**Shell protein composition specified by the lncRNA NEAT1 domains dictates the formation of paraspeckles as distinct membraneless organelles**

Takakuwa, H., Yamazaki, T., Souquere, S. et al. Nat Cell Biol 25, 1664–1675 (2023).

**Presenter:** Pin-Ru Wang **Date**: 2024.3.14 16:10-17:00

**Commentator:** Chi-Wu Chiang, Ph.D. **Location:** Room 601, Med college Building

**Background:**

 Membraneless organelles (MLOs) play a pivotal role in cellular organization, forming through phase separation and contributing significantly to the intracellular compartmentalization of specific biological functions. These dynamic entities comprise specific proteins, with many featuring intrinsically disordered regions (IDRs), and nucleic acids, predominantly RNA. Eukaryotes boast dozens of identified MLOs, such as the nucleolus, nuclear speckles (NSs), and paraspeckles (PSs). However, in contrast to the typical MLOs characterized by a lack of ordered internal structure, paraspeckles stand out as a distinctive example. Paraspeckles with core–shell architecture scaffolded by NEAT1\_2 long noncoding RNAs and harbor several core RNA-binding proteins, including SFPQ, HNRNPF, and BRG1, which collectively orchestrate their structural integrity. Despite these insights, the mechanisms governing NEAT1\_2 domain-specified shell protein composition and the unique formation of paraspeckles as independent MLOs remain unclear.

**Objective:**

To investigate how the specific composition of shell proteins, as dictated by the lncRNA NEAT1 domains, influences the formation and distinctiveness of paraspeckles.

**Results:**

 The authors generated a NEAT1\_2 mutant (mini-NEAT1) to create paraspeckles (mini-PS) with distinct nucleocapsid organization. Through RNA-FISH and immunofluorescence analysis, we observed the integration of mini-PS into NSs. The authors found that the recruitment of key paraspeckle constituent proteins, including HNRNPH1, HNRNPF, BRG1, and SFPQ to mini-PS was significantly reduced. The authors employed an artificial tethering approach by fusing constituent proteins with MCP onto mini-NEAT1 at the 5' shell region. The results demonstrated that shell tethering by SFPQ, HNRNPF and BRG1 facilitates the separation of mini-PSs from NSs. Subsequently, MS2-tethering experiments with MCP-fused SFPQ WT and mutants revealed SFPQ ΔRRM2/NOPS inability to facilitate segregation and assembly of mini-PSs. The SFPQ were determined to be essential for both the segregation of PS from NSs and the assembly of PSs. Then, the RNA subdomain (8–9.8 kb region) of mini-NEAT1 actively promotes the internalization of mini-PS into NSs. Using RNA Pull-down and mass spectrometry analysis, the authors identified more than 100 proteins that interact with this domain, including the U2 snRNP-related proteins. MS2-tethering experiments showed that U2 snRNP components induces the internalization of mini-PSs into NSs. Finally, the authors demonstrated that treating cells with U2 snRNP assembly inhibitors, spliceostatin A (SSA) and pladienolide B (PlaB), resulted in the separation of PSs from NSs. This observation indicates the involvement of the U2 snRNP complex in the internalization of PSs into NSs.

**Conclusion:**

 This study demonstrates that the composition of the paraspeckle shell plays a crucial role in determining the independence of MLOs within the nucleus, providing insights into the significance of the shell in defining the distinctive features of MLOs.