Paraspeckles: nuclear bodies built on long noncoding RNA

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Paraspeckles are ribonucleoprotein bodies found in the interchromatin space of mammalian cell nuclei. These structures play a role in regulating the expression of certain genes in differentiated cells by nuclear retention of RNA. The core paraspeckle proteins (PSF/SFPQ, P54NRB/NONO, and PSPC1 [paraspeckle protein 1]) are members of the DBHS (*Drosophila melanogaster* behavior, human splicing) family. These proteins, together with the long nonprotein-coding RNA *NEAT1* (*MEN-ε/β*), associate to form paraspeckles and maintain their integrity. Given the large numbers of long noncoding transcripts currently being discovered through whole transcriptome analysis, paraspeckles may be a paradigm for a class of subnuclear bodies formed around long noncoding RNA.

Introduction

The cell nucleus, especially in complex eukaryotes, is a highly organized structure. Individual chromosomes occupy discrete territories, and specific proteins and nucleic acids are enriched in subnuclear structures such as nucleoli, Cajal bodies, paraspeckles, and nuclear speckles (Platani and Lamond, 2004). Nuclear organization is linked to genome maintenance and to the control of gene expression and thus influences growth, development, and cellular proliferation. Moreover, disruption of nuclear organization is often correlated with disease states such as the loss of subnuclear promyelocytic leukemia bodies in acute promyelocytic leukemia (Weis et al., 1994).

In this review, we discuss the composition, formation, and function of paraspeckles, one of the most recent subnuclear bodies identified. We describe studies that demonstrate the role of paraspeckles in controlling gene expression by trapping adenosine to inosine (A to I) hyperedited RNA within the nucleus. New evidence suggests that this mechanism may be widely used to coordinate gene expression within a variety of different cellular contexts. We also review the recent findings by several groups that paraspeckles are formed around a long nuclear noncoding RNA (ncRNA), *NEAT1* (Chen and Carmichael, 2009; Clemson et al., 2009; Sasaki et al., 2009; Sunwoo et al., 2009). This finding has increased our knowledge of the functional capabilities of long ncRNAs and has opened up the possibility of more nuclear bodies being formed in this way.

Paraspeckles

Paraspeckles are a relatively newly identified subnuclear body. They were discovered when a putative nucleolar protein was found to localize to nucleoplasmic foci that did not directly overlap with markers for any known subnuclear structure (Andersen et al., 2002; Fox et al., 2002). These foci were named paraspeckles because they were observed in the interchromatin space near to, yet distinct from, the nuclear speckles that are enriched in splicing factors (Fig. 1; Fox et al., 2002). The novel protein was named PSPC1 (paraspeckle protein 1) and has become the standard marker used to identify paraspeckles.

Paraspeckles are restricted to mammalian nuclei and are observed in transformed and primary cell lines, embryonic fibroblasts, tissues, and tumorigenic biopsies (Fox et al., 2002; Prasanth et al., 2005; Clemson et al., 2009; Sunwoo et al., 2009; unpublished data). They are also dynamic structures; for instance, paraspeckles are not present in human embryonic stem cells but only appear upon differentiation (Chen and Carmichael, 2009). These bodies are ∼0.5–1.0 µm in size, and their numbers vary both within cell populations and depending on cell type. For example, HeLa have 13–17 paraspeckles per nucleus, whereas NIH3T3 have 5–10 foci per nucleus (Fig. 1; Fox et al., 2002; Cardinale et al., 2007; Clemson et al., 2009). At the EM level, paraspeckle markers label distinct nuclear structures that are electron dense and rich in RNA (Fig. 1 D; Prasanth et al., 2005; Cardinale et al., 2007). These transmission EM (TEM) paraspeckles correspond to the interchromatin granule–associated zones (IGAZs; Visa et al., 1993). IGAZs are electron-dense fibrillar structures found in close proximity to interchromatin granules/nuclear speckles, whose function has remained unknown since their identification in the early 1990s.

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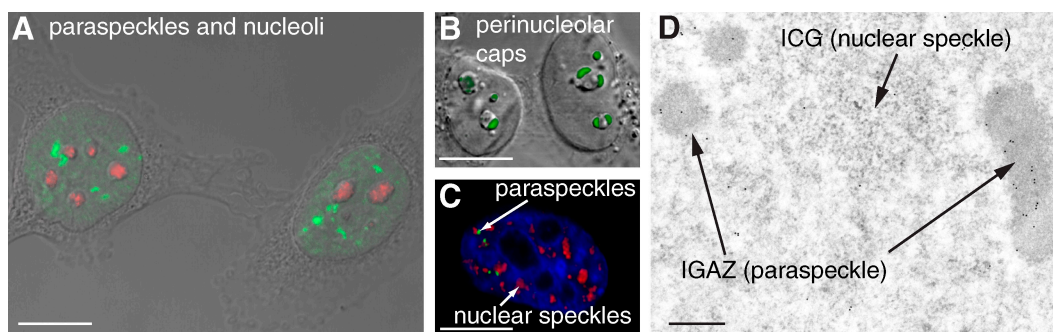
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Figure 1. **Visualizing paraspeckles.** (A) Combined differential interference contrast and fluorescence micrograph of HeLa cells stained with anti-PSPC1 to show paraspeckles (green) as nucleoplasmic foci distinct from nucleoli (stained with B23 antibody; red). (B) HeLa cells showing reorganization of the DBHS protein PSPC1 (green) to perinucleolar caps after treatment with actinomycin D to inhibit RNA Pol II transcription. (C) HeLa cell stained with anti-PSPC1 (green), anti-SC35 (red), and DAPI (blue) to show the relationship between paraspeckles abutting nuclear speckles in the interchromatin space. (D) TEM image of a HeLa cell section immunogold labeled with anti-PSPC1. The labeled IGAZs are usually found in close proximity to the interchromatin granules (ICGs; nuclear speckles). This image was provided by S. Souquere and G. Pierron (Institut André Lwoff, Villejuif, France). Panels B and C are adapted from Fox et al. (2002) with permission from Elsevier. Bars: (A–C) 10 µm; (D) 0.5 µm.

At present, paraspeckles are known to contain a small number of proteins with reported roles in transcription and/or RNA processing (Table I and next section). However, paraspeckles do not directly overlap with sites of active transcription, as measured by bromo-UTP incorporation (Fox et al., 2002; Xie et al., 2006), although they may still form in association with some active genes (discussed in Role of paraspeckles in nuclear retention of RNA and Paraspeckle formation). Nevertheless, paraspeckles are intimately linked with transcription because of the presence of active RNA polymerase II (Pol II) and newly made RNA at their periphery (Xie et al., 2006).

Paraspeckle proteins

Currently, paraspeckle proteins are defined by their colocalization in subnuclear foci with a member of the mammalian DBHS (*Drosophila melanogaster* behavior, human splicing) protein family, consisting of PSPC1, P54NRB/NONO, or PSF/SFPQ. These three members of the DBHS protein family are the most well-studied intrinsic protein components of paraspeckles. Reported interactions between all members of this family suggest that they exist as either homo- or heterodimers in vivo (Myojin et al., 2004; Fox et al., 2005). They share >50% sequence identity within two N-terminal RNP-type RNA recognition motifs and a C-terminal coiled-coil domain. One of these RNP-type RNA recognition motifs and the coiled-coil domain (which mediates dimerization) are required for PSPC1 to be targeted to paraspeckles (Fox et al., 2005). DBHS proteins are dynamic within the nucleus: they cycle between the nucleoplasm, paraspeckles, and the nucleolus under normal conditions and accumulate within perinucleolar cap structures when RNA Pol II transcription is inhibited (Fig. 1 B; Fox et al., 2002; Shav-Tal et al., 2005). This latter finding explains the discovery of PSPC1 in the nucleolar proteome. Knockdown of either of the two highly expressed DBHS proteins (P54NRB/NONO and PSF/SFPQ) in HeLa cells results in the loss of paraspeckles (Sasaki et al., 2009). In contrast, knockdown of the less abundant DBHS protein PSPC1 in HeLa cells has no effect on paraspeckles (Sasaki et al., 2009). Thus, highly expressed DBHS protein dimers are at the core of paraspeckle structural integrity.

Proteins of the DBHS family have been implicated in a wide array of functions. They have been shown to bind to both double- and single-stranded DNA and RNA and have been copurified in numerous different complexes, leading to the catch-all label of "multifunctional nuclear proteins" (for review see Shav-Tal and Zipori, 2002). These functions encompass many aspects of transcription and RNA processing, including transcription initiation (Dong et al., 1993; Yang et al., 1993, 1997), coactivation (Kuwahara et al., 2006; Amelio et al., 2007), and corepression (Mathur et al., 2001; Dong et al., 2005), constitutive and alternative splicing (Patton et al., 1993; Peng et al., 2002; Kameoka et al., 2004; Ito et al., 2008), and transcriptional termination (Kaneko et al., 2007). A further function relevant to paraspeckles is the involvement of PSF/SFPQ and P54NRB/NONO in the nuclear retention of RNA, specifically preventing A to I hyperedited RNA from leaving the nucleus (Zhang and Carmichael, 2001). RNA hyperediting of long double-stranded RNA (optimally 100 bp) occurs in the nucleus and results in the conversion of up to half of all adenosines (A) in the RNA to inosines (I). A to I hyperediting mostly occurs on transcribed repeat elements (as discussed in more detail in Role of paraspeckles in nuclear retention of RNA).

With roles in constitutive processes such as splicing and transcription, the biological implications for the DBHS proteins are wide ranging. One interesting example indicates a conserved role for P54NRB/NONO in mammals and NonA, a DBHS orthologue in *Drosophila*, in the control of circadian rhythms. P54NRB/NONO is required for mammalian circadian rhythm maintenance via association with the PERIOD-1 protein (Brown et al., 2005), and *NonA* mutants are nearly arrhythmic. Brown et al. (2005) speculate that P54NRB/NONO serves to dampen the effects of transcriptional noise on circadian rhythms. DBHS proteins likely carry out their diverse functions by varying their binding partners, posttranslational modification, and subcellular and subnuclear localization (Proteau et al., 2005; for review see Shav-Tal and Zipori, 2002).

Paraspeckle RNAs

After the initial discovery of paraspeckles, several observations suggested that in addition to proteins, paraspeckles would contain

Table I. **Paraspeckle proteins**

Protein	Synonyms	Features	Reference[a]
Core paraspeckle proteins			
P54NRB	NONO, NMT55, NRB54	DBHS; required for paraspeckle integrity in HeLa cells	Fox et al., 2002
PSF	SFPQ	DBHS; required for paraspeckle integrity in HeLa cells	Prasanth et al., 2005
PSPC1	PSP1	DBHS	Fox et al., 2002
Other paraspeckle proteins			
CoAA[b]	PSP2, RBM14, SIP, SYTIP1	Transcriptional/splicing coregulator	Fox et al., 2002
CFIm68	CPSF6, HPBRII-4	RNA 3′ end cleavage factor; also found in nuclear speckles	Dettwiler et al., 2004
SOX9[b,c]	SRA1	Developmental transcription factor	Hata et al., 2008
WTX[b]	NA	Wilms tumor protein, tumor suppressor	Rivera et al., 2009
WT1(+KTS)[b]	WAGR	Wilms tumor transcription factor, partial colocalization with paraspeckles	Dutton et al., 2006
BCL11A[b,c]	CTIP1, ZNF856	Zinc finger transcription factor	Liu et al., 2006
RNA Pol II	NA	Also found associated with chromatin and nuclear speckles	Xie et al., 2006

NA, not applicable.

[a]Localization to paraspeckles was first shown in these studies.

[b]Only overexpressed proteins have been assessed for localization in paraspeckles.

[c]Further studies need to address whether these proteins are genuine paraspeckle components or are retargeting DBHS proteins into different subnuclear locations.

RNA. First, paraspeckles are degraded after incubation with RNase A (which degrades single-stranded RNA); however, DNase I does not affect their structural integrity (Fox et al., 2005; Prasanth et al., 2005). Second, all of the major paraspeckle proteins contain RNA-binding motifs, and many have previously described functions in RNA processing (see previous section and Table I). Third, PSPC1 requires its RNA-binding domains for paraspeckle targeting (Fox et al., 2005). Finally, paraspeckles disassemble in the absence of active Pol II transcription and subsequently reassemble on its restoration, suggesting that their formation may be dependent on RNA production (Fox et al., 2002, 2005). In line with this evidence, two types of RNA have now been identified that specifically localize to paraspeckles, each providing clues to paraspeckle formation and function.
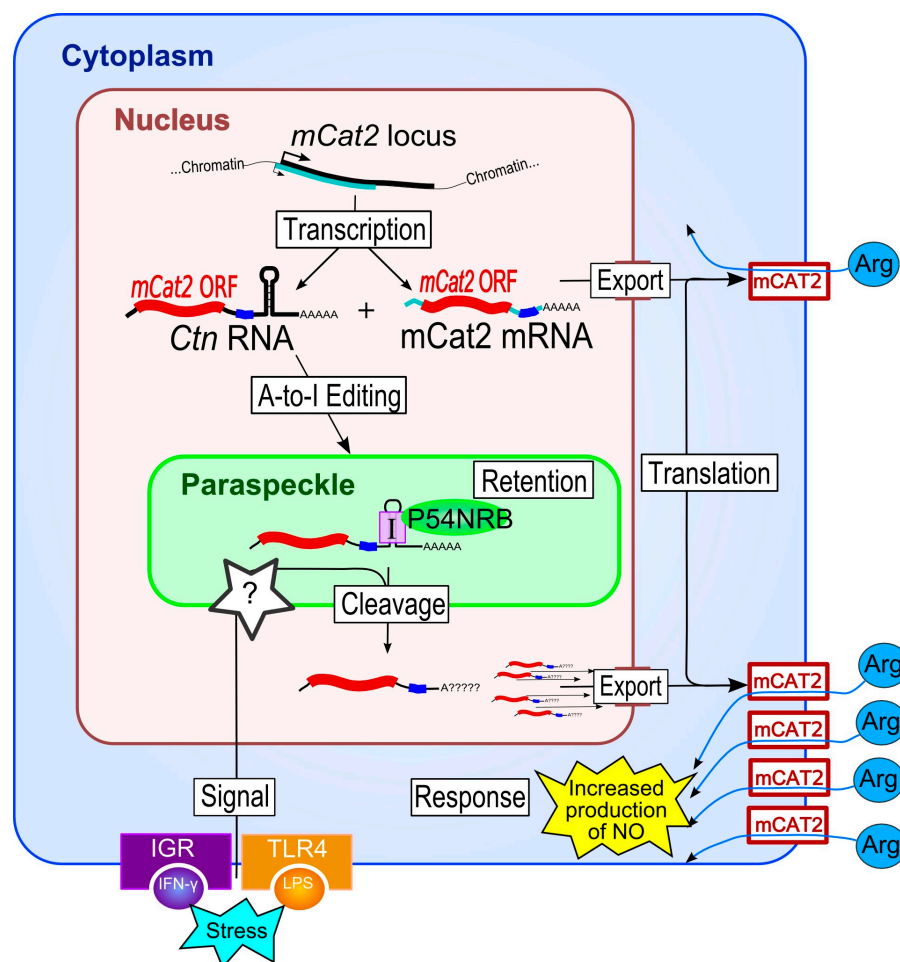
Role of paraspeckles in nuclear retention of RNA. The discovery in mouse of the first paraspeckle RNA revealed how paraspeckles are involved in the control of gene expression through retention of RNA in the nucleus (Prasanth et al., 2005). *Ctn* RNA is an alternative transcript generated from the *mCAT2* gene (encoding the cationic amino acid transporter 2 protein). *Ctn* differs from the canonical *mCAT2* mRNA in that it uses a different promoter and a distal poly(A+) site (producing a much longer 3′ untranslated region [UTR]) and is nuclear enriched (Fig. 2). However, similar to *mCAT2*, *Ctn* is spliced and contains the entire open reading frame of the mCAT2 protein. The fate of the two RNA species is quite different: although *mCAT2* is exported and translated as normal, *Ctn* is retained in the nucleus and within paraspeckles in some cell types (Prasanth et al., 2005). The key to paraspeckle/nuclear retention lies in the long 3′ UTR of *Ctn*, which contains double-stranded RNA hairpins formed by inverted repetitive elements. These RNA hairpins were shown to be A to I hyperedited and associated with DBHS proteins in vivo, which is consistent with the previous study linking nuclear retention of inosine-containing RNA and DBHS proteins (Zhang and Carmichael, 2001). The fate of *Ctn*

does not end in paraspeckles, as the long 3′ UTR is cleaved off (potentially via the paraspeckle-associated cleavage factor CFIm; Table I) as a response to a variety of stress signals. The cleavage event is associated with a concomitant rise in the shorter *mCAT2* mRNA levels in the cytoplasm and a pulse of increased protein production. As the mCAT2 protein mediates uptake of precursors in the nitrous oxide response pathway, this retention–release mechanism allows the cell to rapidly mount a nitrous oxide response to the stress.

Although *Ctn* is not present in humans, evidence supports widespread nuclear retention of RNA because up to half of human transcripts may have extended 3′ UTRs (Iseli et al., 2002). In addition, primates have the largest number of repetitive elements, many of which are inverted, and also have much more A to I hyperediting than other species, the bulk of which takes place at Alu sequences (repetitive elements that make up 10% of the human genome; Levanon et al., 2005; Chen et al., 2008). Moreover, nuclear retention of RNA also takes place when inverted repeats are taken out of their natural biological context and placed downstream of a reporter gene (Chen et al., 2008). P54NRB/NONO was also shown to associate in vivo with these nuclear-retained reporter RNA transcripts (Chen et al., 2008). The nuclear retention mechanism may also be mediated by inverted repeats in 5′ UTRs and retained introns, as many transcripts contain these features. Moreover, because there is at least one other example of a nuclear-retained RNA that does not have inverted repeats (Kay et al., 2005), it is also likely that additional RNA elements are mediating nuclear retention. Until very recently, *Ctn* was the only example of an RNA that underwent cleavage to be released from nuclear retention. However, a bioinformatic study has now shown evidence that many hundreds of human transcripts containing inverted repeats also exist in a shorter form in which the inverted repeats have been excised (Osenberg et al., 2009). This suggests that the excision of inverted repeats may be generally used as a mechanism for release of transcripts from nuclear retention.

Figure 2. **The role of nuclear retention in gene regulation in differentiated cells.** In the example of the *mCat2* gene, two different promoters result in two alternative transcripts: the *mCat2* mRNA, which is exported for translation of the mCAT2 cation transporter protein, and *Ctn* RNA, which is a longer transcript including the mCAT2 protein coding region and an extended 3′ UTR containing inverted repeats. These repeats undergo A to I hyperediting, resulting in *Ctn* binding to DBHS proteins and retention in paraspeckles. Stress signals mediated by IFN-γ–receptor (IFN-γ–IGR) and lipopolysaccharide–toll-like receptor 4 (LPS–TLR4) interaction result in a cleavage event that liberates a shorter *Ctn*, which is exported for translation. This up-regulation of the mCAT2 protein results in increased nitric oxide (NO) production as a response to the cellular stress. Although detail in this figure is specific to mCAT2 protein regulation, recent research hints at a more generic retention–release mechanism that exists for other transcripts containing hyperedited inverted repeats in their 3′ UTR (Chen et al., 2008; Chen and Carmichael, 2009; Osenberg et al., 2009).
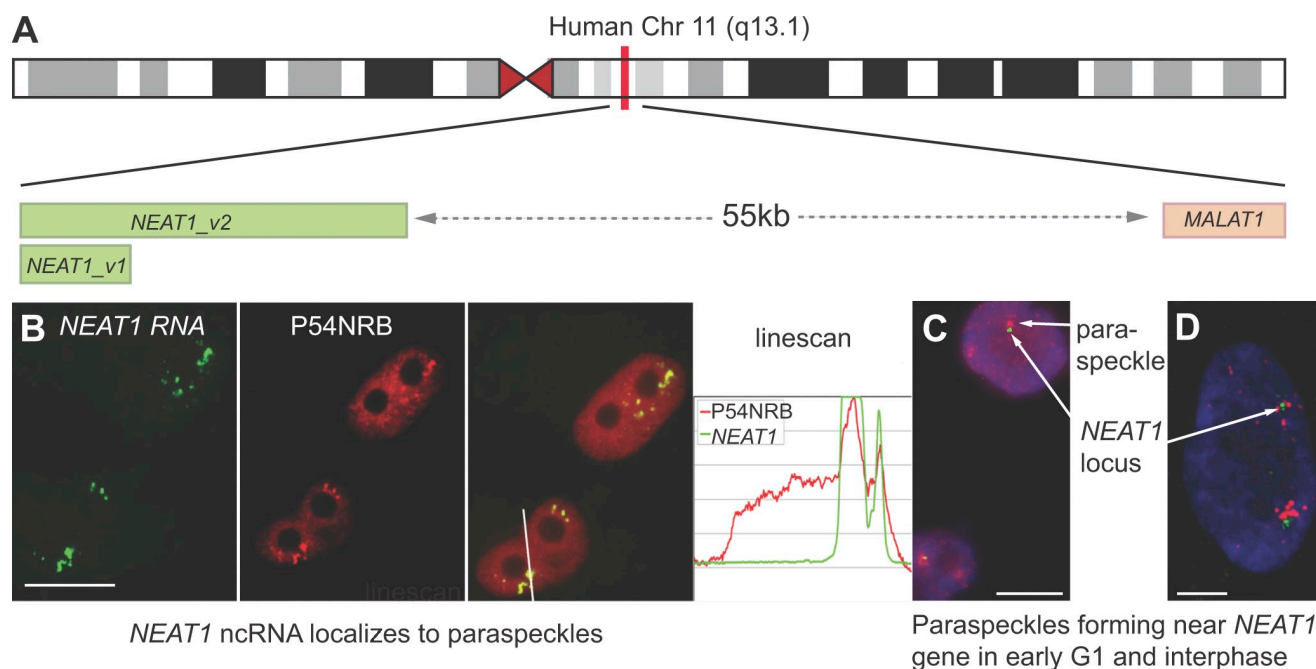
Another very recent study has confirmed the importance of paraspeckles to the nuclear retention mechanism. In cells without paraspeckles such as human embryonic stem cells, mRNAs containing A to I hyperedited inverted repeats were able to efficiently overcome nuclear retention and were present in the cytoplasm at high levels (Chen and Carmichael, 2009). However, with cellular differentiation and the induction of paraspeckles, the ratio of nuclear to cytoplasmic A to I hyperedited mRNAs increased. Interestingly, DBHS protein expression did not vary between the two cellular contexts, instead, paraspeckle induction and increased RNA nuclear retention correlated with the expression of the paraspeckle-specific structural ncRNA species *NEAT1*.

NEAT1: an architectural long ncRNA in paraspeckles. We now know that the majority of our genome is transcribed to generate both protein- and nonprotein-coding RNA (Carninci et al., 2005). The numerous ncRNAs may be derived from introns of protein-coding genes or may be antisense or found between protein-coding genes (Mercer et al., 2009). Although our understanding of the roles and identity of different classes of small ncRNAs is substantial, we are only beginning to understand the varying roles that long ncRNAs may be playing in the cell (Prasanth and Spector, 2007). A long-held view in the field has been that there is a role for nuclear RNA as a structural component of nuclear organization.

In 2007, a study to identify nuclear-enriched RNA transcripts found three major long ncRNA species: the *XIST* RNA (well-known for its role in X chromosome inactivation), and two nuclear-enriched autosomal transcripts termed *NEAT1* (also reported in the literature as *MEN-ε/β* or *VINC-1*) and *MALAT1* (also known as *NEAT2*; Hutchinson et al., 2007). Genes encoding *NEAT1* and *MALAT1* are typically found close together in mammalian genomes, some distance away from the nearest protein-coding gene. Besides both being nuclear-enriched ncRNA, another feature of *NEAT1* and *MALAT1* is that the long RNAs transcribed from each gene are both cleaved at their 3′ ends to produce an unusual small tRNA-like molecule that may be a hallmark of some nuclear ncRNAs (Wilusz et al., 2008; Sunwoo et al., 2009). Like *XIST*, both of these ncRNAs were shown to have defined subnuclear localization, *MALAT1* within nuclear speckles and *NEAT1* in subnuclear foci found abutting nuclear speckles (Hutchinson et al., 2007). Recently several groups have shown that these *NEAT1* foci colocalize with paraspeckles and, moreover, that *NEAT1* RNA is essential for paraspeckle integrity (Chen and Carmichael, 2009; Clemson et al., 2009; Sasaki et al., 2009; Sunwoo et al., 2009).

Two isoforms of *NEAT1* are transcribed, *NEAT1_v1* and *NEAT1_v2* (also known as *MEN-ε* and *MEN-β*; Fig. 3), overlapping in ∼3–4 kb of sequence at the 5′ end, and both transcripts, either endogenous or overexpressed, localize in paraspeckles in

Figure 3. **Paraspeckles contain *NEAT1* ncRNA and form near the *NEAT1* gene.** (A) *NEAT1* and *MALAT1* gene loci on human chromosome (Chr) 11 q13.1. Two transcripts are produced from the *NEAT1* gene, 3.7-kb *NEAT1_v1* and 23-kb *NEAT1_v2* in humans. (B) RNA FISH against *NEAT1* ncRNA (green) and immunofluorescence against P54NRB/NONO (red) shows that they colocalize in paraspeckles. The line scan is taken over a line as indicated in the merged image. (C) HeLa cells in early G1 stained with PSPC1 (red) to mark the first forming paraspeckles and DNA FISH (green) against 11q13. (D) Interphase HeLa cell with *NEAT1* RNA FISH to mark paraspeckles (red) and DNA FISH to mark chromosome 11 q13.1 (green). Panels B–D are reproduced from Clemson et al. (2009) with permission from Elsevier. Bars: (B and C) 10 µm; (D) 5 µm.

mouse and human cells (Fig. 3 B). The confinement of *NEAT1* to paraspeckles is greater than that seen with DBHS proteins such as P54NRB/NONO, which is also abundant in the nucleoplasm (Fig. 3 B). *NEAT1* is essential for the formation and maintenance of paraspeckles: they do not reform in the absence of *NEAT1* after temporary transcription inhibition (Sasaki et al., 2009; Sunwoo et al., 2009), and knocking down *NEAT1* results in loss of paraspeckles (Chen and Carmichael, 2009; Clemson et al., 2009; Sasaki et al., 2009; Sunwoo et al., 2009). Moreover, stable cell lines overexpressing *NEAT1_v1* in NIH3T3 cells have more paraspeckles than control cells, suggesting that *NEAT1* RNA is the limiting factor in paraspeckle formation (Clemson et al., 2009). *NEAT1* is also likely to be the paraspeckle nucleating factor, as paraspeckles are observed forming in early G1 near to the *NEAT1* gene locus and are often found clustered near the *NEAT1* gene in interphase (Fig. 3, C and D; Clemson et al., 2009). Interestingly, in contrast to the essential role for *NEAT1* in paraspeckles, *MALAT1* is not required for nuclear speckle maintenance, as knockdown of *MALAT1* has no effect on these or other subnuclear structures (Clemson et al., 2009).

Like *Ctn*, *NEAT1* associates with DBHS proteins in vivo, as immunoprecipitation of PSF/SFPQ, P54NRB/NONO, and PSPC1 all copurify *NEAT1* RNA to varying levels (Chen and Carmichael, 2009; Clemson et al., 2009; Sasaki et al., 2009; Sunwoo et al., 2009). In contrast to *Ctn*, *NEAT1* shows no evidence of A to I hyperediting, suggesting that the DBHS proteins use more than one mode of RNA binding within paraspeckles. The presence of only very sporadic short regions of conservation between mammalian *NEAT1* sequences raises the possibility

that DBHS proteins bind an RNA structure rather than a sequence. However, a previous study of DBHS proteins binding to U5SnRNA supports both sequence- and structure-based aspects to binding (Peng et al., 2002). There is some discrepancy as to the relative roles of the short and long *NEAT1* isoforms in paraspeckle formation and maintenance: *NEAT1_v1* overexpression alone can increase paraspeckle number in NIH3T3 cells (Clemson et al., 2009) and *NEAT1_v1* is present in paraspeckles when *NEAT1_v2* is knocked down (Sunwoo et al., 2009) and is bound by recombinant DBHS proteins in vitro (Clemson et al., 2009). However, other data suggests that the final 10 kb of *NEAT1_v2* may be required for exogenous rescue of the *NEAT1* knockdown (Sasaki et al., 2009). Detailed studies of the molecular interactions between the DBHS proteins and *NEAT1* should address these issues.

Paraspeckle formation

Now that we have a grasp of the main protein and RNA components of paraspeckles, it is possible to put this information together to form a picture of paraspeckle formation, beginning with the production of *NEAT1* in daughter nuclei after cell division (Fig. 4). Before newly made *NEAT1* has a chance to diffuse away from its gene locus, it is rapidly targeted by DBHS protein dimers, and together, the RNA–protein complex builds up the paraspeckle particle. The finished paraspeckle likely consists of multiple copies of *NEAT1* RNA–DBHS protein complexes, which form a structural scaffold that is nevertheless dynamic, in that individual DBHS protein molecules can exchange with the nucleoplasm. It is possible that the oligomerization propensity of
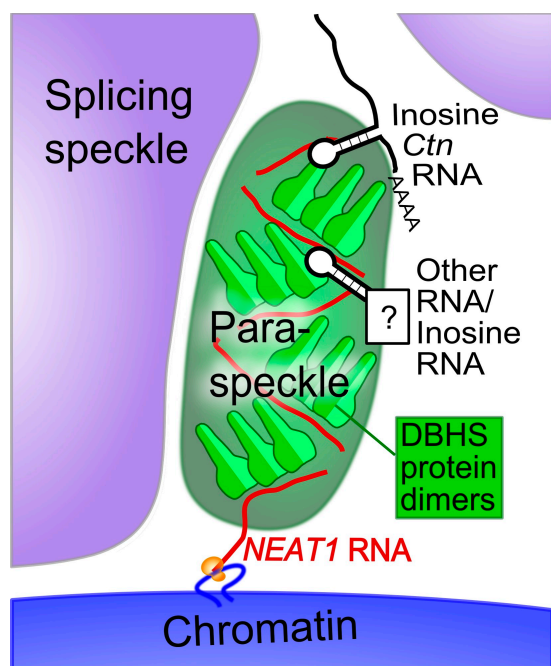
Figure 4. **Model of paraspeckle formation.** The schematic shows a paraspeckle forming in the interchromatin space in between two nuclear speckles. The paraspeckle forms near the *NEAT1* gene locus through the interactions between newly transcribed *NEAT1* RNA and DBHS protein dimers. RNA containing A to I hyperedited inverted repeats, as well as potentially other RNA, is regulated within paraspeckles via interaction with DBHS proteins.

the DBHS proteins (Kiesler et al., 2003; Myojin et al., 2004; Fox et al., 2005) contributes to the paraspeckle structural framework. Interestingly, cytoplasmic P bodies have many parallels with nuclear paraspeckles, including being linked to the control of gene expression through mRNA storage. A recent structural study of Edc3, a protein involved in P-body formation, shows that the dimerization properties of this protein increase the RNA-binding potential of the complex and may also contribute to P-body assembly by linking messenger RNPs together (Ling et al., 2008).

Without the production of *NEAT1* RNA, paraspeckles fail to form, explaining why paraspeckles are not observed when Pol II transcription is inhibited nor in cell types that do not express *NEAT1* or in organisms that do not contain the *NEAT1* gene. Conversely, without abundant DBHS proteins, paraspeckles are also not observed. In light of this model, it is reasonable that, in the future, a molecule may only be labeled as paraspeckle associated once it is shown to localize with both DBHS proteins and *NEAT1*. The first paraspeckles form very close to the *NEAT1* gene locus, and paraspeckles also remain closely associated with the *NEAT1* gene in interphase; however, just how the association is maintained is not known. Interestingly, although there is a tendency for paraspeckles to cluster near the *NEAT1* gene during interphase, it is not an absolute correlation; many nuclei also have paraspeckles at other nuclear locations in addition to a NEAT1 cluster (Fig. 3 D; Clemson et al., 2009). It is possible that these locations are the site of production for RNA species that are being regulated in paraspeckles such as mRNAs containing A to I hyperedited inverted repeats (for example, *Ctn*).

Cellular function of paraspeckles

A recent study brings together the contributions of the three main types of protein and RNA molecules within paraspeckles in an important area of cell biology: pluripotency and differentiation (Chen and Carmichael, 2009). In this study, the authors draw the conclusion that paraspeckle formation is associated with a loss of pluripotency in embryonic stem cells. Furthermore, they suggest that a lack of *NEAT1* and paraspeckles can be used as a marker for pluripotency. Paraspeckles are most likely contributing to differentiation by changing the gene expression profile through nuclear retention of A to I hyperedited mRNA. Interestingly, another study linking *NEAT1* to paraspeckle formation also showed a trend of *NEAT1*/paraspeckle induction with differentiation: in this case, the differentiation of myoblasts into myotubes is associated with a threefold up-regulation of *NEAT1* and an increase in paraspeckle size and number (Sunwoo et al., 2009). It will be interesting in the future to determine how widespread changes in NEAT1 levels and paraspeckle abundance correlate with different models of differentiation and the identity of those transcripts being controlled by nuclear retention in these systems.

Another potential role for paraspeckles in cell biology is in the response to certain viruses. An earlier study reported that *VINC-1* (since shown to correspond to *NEAT1*) is up-regulated in the central nervous system of mice upon infection with Japanese encephalitis or rabies viruses (Saha et al., 2006). Given that many of the aforementioned studies have proven a link between *NEAT1* up-regulation and paraspeckle formation, it is possible that these viruses may trigger an increase in paraspeckle size and number, either as a cellular defense mechanism or for viral processing. The link between a viral response and paraspeckles is consistent with the speculation that HIV RNA may hijack the RNA processing pathways mediated by DBHS proteins, as PSF/SFPQ and P54NRB/NONO bind elements within HIV mRNAs and regulate their processing and nuclear export (Zolotukhin et al., 2003).

It is likely that the most telling analyses of the biological role for paraspeckles will arise from the generation and analysis of a NEAT1-null mouse, which should be devoid of paraspeckles. However, it should also be considered that there may be primate-specific functions for paraspeckles, given the abundance of A to I hyperedited inverted repeats specifically transcribed from primate genomes.

Paraspeckles as a paradigm for a class of subnuclear bodies

Paraspeckles are not the only subnuclear foci formed via the specific interactions between nuclear proteins and nuclear-retained ncRNA. A clear example occurs in myotonic muscular dystrophy, in which RNA transcribed from mutated genes with expanded CTG repeats is bound by the muscleblind-like family of proteins and retained in the nucleus within subnuclear foci (O'Rourke and Swanson, 2009). These myotonic muscular dystrophy foci do not colocalize with paraspeckles (Clemson et al., 2009). However, the similarities between these foci and paraspeckles suggest that the cell has common themes in nuclear retention of RNA that are apparent in both normal cell function and disease.

Paraspeckle markers localize to the IGAZ in TEM sections. Given the heterogeneity in IGAZ composition (Puvion-Dutilleul et al., 1995), it is possible that this compartment seen under EM is actually composed of numerous different subnuclear bodies akin to paraspeckles, i.e., containing distinct structural ncRNA and specific RNA-binding proteins and having different species of RNA regulated/retained within them. Indeed, there already exist two examples of other long ncRNA localizing to unique subnuclear foci, although in these cases, their appearance is very cell type specific, and no corresponding protein partners are known (Royo et al., 2007; Sone et al., 2007). The lack of identified marker ncRNAs and proteins for other subnuclear bodies may have so far prevented their detection and characterization. Further studies on subcellular localization of the many thousands of new long ncRNAs being currently discovered from high throughput genomics analyses will, in the future, provide a fuller picture of these structures and their roles in the cell.

Conclusion

Paraspeckles lie at the nexus of two expanding areas of interest in the control of gene expression in mammals: the roles of functional long ncRNAs and the effects of RNA editing of transcripts. An exciting prospect is finding new subcellular complexes by studying the localization of newly identified ncRNAs. Potentially, paraspeckles can be used as a model system for studying protein–ncRNA interactions and dynamics in such complexes. Within paraspeckles, the mechanism of RNA nuclear retention may be critical for controlling gene expression in a variety of cellular contexts. The discovery of the identity and function of the many key molecules that may be regulated in paraspeckles through this mechanism will be of great interest in the years to come.

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