Shedding light on paraspeckle structure by super-resolution microscopy

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The nuclear body paraspeckle is built on the lncRNA *Neat1* and plays important roles in gene regulation. In this issue, West et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201601071) use super-resolution structured illumination microscopy to show that paraspeckles are organized in a core-shell spheroidal structure composed of *Neat1* and seven proteins.

Mammalian nuclei are compartmentalized into distinct membrane-less nuclear bodies composed of specific proteins and nucleic acids. Paraspeckles are ribonucleoprotein bodies located in the interchromatin space near nuclear speckles (Fox et al., 2002). The long noncoding RNA (lncRNA) *Neat1* is the main structural RNA component of paraspeckles (Chen and Carmichael, 2009; Clemson et al., 2009; Sasaki et al., 2009; Sunwoo et al., 2009). Paraspeckles form in close proximity to the site of *Neat1* transcription (Clemson et al., 2009; Mao et al., 2011) and the *Neat1* lncRNA serves as the platform to recruit proteins for paraspeckle assembly (Mao et al., 2011). Both the *Neat1* RNA itself and the transcription of *Neat1* gene are essential for paraspeckle formation and maintenance (Mao et al., 2011). The first proteins to be identified as part of paraspeckles were the Drosophila behavior and human splicing (DBHS) family members PSPC1, NONO, and SFPQ (Fox et al., 2002). More than 40 proteins are now defined as paraspeckle proteins because of their colocalization with known paraspeckle proteins and *Neat1* (Naganuma et al., 2012; West et al., 2014). Paraspeckles regulate gene expression by sequestrating proteins (such as SFPQ, which prevents its binding to the promoters of specific genes [Hirose et al., 2014; Imamura et al., 2014]) or mRNAs with inverted repeats in their 3′ UTRs. Most of these repeats are *Alu* elements in human, and their retention is mediated by the paraspeckle protein NONO (Prasanth et al., 2005; Chen et al., 2008; Chen and Carmichael, 2009; Mao et al., 2011). mRNAs sequestered in paraspeckles can be released in response to cellular stresses (Prasanth et al., 2005; Elbarbary et al., 2013; Hu et al., 2015) and during circadian rhythm regulation (Torres et al., 2016), leading to altered gene expression. Although these emerging studies have greatly advanced our understanding of paraspeckle functions, how paraspeckle components—both the lncRNA *Neat1* and proteins—get organized into membrane-less compartments that ultimately make functional paraspeckles has remained unclear. In the current issue, West et al. describe the fine core-shell spheroidal architecture of paraspeckles using super-resolution structured illumination microscopy (SIM) and identify FUS as a crucial regulator of paraspeckle assembly (West et al., 2016; Fig. 1).

A previous study of paraspeckles by electron microscopy showed that both *Neat1* isoforms (the less abundant long isoform *Neat1_2*, which is essential for paraspeckle construction, and the abundant, shorter isoform *Neat1_1*, which seems dispensable for paraspeckle assembly) are found at the periphery of paraspeckles, with the central sequence of the *Neat1_2* (*Neat1_mid*) isoform located in the core of paraspeckles (Souquere et al., 2010; Fig. 1). West et al. (2016) designed three probes that individually recognize the 5′ end, the middle, or the 3′ end of *Neat1_2* to visualize paraspeckles using RNA fluorescence in situ hybridization under SIM. SIM observations of the combination of these probes for *Neat1_2* revealed a core-shell spheroidal arrangement of *Neat1_2* (Fig. 1). Consistent with previous electron microscopy observations (Souquere et al., 2010), the middle region of *Neat1_2* formed a solid core that was surrounded by its 5′ and 3′ ends, supporting the hypothesis that SIM is appropriate to visualize paraspeckles in super-resolution. Notably, the majority of paraspeckles appeared as disperse spheroids, whereas a small proportion dimerized and even polymerized to form sausage-like structures.

To address how proteins are assembled into paraspeckles, West et al. (2016) costained *Neat1_2* and seven previously defined paraspeckle proteins (Naganuma et al., 2012). Depending on the extent of their colocalization with different regions of *Neat1_2*, these proteins could be classified into three categories: those that localized to the core, the patch, or the shell of paraspeckles (Fig. 1). SFPQ, NONO, PSPC1, and FUS are the core components of paraspeckles, which exclusively colocalized with the middle region of *Neat1_2* in the center of paraspeckles. The patch proteins RBM14 and BRG1 were mainly found in the core and shell parts of paraspeckles, whereas the shell protein TDP43 was predominantly enriched in the periphery of paraspeckles. Together, the description of *Neat1* and paraspeckle protein arrangement suggests a fine core-shell spheroidal architecture for paraspeckles.

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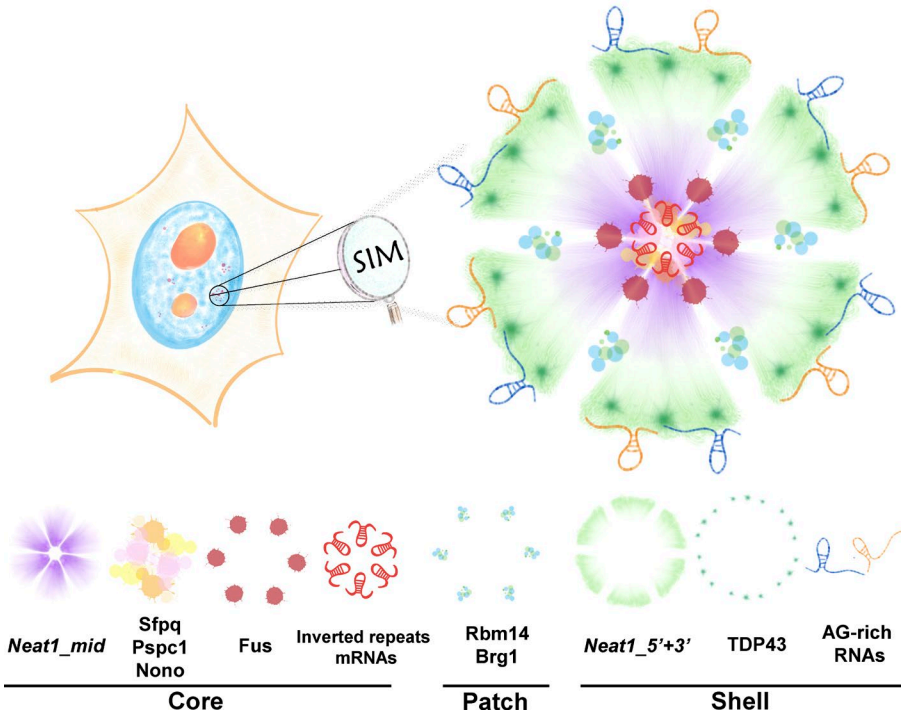

Figure 1. **A schematic drawing shows the transverse section of the core-shell spheroid paraspeckle structure.** Under SIM, the paraspeckle structure can be divided into two parts, the core and the shell. The core of paraspeckles contains the middle region of *Neat1_2* (shown as *Neat1_mid*) and proteins SFPQ, PSPC1, NONO, and FUS. The 5′ and 3′ ends of *Neat1_2* (shown as *Neat1_5′+3′*) and the protein TDP43 are located in the shell of paraspeckles. Proteins RBM14 and BRG1, defined by West et al. (2016) as patch proteins, are located in both the core and the shell of paraspeckles. FUS proteins assemble the paraspeckle-like core units and patch/shell proteins into paraspeckle spheroids. The AG-rich RNAs are enriched in the surface of paraspeckles, whereas the previously reported inverted repeats containing mRNAs interact with the core proteins and are proposed to localize in the middle of paraspeckles.

West et al. (2016) next addressed how these proteins and the *Neat1* RNA get organized inside paraspeckles. Visualizing paraspeckles in FUS knockout (KO) cells extended the study from the descriptive observation of paraspeckles under SIM to an analysis of the mechanisms governing their hierarchical organization. FUS is essential for paraspeckle formation but has little effect on *Neat1* expression (Naganuma et al., 2012). Although by conventional fluorescence microscopy West et al. (2016) still observed paraspeckle-like foci in FUS KO cells, these paraspeckle-like structures appeared remarkably impaired when analyzed by SIM: FUS depletion disrupted the core-shell structure of paraspeckles. The 5′ and 3′ ends of *Neat1_2* no longer surrounded its middle region; instead, the *Neat1_2* 5′ and 3′ ends were aligned in a head-to-end manner, indicating that the *Neat1_2* folds in half with the 5′ and the 3′ regions bundled independently. Measuring the distances between each region of *Neat1_2* and comparing to the diameter of the normal core-shell spheroid, West et al. (2016) proposed as a model that each *Neat1_2* is folded into a V-shaped unit and that many of these units are assembled into the core-shell spheroid by FUS (Fig. 1). This model is supported by the observation that the core proteins SFPQ, NONO, and PSPC1 were still tightly associated with *Neat1_2* and the shell protein TDP43 partially colocalized with *Neat1_2* in FUS KO cells, whereas the patch proteins RBM14 and BRG1 were dissociated with *Neat1_2* when FUS was depleted. These analyses shed some light onto the mystery of paraspeckle assembly—the authors propose that the *Neat1_2* isoform is folded and binds to paraspeckle core proteins to first form paraspeckle-like units, which are bridged together by FUS proteins to form the ordered paraspeckle sphere.

As paraspeckle function requires both its protein and RNA components, West et al. (2016) next investigated which RNAs are located in paraspeckles. They performed capture hybridization analysis of RNA targets (CHART; a technique that captures lncRNA-associated DNAs or RNAs genome-wide) purification of paraspeckle components using antisense oligonucleotides that target the 5′ end of *Neat1_2*. Two types of RNA transcripts were enriched by CHART: spliced mRNAs and spliced introns that both have AG-rich motifs. RNA fluorescence in situ hybridization confirmed that these newly identified RNAs at least partially colocalized with *Neat1_2*. Remarkably, AG-rich RNAs accumulated on the surface of paraspeckles, corresponding to the *Neat1_2* target sequences of the antisense oligonucleotides used in CHART. However, how paraspekles sequester these AG-rich RNAs is unknown.

Overall, SIM analyses allowed West et al. (2016) to delineate the highly ordered core-shell spheroidal architecture of paraspeckles. This work greatly expands our knowledge of the organization of the *Neat*1 lncRNA and the integration of paraspeckle proteins and RNAs into functional paraspeckles. Such a hierarchical assembly raises many questions as to the structural and functional significance of paraspeckles. It is interesting to note that the CHART analysis West et al. (2016) performed in mouse cells did not capture a class of previously reported mRNAs that contain inverted repeats in their 3′ UTRs. In both mouse and human cells, retention of these mRNAs was shown to be mediated by NONO (Prasanth et al., 2005; Elbarbary et al., 2013; Hu et al., 2015), which localizes to the core of paraspeckles (West et al., 2016). This observation indicates that such inverted repeat-containing mRNAs may be sequestered in the middle rather than in the shell of paraspeckles (Fig. 1), hence preventing their precipitation by CHART probes that recognize the periphery of paraspeckles (West et al., 2016). Thus, the unique localization patterns of AG-rich RNAs and inverted repeat-containing mRNAs imply that different classes of RNAs may have distinct geographic distributions within paraspeckles, just like paraspeckle proteins do, as reported by West et al. (2016). In this scenario, it will be of great interest to identify additional RNAs that are sequestered in paraspeckles and to dissect their exact localization.

Furthermore, nuclear retention of inverted repeat-containing mRNAs in paraspeckles is known to change in response to cellular stimulations (Prasanth et al., 2005; Elbarbary et al., 2013; Hu et al., 2015; Torres et al., 2016). Identifying conditions

that alter the retention of AG-rich RNAs may uncover new paraspeckle functions that are associated with these RNAs. Moreover, proteins are highly organized inside paraspeckles (Fig. 1). Hence, elucidating the exact sublocalization of additional proteins, together with retained RNAs, within paraspeckles will provide additional insights into paraspeckle structure and assembly. The formation and function of paraspeckles appear to be associated with *Neat1* transcription (Mao et al., 2011), which varies with cellular stimulations (Imamura et al., 2014; Hu et al., 2015; Adriaens et al., 2016). It will be important to test how *Neat1* transcription affects the structural assembly of paraspeckles mediated by FUS. Finally, West et al. (2016) also reported different states of paraspeckles in cells, i.e., paraspeckle-like units, paraspeckle spheres, and polymerized, large sausage-like paraspeckles. These different-looking paraspeckles may be associated with altered *Neat1* transcription activity and varied protein/RNA components. Future studies are needed to decipher the molecular and biological differences among these states and to determine what dynamically regulates the conformation of paraspeckles, including after cellular stimulations. The combination of live cell imaging and super-resolution microscopy in single cells may be able to address these questions.

Super-resolution microscopy made it possible to visualize paraspeckles with more detail than ever before, providing substantial new insights into their organization and assembly. Technical limits had previously prevented the level of analysis required to define the structure of highly organized nuclear bodies. Newly developed microscopy techniques, including SIM, have extended the application range of fluorescence microscopy beyond the diffraction limit, achieving near 100-nm resolution along the xy axis (Gustafsson, 2000). SIM is user-friendly and has the advantage over other super-resolution microscopy technologies of providing multicolor imaging, as SIM allows the use of the same chemical dyes as wide-field and confocal microscopy (Wegel et al., 2016). However, the achievable resolution of SIM is constrained to ∼100 nm, and the detailed structure below this resolution cannot be distinguished. Improved imaging techniques will be needed to further delineate the detailed structure of paraspeckles and other cellular subcompartments enriched in RNAs and proteins in the future.

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