and colleagues identified an ncRNA by searching for nuclear-abundant ncRNA using microarrays (50). Carmichael and colleagues obtained MENε/β ncRNAs during their research on the nuclear retention of inosine-containing mRNAs (51).

The discovery of MENε/β ncRNAs as structural paraspeckle components opened a new window in the investigation of the roles of long ncRNAs in the intracellular architecture. The MENε/β ncRNAs were originally identified as transcripts produced from the multiple endocrine neoplasia type 1 (MEN I) locus on human chromosome 11 (11q13) [Figure 1B; (52)] and its syntenic mouse chromosome 19 (19qA). The MENε/β ncRNAs are also known as NEAT1 and virus-inducible noncoding RNAs (Vinc) (26, 53). They are composed of two isoforms: the shorter isoform MENε ncRNA (3.7 kb in human and 3.2 kb in mouse) and the longer isoform MENβ ncRNA (23 kb in human and 20 kb in mouse) (Figure 1B). Short noncoding transcripts generated from the 3′ region of MENε are known as TSU and TNC (54, 55). The two isoforms of MENε/β ncRNAs share a common promoter recognized by RNA polymerase II and overlap across 3.7 kb of their 5′-terminal sequence in humans (45, 49). RNA fluorescent *in situ* hybridization (RNA-FISH) clearly showed that both isoforms of MENε/β are exclusively localized to paraspeckles in various cultured cell lines [Figure 1A; (45), (49–51)], except for human embryonic stem cells (hES cells), in which the expression of MENε/β ncRNAs is silenced (51).

The apparent evidence of specific paraspeckle colocalization with abundant MENε/β ncRNAs, combined with the

previous report regarding the RNase-sensitive paraspeckle structure, raised the intriguing possibility that MENε/β ncRNAs serve as structural components of the paraspeckle. Specific elimination of MENε/β ncRNAs by antisense chimeric oligonucleotides or siRNAs confirmed this hypothesis (45, 49–51). The elimination of MENε/β ncRNAs causes paraspeckle disintegration, resulting in the dispersal of paraspeckle protein components in the nucleoplasm. Paraspeckles disassemble upon treatment with the reversible transcription inhibitor DRB and reassemble upon DRB removal. Monitoring of the paraspeckle reassembly process after the elimination of MENε/β ncRNAs revealed that the disassembled paraspeckle proteins are unable to reassemble in the absence of MENε/β ncRNAs (45, 49). Taken together, it can be concluded that MENε/β ncRNAs are required for the maintenance and formation of the intact paraspeckle structure (45, 49–51). This conclusion is consistent with the above evidence that the paraspeckle is undetectable in hES cells in which MENε/β ncRNAs are absent.

Paraspeckle proteins required for intact paraspeckle structure

Most of the identified paraspeckle proteins exhibit characteristics of RNA-binding proteins, raising the possibility that the paraspeckle structure is organized by cooperative interactions between MENε/β ncRNAs and multiple protein components. In addition to the primary discovery of PSP1, PSP2 and p54nrb, cleavage and polyadenylation specificity factor subunit 6 (CPSF6) (56) and splicing factor, proline- and glutamine-rich (SFPQ or PSF) were identified as additional paraspeckle proteins (57). Because the known protein components of paraspeckles are summarized in other reviews (39, 42), this paper mainly focuses on the proteins that are required for intact paraspeckle structure.

The *Drosophila melanogaster* behavior, human splicing (DBHS) protein family includes three paraspeckle proteins, PSP1, p54nrb and PSF (38, 41, 58). They are composed of similar conserved domains, including two RNA recognition motifs (RRMs) and a coiled-coil domain. Orthologues of paraspeckle proteins (p54nrb, PSF and PSP1) with >60% amino acid sequence identities to mammalian paraspeckle proteins are present in other vertebrate species, such as chicken and pufferfish, which suggests that similar nuclear bodies may exist in these vertebrate species. DBHS proteins are multifunctional nuclear proteins that are involved in a wide variety of gene expression events, including transcriptional control, pre-mRNA splicing, mRNA 3′-end processing and DNA repair (39, 42, 59). PSP2/CoAA commonly possesses an RRM and acts as a transcriptional coactivator (38, 41, 59). Elimination of these paraspeckle protein components with siRNAs revealed that p54nrb, PSF and PSP2 are essential, and PSP1 is dispensable, to form the intact paraspeckle structure (49 and our unpublished result).

Elimination of p54nrb and PSF results in the selective disappearance of the MENβ isoform but stable accumulation of MENε (49). Because transcription of the MENε and MENβ isoforms is controlled by the same promoter, the selective elimination of MENβ but not MENα indicates that p54nrb and PSF specifically stabilize the MENβ ncRNA isoform. Our immunoprecipitation results revealed that MENβ is preferentially precipitated with PSF and flag-tagged p54nrb (49). Meanwhile, two other groups reported that p54nrb is coimmunoprecipitated with MENε (45, 50). Taken together, these results show that MENε/β ncRNAs contain multiple binding sites for p54nrb and PSF, which are likely more concentrated in MENβ. However, only the interaction(s) on MENβ stabilize the bound ncRNA and also organize the paraspeckle structure.

Paraspeckles contain several other proteins whose roles in the paraspeckle structural organization remain to be investigated. CPSF6 is a subunit of the pre-mRNA cleavage factor I (CFIm) complex, which is involved in mRNA 3′-end processing (60, 61). CFIm targets UGUA sequences clustered upstream of the cleavage/polyadenylation site (62). This action is followed by recruitment of the catalytic CPSF complex (60). Recently, it was reported that the CFIm complex is involved in regulation of the alternative polyadenylation of various mRNA species (63). Deletion analysis of CPSF6 revealed that an RRM is required for localization in paraspeckles and for interaction with CPSF5/CFIm25. This finding suggests that CPSF6 associates with some RNA components in paraspeckles (56, 64) and raises the interesting possibility that CPSF6 mediates the formation of the 3′-end of paraspeckle-localized RNAs. The MENε/β ncRNAs are comprised of two isoforms that are likely synthesized by alternative 3′-end processing (Figures 1B and 2). Therefore, CFIm, including CPSF6, may be responsible for isoform synthesis of MENε/β ncRNAs. Heterogenous nuclear ribonucleoprotein M (hnRNP M) was recently reported to interact with p54nrb and PSF and to colocalize with defined nuclear structures that are likely paraspeckles (65). Therefore, hnRNP M is a candidate protein for interacting with MENε/β and contributing to paraspeckle formation.

How is paraspeckle formation initiated?

Having described the RNA and protein components that are required for formation of the intact paraspeckle structure, we next consider the prerequisite steps of paraspeckle construction from the multiple components. Paraspeckle is an RNase-sensitive nuclear body that is built on MENε/β ncRNAs; therefore, it can be considered a massive ribonucleoprotein complex. Electron microscopic observations by Pierron and colleagues indicated that the average size of paraspeckles is ~0.36 μm in diameter (43), which is more than 1500 times larger than that of the ribosome.

Ribosome biogenesis occurs in the nucleolus and is extremely complicated. The process begins with transcription of the pre-rRNA by RNA polymerase I and is followed by the processing and modification of pre-rRNA (66). Multiple ribosomal proteins may assemble onto the pre-rRNA molecule cotranscriptionally and/or post-transcriptionally in the defined order (66). Transcription of pre-rRNA by RNA polymerase I

on the rDNA locus also initiates the on-site construction of the nucleolar structure for its function. The triggering mechanism to form the nuclear body initiated by transcription of specific RNA species also can be applied to paraspeckle formation.

Paraspeckle formation is initiated by the transcription of MENε/β ncRNAs by RNA polymerase II from the corresponding locus on human chromosome 11. Using combined DNA-FISH and RNA-FISH analyses, Lawrence and colleagues showed that subpopulations of MENε/β ncRNAs localize to the MENε/β genomic locus in interphase HeLa cell nuclei (50). This result indicates that the initial stage of paraspeckle assembly occurs in close proximity to the MENε/β chromosomal locus [Figure 2; (50)]. Spector and colleagues recently demonstrated that the ongoing transcription of MENε/β gene is required for paraspeckle formation (67). They employed an artificial MENε/β gene conjugated with a LacO array that was integrated onto a specific chromosomal locus. The MENε/β ncRNAs synthesized from the artificial gene were monitored by utilizing MS2 stem-loop repeats and fluoresecently labeled MS2 coat protein. Paraspeckle formation was observed on the chromosomal locus, which was visualized by the binding of a fluorescent protein onto the LacO array, and the binding of MS2 coat protein to MS2-tagged MENε/β ncRNAs, when the transcription of the MENε/β ncRNAs was induced from a tetracycline-inducible promoter. Importantly, the transcription of MENε/β RNAs per se instead of MENε/β transcripts was required for paraspeckle maintenance because cells treated with the transcriptional inhibitor DRB exhibited paraspeckle disintegration even though substantial levels of MENε/β ncRNAs still accumulated. Based on these data, the authors concluded that ongoing transcription of MENε/β ncRNAs is required for initial paraspeckle formation on the corresponding chromosomal locus.

Some paraspeckle foci are detected outside the MENε/β chromosomal loci. This observation indicates that the assembled paraspeckles dissociate from the MENε/β chromosomal locus. The locations of the dissociated paraspeckles remain to be determined; however, they can be transported to a certain location in the nucleoplasm. Pursuing the intranuclear movement of the assembled paraspeckles and identifying their locations and dynamics under various conditions may provide important insights for understanding paraspeckle function.

Possible significance of the unique features of MENε/β ncRNAs

Each MENε/β isoform may play a distinct role in paraspeckle formation and function. As described above, the elimination of p54nrb or PSF suggests that MENβ is an essential RNA component to construct the paraspeckle structure but MENε is dispensable. As supportive evidence, we only observed paraspeckles in a limited number of cells of adult mouse tissues where MENβ was expressed (44). In mammalian cultured cells, MENε is usually more abundant than MENβ (e.g., ~3 times more in HeLa cells) (49).

The results of *in situ* hybridization combined with electron microscopy suggested that MENε ncRNA is mainly localized to the paraspeckle periphery (43), which raises the possibility that MENε ncRNA may be required for functional execution at the periphery rather than for paraspeckle structural organization. Electron microscopic observations also indicated that the 3′-terminal region of MENβ ncRNA localizes to the paraspeckle periphery, whereas the middle region of MENβ ncRNA is localized to the interior [Figure 1A; (43)]. These observations argue that the paraspeckle is not a random aggregate, but rather is constructed via defined RNA-protein interactions and a subsequent spatially defined arrangement of RNP subparticles. In this process, the MENβ-specific region may serve as the interaction platform for p54nrb and PSF, permitting the formation of the primary paraspeckle structural core. MENε subsequently joins and moves to the periphery of the paraspeckles.

By contrast, Shevtsov and Dundr (2011) reported that the tethering of MENε at a specific chromosomal site triggered the on-site formation of the paraspeckle (68). Clemson et al. (2009) reported that MENε overexpression in a stable cell line increased the number of nuclear paraspeckles (50). We observed a similar effect by MENε overexpression, although MENβ overexpression was more effective at increasing the number of paraspeckles in the cell (our unpublished result). These experiments were performed in cells possessing intact paraspeckles with endogenous MENβ. Therefore, it is likely that locally concentrated MENε captured the pre-existed paraspeckles or their subparticles containing MENβ, which resulted in the formation of paraspeckles containing exogenous MENε. To clarify this discrepancy, it will be important to attempt the rescue experiment by using either the MENε- or MENβ-expression plasmid in MENε/β-eliminated cells.

MENε/β ncRNAs are products of RNA polymerase II that are similar to protein-coding mRNAs. Intriguingly, they have several unique features that are distinct from mRNAs, which may be required for their architectural role in the nucleus: 1, both MENε/β ncRNAs are transcribed as intronless single-exon transcripts; 2, MENε/β ncRNAs are exclusively retained in the nucleus; and 3, MENβ ncRNA is matured via a unique 3′-end processing pathway to form a non-polyadenylated transcript (Figure 2).

In mammalian cells, >90% of protein-coding genes are divided by at least one intron, and the primary mRNA transcripts are matured via pre-mRNA splicing (69–71). In particular, MENβ, which extends 23 kb without interruption by any intron, is an extremely unusual case, suggesting that a specialized mechanism exists to prevent commitment to the splicing pathway. During the reaction of pre-mRNA splicing, the dynamic remodeling of ribonucleoprotein on the mRNAs results in the recruitment of factors involved in nuclear export (69–71). The unique structure of MENε/β ncRNAs may be required to avoid the pathway responsible for producing RNAs that need to be retained in the nucleus. It would be intriguing to elucidate the cotranscriptional assembly process of ribonucleoproteins onto nascent MENε/β ncRNAs, which avoid the canonical mRNA biogenesis pathway.

Alternatively, the nuclear export factor is also recruited onto mRNA through its association with the cap-binding complex (CBC) that directly binds to the 5′-terminus of mRNAs

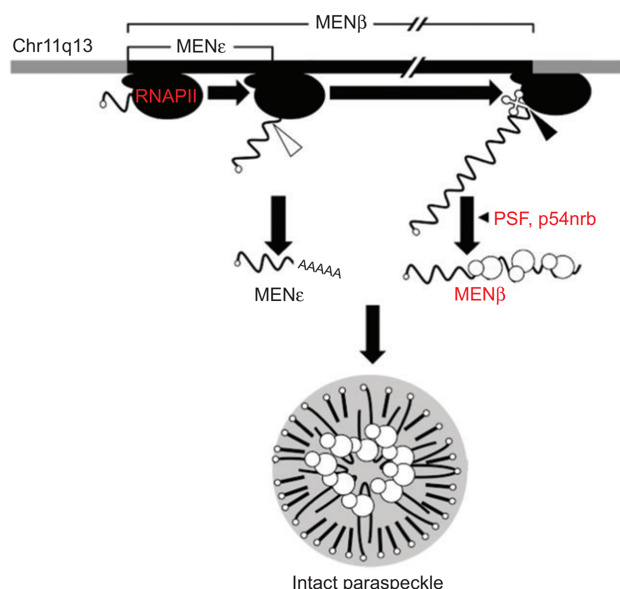

Figure 2 Model of intact paraspeckle formation.
Essential steps are schematized, including (1) ongoing transcription of MENε/β by RNA polymerase II (RNAPII), (2) MENβ synthesis by alternative 3′-end processing, and (3) MENβ stabilization by p54nrb and PSF. The 3′-ends of MENε and MENβ are formed by distinct mechanisms: canonical polyadenylation (open triangle) and RNase P cleavage (closed triangle).

(72, 73). Although MENε/β ncRNAs are believed to possess canonical cap structures that are autonomously added to the 5′-terminus of any RNA polymerase II transcript, this fact has not been experimentally validated. It would be interesting to determine whether there is a distinct 5′-terminal structure of MENε/β that prevents the association of nuclear export factors. The MENε ncRNA isoform is polyadenylated at its 3′-end through its canonical polyadenylation signal (PAS), whereas the MENβ ncRNA isoform lacks the canonical poly(A) tail and the PAS. Instead, the 3′-terminus of MENβ ncRNA is reportedly processed by the tRNA-processing endoribonuclease RNase P, which recognizes a tRNA-like structure located downstream of the 3′-end of MENβ ncRNA (45). A similar mechanism is also employed for the 3′-end processing of adjacent Malat-1 ncRNAs, which are transcribed from 55 kb of the MENε/β ncRNA gene (74). The processed 3′-termini of MENβ ncRNA and Malat-1 include a stable secondary structure with a genomically-encoded short A-rich sequence that may be required for the stable accumulation of these ncRNAs in the nucleus. The downstream tRNA-like structure is processed by RNase P and RNase Z yielding a small RNA that is smaller than tRNA and is not aminoacylated (74).

The mechanism of RNA stability control in the nucleus largely remains to be investigated. Recently, Mattick and colleagues reported that MENε/β ncRNAs are extremely short-lived RNAs, with an estimated half-life of ~30 min in mouse fibroblasts (75). This half-life is much shorter than that of human MENε/β, ~6 h, measured in human B-cells (76). Non-polyadenylated MENβ, whose half-life was estimated to be ~60 min, was found to be more stable than MENε. Therefore, the respective 3′-terminal structures of these isoforms may influence the rapid turnover of MENε/β ncRNA, which in turn may contribute to the highly dynamic nature of paraspeckles (75). However, these reported RNA half-lives were measured after transcriptional arrest by actinomycin D, which triggers rapid paraspeckle disintegration and, thus, dissociation of paraspeckle proteins from MENε/β. Indeed, Akimitsu and colleagues reported a longer half-life of MENε/β (3.3 h) using a pulse-and-chase experiment with the nucleotide analogue BrU (77). Although the significance of the non-canonical 3′-end processing of MENβ ncRNA remains to be elucidated, such processing would be critical to alter the stability of MENβ ncRNA specifically under certain conditions.

To understand the architectural roles of MENε/β ncRNAs, it is critical to determine the RNA regions that are responsible for their architectural function. In mouse NIH3T3 cells in which MENε/β ncRNAs were eliminated, the defect of paraspeckle formation could not be rescued by the introduction of human MENε ncRNA or a truncated 13 kb-MENβ ncRNA lacking the 10-kb 3′-terminal region (49). Future experiments using full-length MENβ ncRNA, and its deletion constructs should identify the RNA region responsible for architectural function.

Intermolecular interactions important for paraspeckle formation

To understand the architecture of the paraspeckle structure, one must identify the essential intermolecular interactions sustaining the intact paraspeckle structure. Immunoprecipitation experiments have revealed that two DBHS proteins (PSF and p54nrb) selectively bind MENβ ncRNA. These data are consistent with data showing that elimination of these proteins obliterates MENβ and results in paraspeckle disintegration. Therefore, interactions between MENβ ncRNA and DBHS proteins appear to sustain the paraspeckle structure [Figure 2; (49)]. Meanwhile, Spector and colleagues reported that p54nrb coimmunoprecipitates with both MENε and MENβ ncRNAs (45). An *in vitro* binding study identified three p54nrb-binding sites in the MENε/β overlapped region. One of these sites (PIR-1) was located near the 5′-end, whereas the other two (PIR-2, PIR-3) were located near the 3′-terminus of the MENε/β overlapped region (78). It is possible that the MENε/β ncRNAs provide multiple binding sites for p54nrb and PSF, in which subsets of the binding sites in the MENβ-specific region may serve as platforms for the paraspeckle core. Others may be dispensable to paraspeckle structure but vital for paraspeckle function.

The assignment of the functions of the MENε/β isoforms in paraspeckle formation leads to consideration of the importance of alternative 3′-end processing for the synthesis of the MENε/β ncRNA isoforms. As described above, paraspeckle-localized CPSF6 is presumably involved in the production of the MENε/β ncRNA isoforms, through facilitating the processing of either 3′-end. It would be intriguing to address the detailed mechanism of the alternative 3′-end processing,

especially how the ratio of the two isoforms is determined. Our careful observations of paraspeckles and the related accumulation level of MENβ ncRNA suggest that a few cultured cell lines exhibit an absence of intact paraspeckles, despite the substantial accumulation of MENβ ncRNA. This observation suggests that MENβ ncRNA accumulation is required but not sufficient for paraspeckle formation (our unpublished observation). Essential factors in addition to those involved in MENβ accumulation may be required to mediate the assembly of individual ribonucleoprotein subcomplexes containing either MENβ or MENε ncRNA (Figure 2).

In addition to the RNA-protein interactions described above, intermolecular interactions between paraspeckle proteins are implicated in paraspeckle formation. Indeed, three paraspeckle DBHS proteins are capable of forming heterodimer combinations (PSP1/p54nrb, p54nrb/PSF and PSP1/PSF) that likely serve as part of the paraspeckle core (47, 79). The functional domains of the DBHS proteins required for their interaction and localization have been identified: p54nrb binds PSF through its C-terminal coiled-coil domain (80, 81), whereas PSP1 binds p54nrb through its coiled-coil domain (47). The region containing RRMs and the coiled-coil domain is required for the paraspeckle localization of PSP1 (47). The second RRM is required for the localization of PSF in paraspeckle-like foci (81). The recently solved crystal structure of the heterodimer of human p54nrb and PSP1 supports the previous protein interaction data. In particular, the crystal data support the possibility that the antiparallel coiled-coil emanating from either end of the DBHS protein dimer leads to oligomerization and is required for formation of the paraspeckle core (82, 83).

Post-translational modifications, such as phosphorylation, acetylation, and methylation, can modulate characteristics of the paraspeckle proteins, such as properties related to their interactions with MENε/β ncRNAs or other paraspeckle proteins. Indeed, various post-translational modifications have been reported in p54nrb, PSF, PSP1 and CoAA paraspeckle proteins (84–88). These modifications can be the critical regulatory step for modulating paraspeckle structure and functions under certain conditions.

Possible regulatory steps for paraspeckle formation

Paraspeckles are commonly observed in various cultured cell lines from human and mouse; however, they are undetectable in hES cells (37, 51). This result is a consequence of the specific silencing of MENε/β ncRNA expression in hES cells, which indicates that the regulated MENε/β ncRNA expression underlies paraspeckle dynamics. Using the system for *de novo* paraspeckle formation, Spector and colleagues showed that paraspeckle formation/maintenance was dependent on the active transcription of MENε/β ncRNAs (67). Interestingly, when the transcription of MENε/β RNAs continued, more MENε/β transcripts were generated. The *de novo* assembled paraspeckles increased in size and eventually bud or split into a cluster of paraspeckles around the transcription sites (67). The expression of the endogenous MENε/β ncRNAs was upregulated 3-fold during the differentiation of a C2C12 mouse myoblast cell line into myotubes, and upregulation also led to paraspeckle enlargement in the differentiated myotubes (45). The expression of MENε ncRNA has been shown to be induced in the mouse brain during infection by Japanese encephalitis virus (JEV) and rabies virus (53). Thus, it is expected that the expression of MENε/β ncRNAs is differentially maintained under various external and internal conditions, which results in various sizes and numbers of the nuclear paraspeckles.

Nakagawa and colleagues reported that MENε was not ubiquitously expressed in adult mouse tissues, and MENβ expression was restricted to a limited population of cells in particular tissues (44). For example, in digestive tissues, such as the stomach and colon, strong MENβ expression and prominent paraspeckle formation were observed in regions where natural cell loss occurred. Expression was especially evident in presumptive preapoptotic cells at the surface-most region of the epithelium facing the lumen. By contrast, MENε expression was observed in a more broad range of the corresponding tissue. These observations indicate that the ratio of MENε to MENβ can be controlled in a cell population-specific manner, which may be due to the specific regulation of MENε/β isoform synthesis by alternative 3′-end processing or selective RNA degradation of specific isoforms.

RNA-protein interactions within paraspeckles may be controlled under certain conditions. As a common feature of the paraspeckle proteins, treatment with transcriptional inhibitors leads to paraspeckle disintegration and subsequent relocation to the perinucleolar caps. During this process MENε/β ncRNAs diffuse into the nucleoplasm and never relocate to the perinucleolar caps, suggesting that RNA-protein interactions between MENε/β ncRNAs and paraspeckle proteins are broadly and rapidly dissolved after drug treatment. The disintegration cannot account for the rapid elimination of MENε/β ncRNAs upon transcriptional arrest, because disintegration occurs very early (within 3 h) after transcriptional arrest, when MENε/β ncRNAs are still detectable. This finding implies a possible mechanism for the dissociation of paraspeckle proteins from MENε/β ncRNAs, such as through post-translational modification(s) of the paraspeckle proteins.

It is important to clarify whether paraspeckle disintegration is triggered by the dissolution of a pivotal interaction with a certain paraspeckle protein or by the simultaneous dissolution of multiple interactions with paraspeckle proteins. It also remains unknown how the dissociated paraspeckle proteins translocate to the distinct perinucleolar area and form cap structures, as well as how the perinucleolar cap structures are disintegrated upon removal of a reversible transcriptional inhibitor such as DRB. Paraspeckles are highly dynamic nuclear bodies whose sizes and compositions vary depending on the conditions to which the cells are exposed. The dynamics are controlled mainly by MENε/β ncRNA biogenesis. Higher expression levels of MENε/β, especially of MENβ, increase the paraspeckle size through the association of multiple paraspeckle proteins. Moreover, the status of paraspeckle proteins (e.g., post-translational modification) may affect the paraspeckle integrity and size.

What are the biological means reflected by paraspeckle size? These may depend on whether the paraspeckle-bound paraspeckle proteins are functionally active or inactive. When the paraspeckle serves as the site of a certain biological event (e.g., the regulation of gene expression), the paraspeckle-bound form of the paraspeckle proteins is considered to be the functional form. Therefore, an enlarged paraspeckle would mirror an activated status, in which more paraspeckle proteins are recruited to join the event. By contrast, if the paraspeckle-bound form of the paraspeckle proteins is non-functional, then the paraspeckle can be considered to be the reservoir of protein factors. This case is analogous to the proposed role of nuclear speckles (14). Because the paraspeckle-bound forms are implicated to be inactive, paraspeckle enlargement can be considered to imply negative regulation in events involving the paraspeckle proteins (Figure 3). Thus, the biological function of the paraspeckle and the significance of the ncRNA core of this nuclear body remain largely enigmatic.

Several reports indicate that paraspeckles are involved in unique regulatory mechanisms of gene expression through the nuclear retention of specific mRNAs, suggesting that the paraspeckle serves as a site for regulatory events (Figure 3). Knockout mice lacking MENε/β expression, and thus paraspeckles, are viable and fertile. Conventional histological analyses fail to detect any obvious abnormalities in these mice (44). These observations suggest that paraspeckle functionality may be visible under certain internal and/or external conditions, which is consistent with the above evidence that MENε/β ncRNAs are inducible under certain conditions (44, 45, 51, 53).

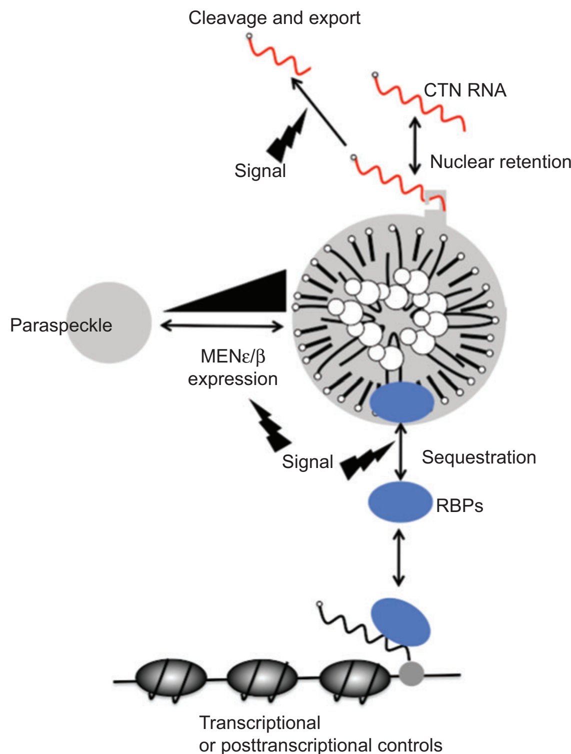

Figure 3 Model of paraspeckle functions.
The paraspeckle size is dynamically altered depending on the expression levels of MENε/β ncRNAs (filled triangle), which are controlled by certain signals. Above: Paraspeckle serves as the site of nuclear retention of a specific mRNA (e.g., CTN RNA) that is cleaved and exported to the cytoplasm upon exposure to certain signals. Below: Paraspeckle modulates the nucleoplasmic pool of RNA-binding proteins (RBPs), which play regulatory functions in the transcriptional and/or post-transcriptional regulation of the target genes occurring away from the paraspeckles.

Paraspeckles as nuclear retention sites of mRNAs

Most mRNAs are transported to the cytoplasm once they are properly processed into their mature form. It has not been recognized that the transport of mature mRNAs is a regulatory step of gene expression. Some virus-produced double-stranded RNAs (dsRNAs) are retained in the nucleus (89). Viral RNAs commonly contain inosine nucleotides that are produced by the deamination of specific adenosine nucleotides with the enzyme adenosine deaminase acting on RNA (ADAR) (90). Carmichael and colleagues addressed the mechanism of the nuclear retention of inosine-containing 'hyper-edited RNAs'. They identified a protein complex containing a major paraspeckle protein (p54nrb) that specifically binds to inosine-containing RNAs (91). A search of potential dsRNA structures in genomically encoded mRNAs revealed that 333 mRNAs contained the putative dsRNA regions formed by the inverted repeat (IR) Alu element (IR*Alu*) in their 3′-UTRs (92), which suggests that they would potentially be recognized by ADARs and retained in the nucleus. After IRs were inserted into the 3′-UTR of GFP reporter constructs, the GFP mRNA was localized to the paraspeckles, which suggests that the paraspeckles served as nuclear retention sites of the IR-containing mRNAs (92).

Spector and colleagues identified cationic amino acid transporter 2 transcribed nuclear RNA (CTN-RNA) as an RNA component enriched in the purified nuclear speckle fraction. Subsequent RNA-FISH analyses revealed that CTN-RNA was specifically localized to paraspeckles (57). CTN-RNA is the first identified natural paraspeckle-localized RNA; however, in contrast to MENε/β ncRNAs, CTN-RNA is not capable of paraspeckle formation (57). CTN-RNA has several unique features. It is a 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., with interferon gamma or lipopolysaccaride). Cleavage produces the processed mCat2 mRNA with a shorter 3′-UTR, which is exported to the cytoplasm for translation (57). Consistently, CTN-RNA possesses a long IR in its 3′-UTR that forms a long stem-loop structure that is subjected to A-to-I RNA editing, and p54nrb has been shown to bind to CTN-RNA (57). Moreover, artificial reporter mRNA containing the IR of CTN-RNA was shown to be retained in the nucleus. Intriguingly, it was endonucleolytically cleaved at its 3′-UTR in response to external stimuli,

and the processed reporter mRNA was likely polyadenylated and exported to the cytoplasm where it was translated [Figure 3; (57)].

Using human ES cells, Carmichael and colleagues reported that intact paraspeckle is required for the nuclear retention of several mRNAs that contain IRs in their 3′-UTRs (51). Lin28 mRNA plays crucial roles in ES cells, where it was found to be exported to and translated in the cytoplasm. Consistent with the status of Lin28 mRNA, MENε/β ncRNAs were poorly expressed in hES cells, and paraspeckles were not formed. However, when the hES cells were induced to differentiate into trophoblast cells in which MENε/β ncRNAs were substantially expressed, intact paraspeckles were formed and Lin28 mRNA was retained in the nucleus (51). These data provide the important insight that paraspeckle formation is regulated under various physiological conditions, mainly through the regulated expression of MENε/β ncRNAs, and that the nuclear retention of IR-containing mRNAs depends on the status of paraspeckle formation.

Does the ncRNA-mediated sequestration of protein factors serve as a possible common regulatory mechanism?

In contrast to its role supporting regulatory events, the paraspeckle may also serve as a site for the sequestration of specific protein factors to suppress their activities. This possibility comes from the analogy of functional insights into other cellular bodies containing RNAs. The stress-inducible nSBs described above assemble on specific pericentromeric heterochromatic domains containing SatIII DNA, which is marked by the prominent localization of heat shock factor protein 1 (HSF1) and a specific subset of RNA-binding proteins, such as the pre-mRNA splicing factor SRSF1 and the hnRNP-like protein scaffold attachment factor B (Saf-B). In response to stress, heterochromatin changes to euchromatin, which leads to the transcription of polyadenylated SatIII RNAs that, subsequently, associate with nSBs (31, 32). The elimination of SatIII RNAs with antisense oligonucelotides affects the recruitment of pre-mRNA splicing factors to nSBs, without altering the association of HSF1 with these structures (31).

Recently, Dundr and colleagues reported that the tethering of MS2-tagged SatIII RNA could initiate *de novo* formation of nSBs (68), indicating that SatIII RNAs play a major role in the formation of functional nSBs. Although the role of nSBs remains elusive, they may act as transcriptional and splicing factors. Pre-mRNA splicing is globally arrested during thermal stress; therefore, nSB may be involved in the fundamental regulation of splicing and modulation of alternative splicing by capturing a specific factor that determines the specific splice site selection (31, 93). Another intriguing possibility is that the nSBs store and help to degrade aberrant RNAs that were blocked at the early stages of maturation (31).

Recently, Lee and colleagues reported that several ncRNAs transcribed from the intergenic spacers (IGSs) of ribosomal DNA clusters in the nucleolus (94). The nucleolus modulates the stress response process by sequestering or releasing proteins under specific stressed conditions (6). For example, under the hypoxic condition, von Hippel-Lindau (VHL) proteins that facilitate the degradation of hypoxia-inducible factor-α (HIF1α) under normal oxygen tension are sequestered into the nucleolus via a specific ncRNA, IGS28 RNA (94). IGS28 RNA is transcribed by RNA polymerase I from 28 kb downstream of the rRNA transcription start site, where it is transcriptionally silent under the normal condition. Upon hypoxic stress, expression of IGS28 RNA is induced and further processed into a non-polyadenylated ncRNA of ~300 nt that is localized to the nucleolus (94). The sequestered VHL is not shuttled and completely immobile in the nucleolus. The relieved HIF1α may be stabilized and act as the active transcription factor for hypoxia-responsive genes. IGS28 RNA also sequesters the catalytic subunit of DNA polymerase.

Furthermore, other stress signals reportedly activate ncRNAs from different IGS regions, and each IGS RNA interacts specifically with a particular protein for its nucleolar immobilization. IGS16 and IGS22 RNAs are induced by thermal stress and control nucleolar detention of HSP70 (94). IGS20 induced by transcriptional stress is involved in the nucleolar immobilization of HDM2, which relieves nucleoplasmic p53 from HDM2-mediated degradation. Importantly, transcriptional activation of individual IGS RNA is programmed by a specific stress signal. Different stress stimuli induce nucleolar immobilization of common proteins through their interaction with different IGS RNA (94).

SatIII RNA and IGS RNAs exhibit several common features that are analogous to the roles of MENε/β ncRNAs through paraspeckle functions. The transcriptions of SatIII and IGS RNAs are commonly induced from the normally silent chromosomal regions upon certain stress signals. Synthesized RNAs are retained at or near the transcribed sites, where the RNAs associate with specific proteins to immobilize them. This mechanism may conditionally inactivate the proteins that are sequestered by the ncRNAs. In the case of paraspeckles, the MENε/β ncRNAs are induced under certain conditions (e.g., viral infection and cell differentiation). The MENε/β ncRNAs are retained at the transcribed chromosomal sites, where they associate with multiple proteins to form paraspeckles. According to the analogy to SatIII RNA and IGS RNAs, the paraspeckles can be considered a detention site for multiple RNA-binding proteins (Figure 3). However, at least one paraspeckle protein, PSP1, actively shuttles between the paraspeckle and nucleolus (37). Spector and colleagues showed that approximately 30%–40% of paraspeckle proteins were immobile in *de novo* formed and endogenous paraspeckles; however, the remaining paraspeckle proteins (e.g., PSP1) were rapidly exchanged with the nucleoplasmic pool (67). It was recently reported that mouse MENε/β ncRNAs are rapidly turned over, and that this unusual feature of ncRNA metabolism may be related to the active exchange of paraspeckle-localized proteins (75). These reports suggest that the paraspeckle may be the site of sequestration of specific proteins. However, this sequestration is not a static immobilization, but rather is an active exchange process to modulate the nucleoplasmic pool of a specific factor under various conditions. This conclusion raises the intriguing

possibility that paraspeckles might negatively regulate the function of paraspeckle-localizing proteins, which regulate distinct nuclear events outside the paraspeckles (Figure 3).

The RNA-dependent sequestering of nuclear RNA-binding proteins is a major cause of certain neurologic diseases (95). Myotonic dystrophy is caused by the sequestration of functional MBNL and CUGBP1 RNA-binding proteins by expanded CUG repeats, resulting in the formation of prominent nucleoplasmic RNA foci (95). Using individual nucleotide-resolution ultraviolet cross-linking and immunoprecipitation (iCLIP), Ule and colleagues observed RNA binding by TDP-43 in brains from patients with frontotemporal lobar degeneration. The greatest increase in binding was to MENε/β and Malat-1 ncRNAs (96). TDP-43 was also found to bind pre-mRNAs and to influence alternative splicing, which suggests that alternative mRNA isoforms regulated by TDP-43 encode proteins that regulate neuronal development or have been implicated in neurological diseases (96). The intriguing possibility is that the specific elevation of TDP43-binding to long ncRNAs, such as MENε/β, in disease affects alternative splicing patterns.

The paraspeckle is a unique nuclear body that is constructed on specific ncRNAs. The biogenesis of ncRNAs may be a key to determining the shape and functionality of this nuclear body, presumably by modulating the population of associated RNA-binding proteins. Paraspeckle-localized RNA-binding proteins have their own target mRNAs with which they participate in transcription, mRNA processing and/or transport. Therefore, paraspeckles can be considered as the active nuclear hub for the precise modulations of the functionality of various RNA-binding proteins, which are mainly mediated by MENε/β ncRNAs. The ncRNA-dependent regulatory mechanism may be linked to various physiological events, including stress response, development, and diseases. The elucidation of the precise functions of paraspeckles and their underlying molecular mechanisms may open new windows for research on the still enigmatic nuclear bodies of the RNA and on roles by the RNA for the RNA.

Expert opinion

The paraspeckle is a unique nuclear body that is formed on specific long ncRNAs. Paraspeckles are massive ribonucleoprotein complexes that are estimated to be more than 1000 times larger than the ribosomes. Because few components of paraspeckles have been identified, additional protein factors are expected to aid in the construction of intact paraspeckles. It will be important to identify additional paraspeckle proteins, to further understand the process of intact paraspeckle formation, their detailed structure, and their biological functions. RNA-protein interactions between MENβ ncRNA and the DBHS proteins are required for paraspeckle construction. It is important to understand how these DBHS proteins contribute to the selective stabilization of the MENβ isoform. Furthermore, the precise mapping of protein-binding sites on MENβ ncRNA will provide important insights into the 'functional RNA units' for the architectural function of MENβ ncRNA. Our preliminary observations indicate that some paraspeckle proteins are essential for paraspeckle construction without affecting MENβ ncRNA levels, and that MENβ accumulation is required but not sufficient for paraspeckle construction. Thus, multiple steps, including those for MENβ accumulation and additional essential step(s) that do not affect MENβ expression, are required for the formation of such tremendously large ribonucleoprotein complexes. Paraspeckle dynamics are achieved through the differential expression of MENε/β ncRNAs and, presumably, conditional changes of RNA-protein interactions within the paraspeckles. It is important to elucidate the mechanism by which the paraspeckle size is determined. This research can be accomplished through an investigation of the mechanisms of MENε/β biogenesis and of the modulation of RNA-protein interactions.

The dynamic nature of paraspeckles suggests that this nuclear body plays particular roles under transient environmental and developmental conditions. Given the lack of apparent phenotypes in MENε/β knockout mice, paraspeckle is not an essential nuclear body under normal laboratory conditions. What are the physiological functions of paraspeckles, if any? In the esophagus, stomach, intestine and colon, paraspeckle formation is found in the outermost, lumen-facing region of the digestive epithelium, where active programmed cell death occurs (44). Strong MENε/β expression is also observed in the atretic follicle cells in female ovaries. In these particular cells, programmed cell death initiates from the innermost cells neighboring the oocytes and progresses into the outer region. Interestingly, cells expressing MENε/β are located along the outer side of the cells expressing the apoptotic cell marker cleaved caspase 3 (our unpublished observation). This finding suggests that MENε/β expression precedes the activation of the effector caspase during the process of programmed cell death. Moreover, these observations raise the intriguing possibility that paraspeckle formation may participate in, if it is not essential for, certain cellular processes leading to programmed cell death or an irreversible senescent/terminal differentiation pathway in living organisms.

Paradoxically, however, paraspeckles are ubiquitously found in most cultured cell lines, which are highly proliferative and presumably undifferentiated. It might be possible that most of these cultured cells had once initiated programmed cell death or senescent pathways, with the accompanying formation of paraspeckles, during their establishment processes. However, these pathways may have been subsequently cancelled by malignant mutations in oncogenes or tumor suppressor genes. This hypothesis is consistent with the observation that totipotential ES cells do not express MENε/β and, thus, do not possess paraspeckles. Alternatively, paraspeckles in terminally differentiated/senescent cells *in vivo* and proliferating cells *in vitro* may play completely distinct roles via distinct paraspeckle components. Regardless, it is necessary to identify the physiological conditions in which the paraspeckle becomes essential (i.e., MENε/β knockout mice show obvious phenotypes) to fully understand the function of this elusive nuclear structure.

Outlook

Over the next decade, various functions of long ncRNAs are expected to be unveiled. Currently, epigenetic histone modifications are the only molecular events in which a subset of long ncRNAs is known to be commonly involved. Future progress in understanding the functions of ncRNAs will provide additional ncRNA subsets that participate in common molecular mechanisms. The architectural function will be categorized as a common function involving an ncRNA subset. Because architectural ncRNAs are localized to some subcellular structures, it would be straightforward to search for additional architectural ncRNA candidates through the RNA-sequencing of enriched cellular body fractions (termed as subcellular RNomics). Because some cellular bodies are cell type-specific or stress-inducible, more varieties of architectural ncRNAs will be identified if subcellular RNomics are applied to various cell types and conditions.

The dissection of the functional RNA units required for architectural functions will provide important insights into the mechanisms of the RNA-mediated construction of subcellular structures. Using the pieces of the functional RNA units, artificial subcellular structures will be able to be constructed at certain intracellular sites and applied for the sequestration of specific factors, such as disease-related proteins. Even in the functionally significant MENε/β ncRNAs, conservation of the nucleotide sequence is relatively low. Thus, the functionality of the ncRNAs depends on more than just the primary nucleotide sequence. The identification of various functional RNA units will establish the new fundamental rules for functional ncRNAs.

Finally, Mattick proposed that the biological complexity of various organisms is correlated with the complexity of non-coding transcripts from the intergenic regions in each organism (97). Orthologues of MENε/β ncRNAs have been identified in only mammalian species. Therefore, it is strongly suggested that the presence of paraspeckles is limited to mammalian species. Elucidation of the biological significance of the paraspeckle may account for the advantage of the acquisition of MENε/β ncRNAs to the architecture of the nuclear bodies.

Highlights

- Paraspeckles are RNase-sensitive nuclear bodies that are ubiquitously observed incultured cells but are cell type-specific in mouse adult tissues.
- Paraspeckles are built on specific ncRNAs, MENε/β.
- Active transcription and subsequent biogenesis of MENε/β ncRNAs are required for paraspeckle formation.
- Interactions between MENε/β ncRNAs and specific paraspeckle RNA-binding proteins sustain the intact paraspeckle structure.
- Paraspeckle size and number change under various developmental and environmental conditions, which are mainly reflected by the expression levels of MENε/β ncRNAs.
- Paraspeckles serve as a retention site of mRNA subsets that are hyperedited.
- Paraspeckles may control the nucleoplasmic pool of functional RNA-binding proteins through local interactions with MENε/β in paraspeckles.
- MENε/β knockout mice are viable and fertile, and show no apparent phenotypes in the laboratory condition, suggesting that paraspeckles may play roles under particular environmental conditions.

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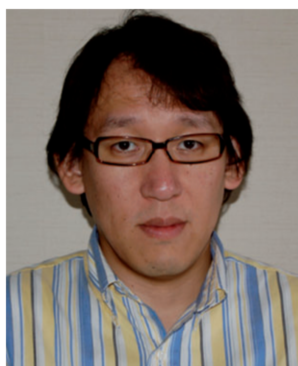

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