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Title	Chromatin remodeling complexes in the assembly of long noncoding RNA-dependent nuclear bodies
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Citation	Nucleus, 6(6), 462-467 https://doi.org/10.1080/19491034.2015.1119353
Issue Date	2015
Doc URL	https://hdl.handle.net/2115/63974
Rights	This is an Accepted Manuscript of an article published by Taylor & Francis in Nucleus on 2015, available online: http://www.tandfonline.com/10.1080/19491034.2015.1119353
Type	journal article
File Information	589_1_merged_1446606234.pdf

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Nucleus (EXTRA VIEW)

Chromatin remodeling complexes in the assembly of long noncoding RNA-dependent nuclear bodies

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Paraspeckles are subnuclear structures that assemble on nuclear paraspeckle assembly transcript 1 (NEAT1) long noncoding (lnc)RNA. Paraspeckle formation requires appropriate NEAT1 biogenesis and subsequent assembly with multiple prion-like domain (PLD) containing RNA-binding proteins. We found that SWI/SNF chromatin remodeling complexes function as paraspeckle components that interact with paraspeckle proteins (PSPs) and NEAT1. SWI/SNF complexes play an essential role in paraspeckle formation that does not require their ATP-dependent chromatin remodeling activity. Instead, SWI/SNF complexes facilitate organization of the PSP interaction network required for intact paraspeckle assembly. SWI/SNF complexes may collectively bind multiple PSPs to recruit them onto NEAT1. SWI/SNF complexes are also required for Sat III (Satellite III) lncRNA-dependent formation of nuclear stress bodies under heat shock conditions. Organization of the lncRNA-dependent omega speckle in Drosophila also depends on the chromatin remodeling complex. These findings raise the possibility that a common mechanism controls the formation of lncRNA-dependent nuclear body architecture.

Key words: Long noncoding RNA, nuclear body, paraspeckle, nuclear stress body, omega speckle, chromatin remodeling complex, RNA-binding protein, prion-like domain (PLD).

Abbreviations: lncRNA, long noncoding RNA; NEAT1, nuclear paraspeckle

assembly transcript 1; nSB, nuclear stress body; nt, nucleotide; PSP, paraspeckle protein; RNP, ribonucleoprotein; RRM, RNA recognition motif; Sat III, Satellite III; SWI/SNF, SWItch/Sucrose Non-Fermentable.

Introduction

The nucleus is a highly compartmentalized organelle in eukaryotic cells containing genomic DNA that is packaged into chromatin and configured in specific chromosome territories. Numerous large granular structures known as nuclear bodies exist in the interchromatin space [1-3]. Nuclear bodies, comprising specific proteins and (usually) RNAs, are involved in specific nuclear events including the biogenesis of macromolecular machinery such as ribosomes and spliceosomes, transcription, RNA processing, protein modification, and protein degradation [1-3]. Nuclear bodies are present at a steady state and dynamically respond to various external and/or intrinsic signals. These structures are thought to form through stochastic assembly or ordered assembly of nuclear body components. In addition, a seeding mechanism has also been proposed for nuclear body assembly and maintenance [1].

Recent studies have revealed that some nuclear bodies are built on specific long noncoding RNAs (lncRNAs) as their seeds or scaffolds; lncRNAs that function as scaffolds of nuclear bodies are referred to as “architectural RNAs (arcRNAs)” [4]. To date, five types of lncRNA have been classified as arcRNAs [4]: nuclear paraspeckle assembly transcript 1 (NEAT1) lncRNA in the paraspeckle [5-8], ribosomal intergenic spacer lncRNAs in the nucleolar detention center [9], human Sat III lncRNAs in the nuclear stress body (nSB) [10], Drosophila

heat shock RNA (hsr) omega in the omega speckle[11], and fission yeast meiosis-specific RNA species, meiRNA, in the Mei2 dot [12]. Thus, arcRNAs are widely present in eukaryotes and function in nuclear body construction in conjunction with multiple proteins. This article mainly describes the building process of the paraspeckle nuclear body that is seeded by and built on architectural NEAT1 lncRNA. In particular, we focus on SWI/SNF chromatin remodeling complexes, which we identify as essential factors for arcRNA-dependent assembly of nuclear bodies.

Paraspeckle components and their roles in paraspeckle assembly

Paraspeckles were initially defined as interchromatin granule-associated zones, which are frequently located adjacent to interchromatin granules, as observed by electron microscopy (EM) [13]. Paraspeckles were later defined as nuclear bodies enriched in characteristic RNA-binding proteins, including non-POU domain containing octamer binding (NONO) protein, paraspeckle component 1 (PSPC1), and splicing factor proline/glutamine-rich (SFPQ) protein, all of which belong to the Drosophila behavior and human splicing (DBHS) protein family[14-16]. Paraspeckles are RNase sensitive, suggesting that maintenance of the intact paraspeckle structure requires RNA(s)[15]. In 2009, four groups independently discovered that NEAT1 lncRNA is an essential architectural component of paraspeckles[5-8]. Paraspeckles average ~360 nm in diameter[17], indicating that they can be considered to represent huge ribonucleoprotein (RNP) particles, which are more than 1000 times larger than ribosomes.

NEAT1 lncRNA comprises two isoforms, 3.7 knt NEAT1_1 and 23 knt NEAT1_2, both of which are transcribed by RNA polymerase II from a common promoter [6,7]. The 3' end of NEAT1_1 is processed by cleavage and polyadenylation specificity factor (CPSF) 5/6 complex to create the canonical 3' terminus, whereas NEAT1_2 is subjected to non-canonical 3' end processing by RNase P to create a non-polyadenylated terminus with the characteristic triple helix structure [7, 18-20] NEAT1_2, but not NEAT1_1, is essential for paraspeckle formation. EM analysis revealed that the central region of NEAT1_2 is located in the interior part of the paraspeckle and that both the 5' and 3' termini of NEAT1_2, as well as NEAT1, are confined to the periphery of the paraspeckle, suggesting that the organization of NEAT1 transcripts constrains the geometry of these bodies[17].

More than 40 paraspeckle proteins (PSPs) have been identified, most of which possess characteristic RNA-binding domains including RNA Recognition Motif (RRM), K-Homology motif, and RGG box [3,18]. Further functional analyses revealed that seven PSPs (NONO, SFPQ, RNA binding motif protein 14 (RBM14), heterogenous nuclear RNP K (HNRNPK), fused in sarcoma (FUS), DAZ-associated protein 1 (DAZAP1), and HNRNPH3) are essential for paraspeckle formation. Functional assignment of the essential PSPs revealed that paraspeckle formation proceeds via two distinct steps; first via NEAT1_2 biogenesis and second via further assembly of the intact paraspeckle from the NEAT1_2 subcomplexes (**Fig. 1**). Direct visualization of NEAT1 and PSPs showed that paraspeckles are formed on the NEAT1 chromosomal locus during transcription of NEAT1 by RNA polymerase II [21], suggesting that paraspeckle

formation is initiated co-transcriptionally (**Fig. 1**). HNRNPK (category 1A protein in **Fig. 1**) accelerates the synthesis of the essential NEAT1_2 isoform by interfering with the polyadenylation of NEAT1_1 (**Fig. 1**) [18]. NONO, SFPQ, and RBM14 (category 1A proteins in **Fig. 1**) stabilize the synthesized NEAT1_2. FUS, HNRNPH3, and DAZAP1 (category 1B proteins in **Fig. 1**) scarcely affect NEAT1_2 biogenesis, but are required for assembly of the intact paraspeckle from NEAT1 subcomplexes (**Fig. 1**) [18]

Novel function of SWI/SNF complexes in paraspeckle assembly

In a search for factors that interact with the identified PSPs, we found several subunits of SWI/SNF complexes that interact with a number of PSPs[22]. A subpopulation of SWI/SNF complexes is diffusely localized to the nucleoplasm, while another subpopulation is enriched in paraspeckles. EM observation revealed that SWI/SNF complexes are distributed in distinct, patchy subdomains in paraspeckles [22].

SWI/SNF complexes reposition nucleosomes for transcriptional control, DNA repair, recombination, and chromosome segregation. ATP hydrolysis by the catalytic subunits BRG1 and BRM is required for their nucleosome remodeling activity [23]. However, CRISPR/Cas9-mediated mutagenesis of the ATPase motif of BRG1 revealed that the ATP-dependent chromatin remodeling activity of BRG1 is not required for the function of the SWI/SNF complex in paraspeckle assembly [22]. This finding suggests that the role of SWI/SNF complexes in paraspeckle assembly is novel and distinct from its well-characterized role in chromatin remodeling. EM observation supports this argument, since BRG1 is

concentrated in interior paraspeckle subdomains depleted of chromatin [22].

How SWI/SNF complexes facilitate paraspeckle assembly remains largely elusive. Molecular interaction studies have indicated that both BRG1 and BRM directly interact with NEAT1_2 via RNA-protein interactions, and they also interact with a number of essential PSPs via protein-protein interactions [22]. Functional depletion of SWI/SNF complexes results in disassembly of paraspeckles, likely due to reduced interactions between the essential PSPs, but not NEAT1-PSP. Thus, SWI/SNF complexes may represent the molecular hub that integrates the assembly of multiple PSPs onto the architectural NEAT1_2 lncRNA (**Fig. 2**) [22]. Recently, the prion-like domain (PLD), which resides in several essential PSPs (e.g., FUS and RBM14), was shown to be required for paraspeckle assembly, presumably via the formation of a protein-protein interaction network (**Fig. 2**) [24]. An interesting possibility is that SWI/SNF complexes may facilitate interactions between PLDs in PSPs that may trigger liquid-liquid phase separation to drive larger droplet-like granule formation [25]. SWI/SNF complexes remain associated with essential PSPs, even when the intact paraspeckle structure is disrupted by depletion of NEAT1, suggesting that SWI/SNF complexes collect PSPs outside the paraspeckle and then recruit them onto NEAT1_2 (**Fig. 2**) [22].

BRG1 and BRM lack any canonical RNA-binding motifs, suggesting that novel domain(s) are involved in their interaction with NEAT1_2. Which SWI/SNF subunits directly interact with the essential PSPs remains unknown. In addition to BRG1 and BRM, at least four subunits of SWI/SNF complexes (BAF170, BAF155, BAF57, and BAF47) were experimentally shown to contribute to

paraspeckle formation; however, the role of each subunit remains uncharacterized. In particular, the subunit composition of paraspeckle-localized SWI/SNF complexes has been poorly investigated. An intriguing possibility is that the specific subunit composition of paraspeckle-localized SWI/SNF complexes enables them to efficiently gather the PSPs onto NEAT1_2 lncRNA.

Chromatin remodeling complexes are required for the formation of several distinct lncRNA-dependent nuclear bodies

SWI/SNF complexes are also essential for the formation of another lncRNA-dependent nuclear body, the nSB. The nSBs form on Sat III lncRNAs that are transcribed from the pericentromeric Sat III repeats located on human chromosomes 9, 12, and 15 in response to heat shock and to various extracellular stresses [10]. These nuclear bodies act to sequestrate several splicing-related RNA-binding proteins (e.g., SRSF1, SRSF7, TARDBP, and SAFB) and transcription factors (e.g., HSF1 and NFAT5) [10]. We showed that SWI/SNF complexes localize to the nSB, where they play an essential role in nSB formation upon heat shock [22]. Analogous to its effect on paraspeckle formation, SWI/SNF depletion does not affect Sat III lncRNA expression, suggesting that SWI/SNF complexes function in the assembly of nSBs from individual Sat III RNP complexes. Further study of the interaction between SWI/SNF complexes and nSB components, including Sat III lncRNA, will clarify the commonality of the functions of SWI/SNF complexes in the assembly of these two lncRNA-dependent nuclear bodies.

Omega speckles are nuclear bodies in Drosophila that form on

hsr-omega lncRNA in response to heat shock stress [11]. Omega speckles contain several RNA-binding proteins including NonA, Hrb87F/Hrp36, Hrb57A/Bancal, Hrp59/Rumpelstiltskin, and Hrp40/Squid. NonA, Hrb87F, Hrb57A, and Hrp59 are Drosophila homologs of mammalian DBHS proteins (PSPC1, NONO, and SFPQ), HNRNPA1, HNRNPK, and HNRNPM, respectively, some of which also possess PLDs as well as RRMs. Additionally, Imitation SWI (ISWI) chromatin remodeling complex was identified as the factor that genetically interacts with hsr-omega lncRNA [26]. Indeed, ISWI physically interacts with hsr-omega lncRNA and acts as the essential factor for omega speckle formation [26]. Thus, paraspeckles, nSBs, and omega speckles share common features, i.e., their subunit compositions and the requirements for their construction. As expected, arcRNAs, PLD-containing RNA-binding proteins, and chromatin remodeling complexes are common factors required for their structural formation (**Table 1**). The missing pieces are the PLD-containing RNA-binding proteins required for nSB formation. The PLD-containing RNA-binding protein TARDBP was reported to localize to nSBs; however, its role remains to be examined. Further analysis will reveal the PLD-containing RNA-binding protein that functions in nSB formation.

Outlook

LncRNA-dependent nuclear bodies have been discovered, and the architectural roles of lncRNA were revealed as a new, distinct function of lncRNA. As described above, a number of arcRNAs possess similar architectural functions for building specific nuclear bodies. Together with arcRNAs, a number

of proteins, including PLD-containing RNA-binding proteins and chromatin remodeling complexes, are involved in nuclear body formation. Our investigation revealed that the reciprocal interactions between arcRNA, PLD-containing RNA-binding proteins, and chromatin remodeler lead to the building of massive RNP complexes comprising nuclear bodies. In the case of paraspeckle formation, the chromatin remodeler may act as the hub to recruit PLD-containing RNA-binding proteins onto arcRNA. It is currently unclear how SWI/SNF complexes specifically target NEAT1 lncRNA. Considering that paraspeckle formation is initiated co-transcriptionally on the NEAT1 chromosome locus in conjunction with NEAT1 transcription, it is possible that SWI/SNF complexes recognize the NEAT1 chromatin region as well as NEAT1 nascent transcripts. Indeed, we have preliminary data suggesting that the association of SWI/SNF complexes on the NEAT1 chromosome locus depends on RNA polymerase II transcription. It would be interesting to pursue the detailed mechanism determining the specific recognition of SWI/SNF complexes to form paraspeckles, as well as other nuclear bodies.

The arcRNA-dependent nuclear bodies act to sequestrate specific RNA-binding proteins with affinity for the respective arcRNAs. The sequestrated proteins are thought to lose their ability to regulate transcription and/or RNA processing; therefore, these nuclear bodies are described as the “molecular sponges” of gene regulation [27, 28]. Paraspeckles also sequestrate specific mRNA species[16]. It would be intriguing to identify the components that are responsible for sequestering respective factors onto paraspeckles and other nuclear bodies. Meanwhile, paraspeckles attach to numerous chromosome regions that are

usually transcriptionally active [29]. This observation raises the interesting possibility that SWI/SNF complexes localized in paraspeckles affect the activity of genomic loci that are attached to these paraspeckles. Neat1 knockout mice are normally viable, but they have defects in the development of corpus luteum and mammary glands, resulting in reduced female fertility and pup growth [30, 31]. Additionally, NEAT1 is regulated by estrogen receptors and is the critical modulator of prostate cancer [32]. Elucidating the molecular mechanisms underlying these NEAT1-related physiological events and diseases will reveal a great deal about the importance of the interaction of arcRNA with chromatin remodeler in novel types of gene regulatory mechanisms.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

TH was supported by the Funding Program for the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT).

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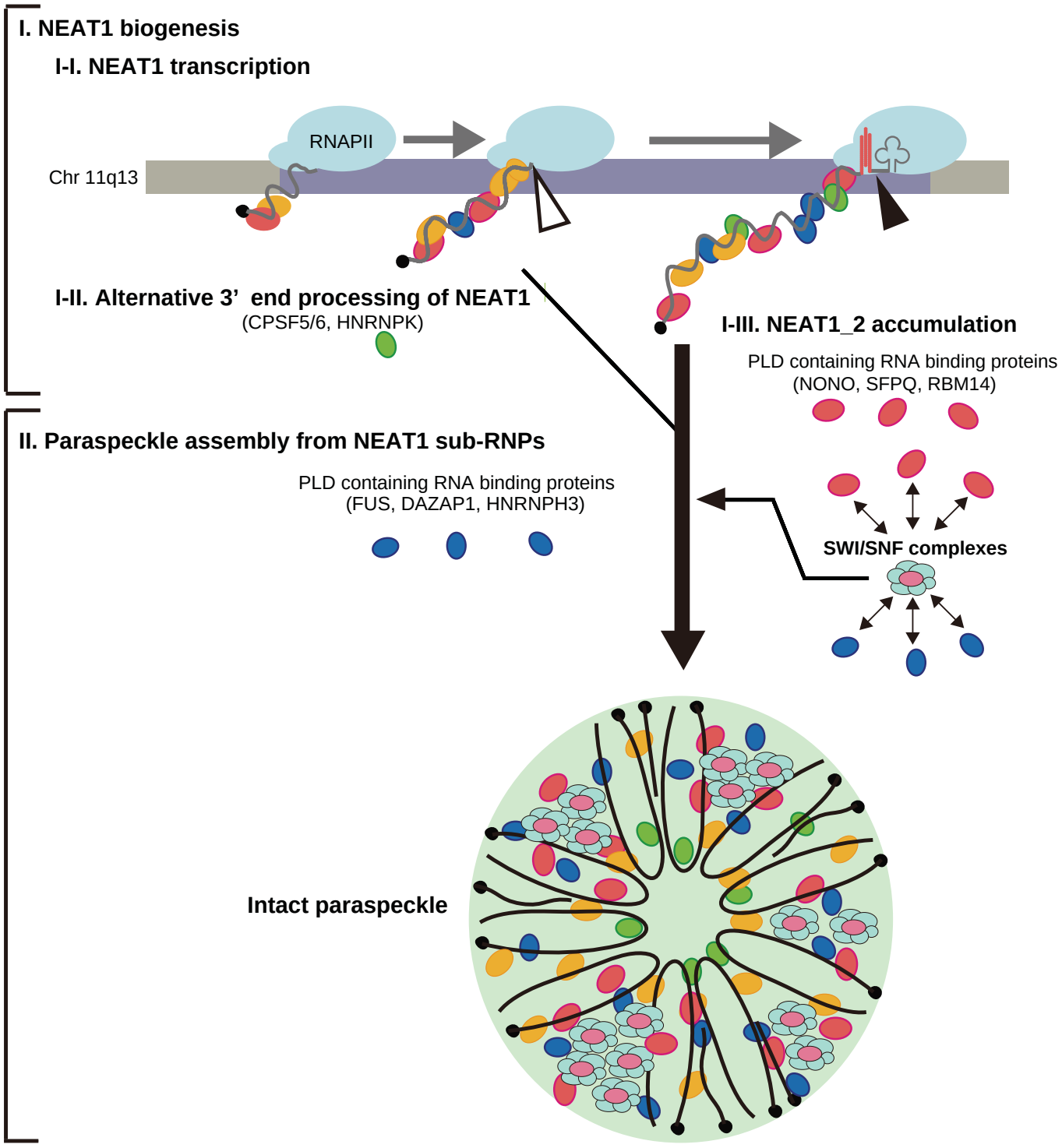
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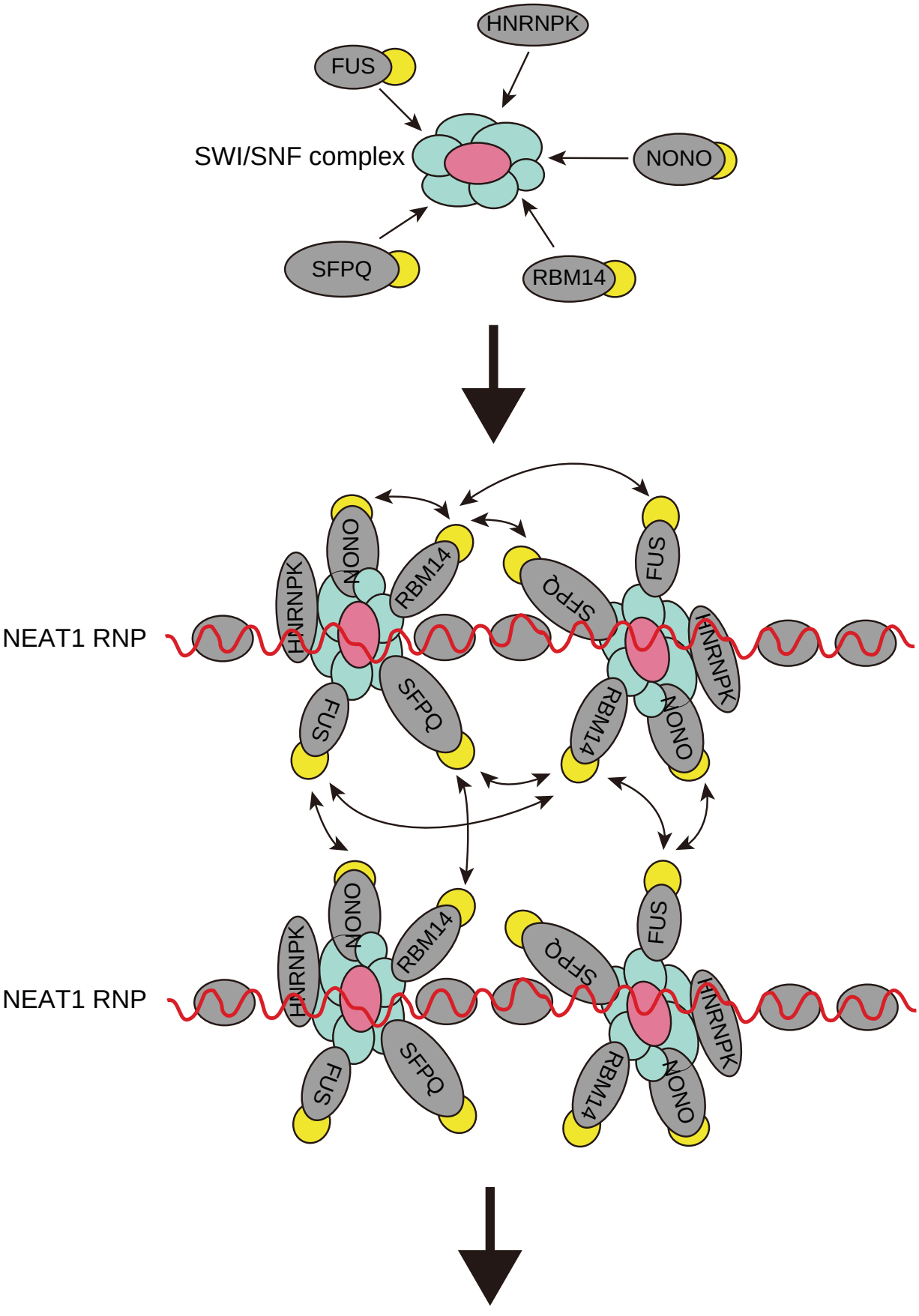
Figure 1. The essential steps in paraspeckle formation. Two essential steps (I and II) in paraspeckle formation are shown. In step I, NEAT1 biogenesis comprises three events (I-I, I-II, I-III) that occur continuously. Paraspeckle formation is initiated by NEAT1 transcription by RNA polymerase II (RNAPII) from the human NEAT1 gene locus at Chr 11q13 (I-I). NEAT1 isoforms in the diagram are not drawn to scale. The black, filled circle at the end of NEAT1 represents the cap structure. The red lines and the clover leaf at the 3' end of NEAT1_2 represent the triple helix structure and tRNA-like structure, respectively, that are required for formation of the non-polyadenylated 3' end of NEAT1_2. NEAT1_1 and NEAT1_2 are processed by canonical polyadenylation (open triangle) and RNase P-mediated processing (closed triangle), respectively (I-II). HNRNPK (green circle) is an essential category 1A factor in NEAT1_2 synthesis that interferes with CPSF5/6-dependent NEAT1_1 polyadenylation. Three category 1A PLD-containing RNA-binding proteins (NONO, SFPQ, and RBM14) (red circles) stabilize NEAT1_2 (I-III). In step II, SWI/SNF complexes and three category 1B PLD-containing RNA-binding proteins (FUS, DAZAP1, and HNRNPH3) (blue circles) act to assemble the intact paraspeckle from the NEAT1 sub-RNPs synthesized in step I. SWI/SNF complexes interact with both category 1A and 1B proteins that may be recruited during paraspeckle assembly. Non-essential paraspeckle proteins are represented by yellow circles.

Figure 2. Model of the role of SWI/SNF complexes in paraspeckle assembly. SWI/SNF complexes bind to the essential PSPs outside the paraspeckle and then recruit them onto NEAT1 lncRNA (red wavy lines) to form NEAT1 RNPs. The spatially concentrated NEAT1 RNP may build massive RNP complexes via interactions between PLDs (yellow ovals) and other domains.

Table 1. Attributes of lncRNA-dependent nuclear bodies in different animal kingdoms

Nuclear bodies	ArcRNAs	PLD RNA binding proteins	Chromatin remodeller	Inducing signals	Species detected
paraspeckle	NEAT1	NONO, SFPQ, RBM14, FUS, DAZAP1 etc	SWI/SNF	virus infection, proteasome inhibition	mammalian speceis
nuclear stress body	Satellite III	TARDBP	SWI/SNF	heat shock, osmotic stress, heavy metal	primate species
omega speckle	hsr-omega	Nona, Hrb87F, Hrb57A etc	ISWI	heat shock	Drosophila


I. NEAT1 biogenesis
I-I. NEAT1 transcription
RNAPII
Chr 11q13
I-II. Alternative 3' end processing of NEAT1
(CPSF5/6, HNRNPK)
I-III. NEAT1_2 accumulation
PLD containing RNA binding proteins
(NONO, SFPQ, RBM14)
SWI/SNF complexes
II. Paraspeckle assembly from NEAT1 sub-RNPs
PLD containing RNA binding proteins
(FUS, DAZAP1, HNRNPH3)
Intact paraspeckle


HNRNPK
FUS
SWI/SNF complex
NONO
SFPQ
RBM14
NEAT1 RNP
NEAT1 RNP
Intact paraspeckle