News Release

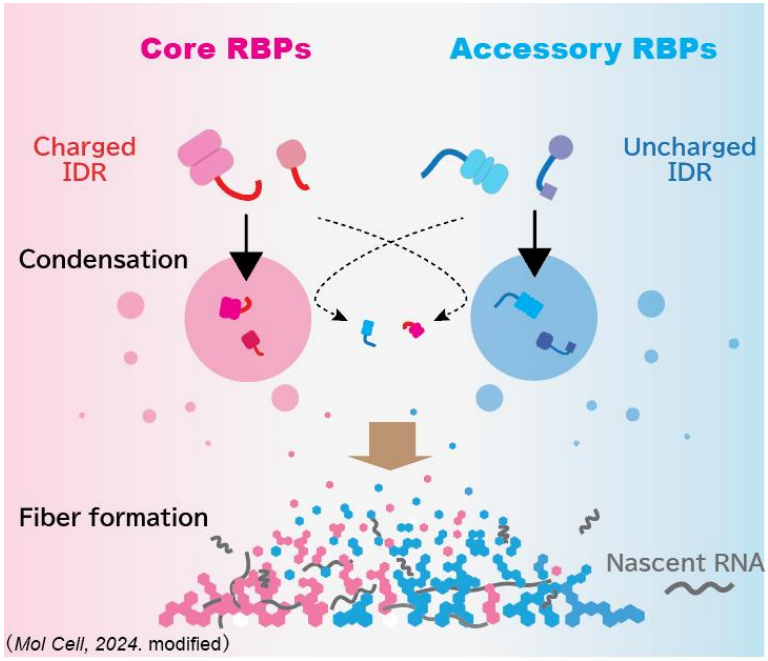
Identification of a novel nuclear structure composed of RNA and RNA-binding proteins to conduct RNA splicing

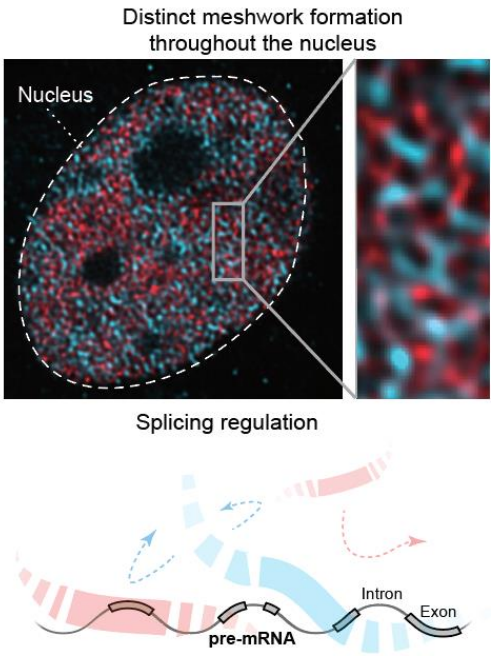
Key Points

•RNA binding proteins (RBPs) form meshworks in the nucleus to drive RNA splicing

•Charged/uncharged intrinsically disordered regions (IDRs) in the RBPs promote mutual exclusion of their meshworks on RNA

•Impaired formation of RBP meshworks leads to aberrant splicing associated with neurodegeneration





Summary

In eukaryotes, RNA processing, including RNA splicing, occurs primarily in the nucleus to produce mature mRNA, which is essential for protein synthesis. Splicing is a complex process catalyzed by a multi-megadalton machinery, the spliceosome. It comprises five small nuclear ribonucleoproteins (U-snRNPs) and hundreds of proteins, including core spliceosomal RNA-binding proteins (RBPs) and accessory RBPs. However, specific nuclear structures associated with the spatiotemporal regulation of splicing remain elusive.

Here, we found that core RBPs and accessory RBPs form two distinct meshworks that are adjacently but distinctly distributed throughout the nucleus. Core RBPs and accessory RBPs harbor charged and uncharged intrinsically disordered regions (IDRs), respectively, which mutually exclude

each other during their condensation. In the nucleus, the binding of these RBPs to nascent RNA facilitates the formation of fibrous structures. Cross-linking and immunoprecipitation (CLIP)-seq and RNA-seq analyses revealed that the fibers of core RBPs and accessory RPBs compete for space occupancy on nascent RNA to drive splicing. Furthermore, the optogenetic enhancement of the RBP meshwork resulted in aberrant splicing of genes associated with neurodegenerative diseases. We identified the spatial organization of RBP networks to regulate RNA splicing.

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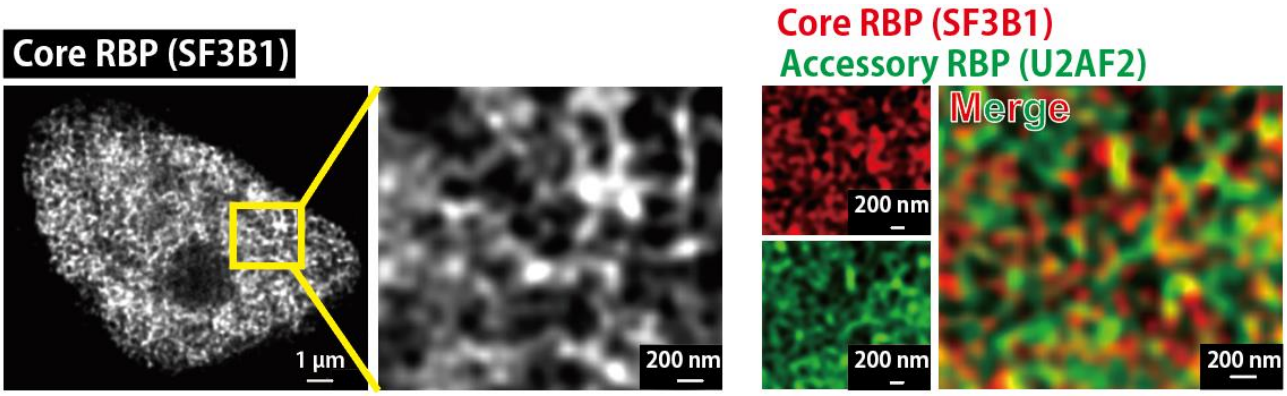
Research Background

The eukaryotic nucleus is an intricated structure where DNA, RNA, and proteins are organized in a spatiotemporal manner. Following transcription of pre-mRNA, RNA processing, such as RNA splicing, 5’ capping. 3’ end processing, and RNA modification, occur in the nucleus to produce mature mRNA. Then, the mRNA is transported to the cytoplasm for translation in the ribosome. Importantly, RNA processing increases proteome diversity by generating multiple mRNA isoforms from a gene sequence encoded in DNA.

Splicing is a complex process facilitated by the spliceosome, a large machinery composed of small nuclear ribonucleoproteins and hundreds of proteins, including core RNA-binding proteins (core RBPs). Accessory RBPs modulate spliceosome activity by binding to specific sites in pre-mRNA. Although splicing is conducted in a spatiotemporal manner, specific nuclear structures associated with the spatiotemporal regulation of splicing remain elusive.

Research Results

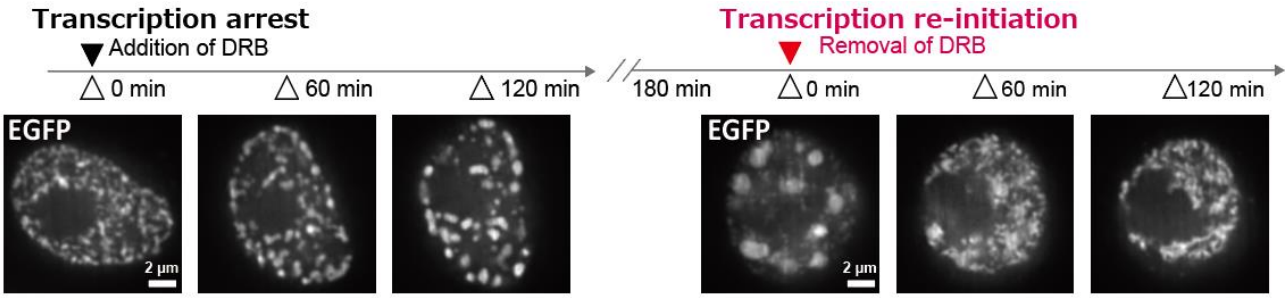
We analyzed the subcellular distribution of RBPs using super-resolution microscopy strategies, of which resolutions are beyond the optical diffraction limit. We observed that at least 13 RBPs were distributed in meshworks throughout the nuclei of cells in various human and mouse tissues (Fig. 1). Although core RBPs and accessory RBPs form distinct meshworks, they were adjacent and intertwined.



【Fig. 1】 Formation of RBP meshworks in the nuclei of human and mouse cells

(Left grayscale images) Formation of meshworks by the core RBP (SF3B1) in the nucleus of a human brain neuron.
(Right color images) Formation of meshworks by the core RBP (SF3B1, red) and the accessory RBP (U2AF2) in the nucleus of a N2A cell.

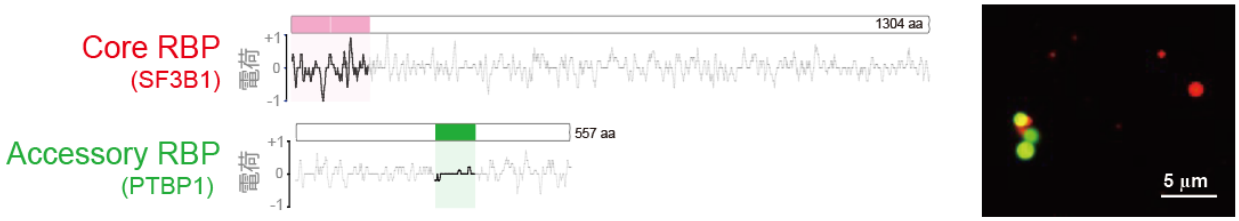
Live imaging analysis of cells expressing EGFP-tagged MATR3 (accessory RBP) showed that transcription arrest by DRB treatment dissipated the MATR3 meshwork and formed large droplets (Fig. 2). Upon the removal of DRB, the large droplets reverted to small patches to form meshworks, suggesting the requirement of nascent RNA for the formation of the RBP meshwork.



【Fig. 2】 Transcription-dependent formation of the RBP meshwork

Live imaging analysis of N2A cells expressing EGFP-tagged MATR3 (accessory RBP). (*Mol Cell, 2024*, modified)

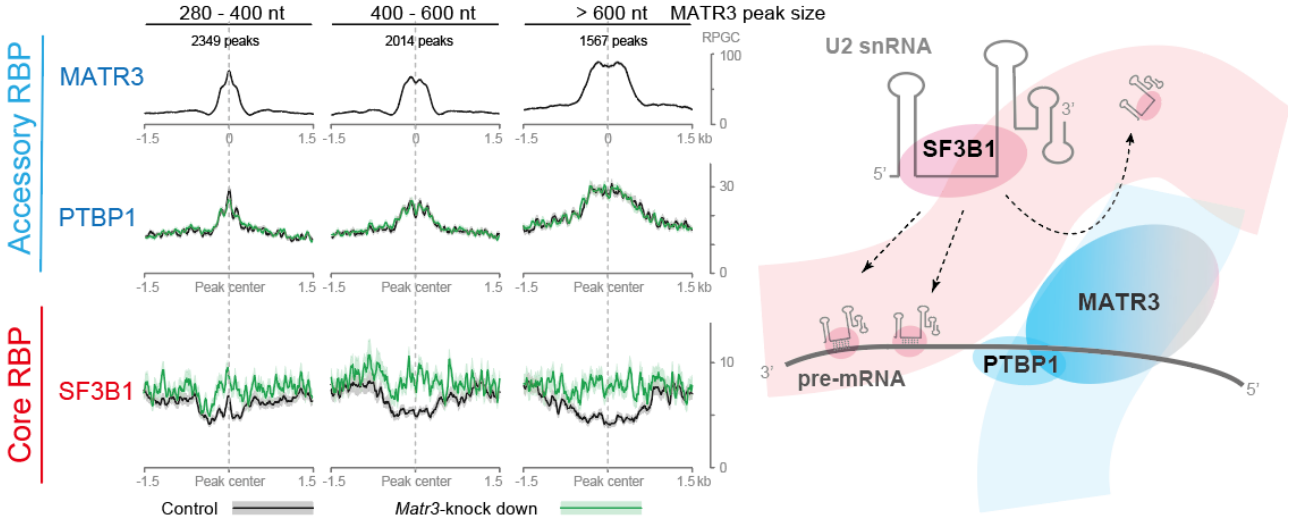
In silico analysis of the amino acid sequences of RBPs revealed that core RBPs and accessory RBPs harbor distinct classes of charged uncharged and charged intrinsically disordered regions (IDRs), respectively (Fig. 3, left). *In vitro* analysis with the recombinant proteins and *in cellulo* analysis with overexpression of the IDRs showed that the charged and uncharged IDRs in RBPs form separating condensates (Fig. 3, right).



【Fig. 3】 Distinct droplet formation by the recombinant proteins of the charged IDR and uncharged IDR in the core and accessory RBPs, respectively

(Left) Amino acid charge distributions of the core RBP (SF3B1) and accessory RBP (PTBP1). IDRs are color indicated.
(Right) *In vitro* droplet formation of the recombinant proteins containing the IDRs in the core RBP (SF3B1, red) and accessory RBP (PTBP1, green). (*Mol Cell*, 2024, modified)

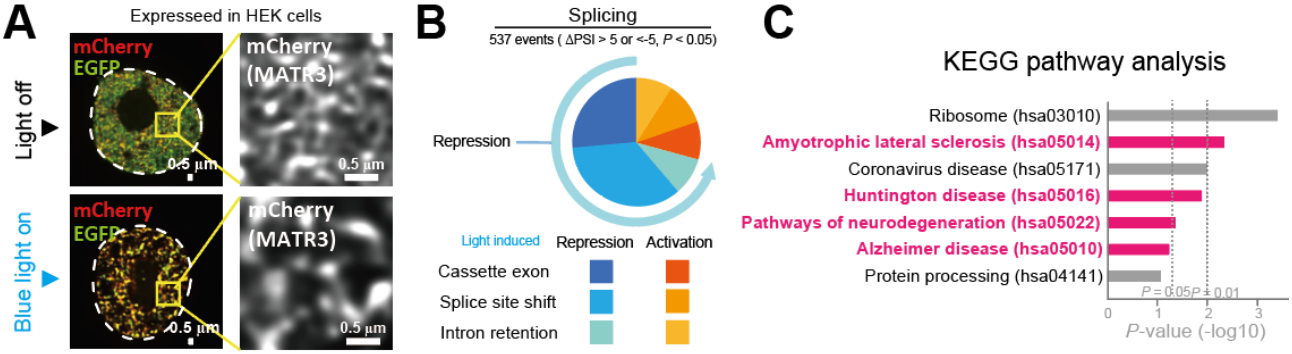
We next performed cross-linking and immunoprecipitation (CLIP)-seq to understand the role of RBP meshworks in RNA binding. Our analysis demonstrated that the local accumulation of the accessory RBP with an uncharged IDR (MATR3) prevents the binding of the core RBP with a charged IDR (SF3B1), but not of the accessory RBP with an uncharged IDR (PTBP1), to pre-mRNA (Fig. 4). These results suggest that the meshworks of core RBPs and accessory RBPs compete for spatial occupancy on pre-mRNA.



【Fig. 4】 Core RBPs and accessory RBPs compete for spatial occupancy on RNA

(Left) Accumulations of MATR3-RNA interactions (top), PTBP1-RNA interactions (middle), and SF3B1-RNA interactions (bottom) around the regions enriched with MATR3-RNA interactions. MATR3-CLIP was performed using naïve N2A cells. PTBP1-CLIP and SF3B1-CLIP were performed using *Matr3*-silenced (green) or control (black) N2A cells.
(Right) Schematic showing the repelling of SF3B1 but not PTBP1 by a MATR3 cluster on pre-mRNA.
(*Mol Cell*, 2024, modified)

Finally, we investigated the role of meshwork formation in alternative splicing regulation using an optogenetic system. We confirmed that blue light irradiation induced the condensation of overexpressed MATR3, coarsening its meshwork (Fig. 5A). RNA-seq analysis showed that the artificial coarsening of the MATR3 meshwork represses splicing of genes associated with neurodegenerative diseases (Fig. 3 BC). In addition, we observed that the introduction of an ALS-causative mutation of MATR3 into the genomic DNA of N2A cells by CISPR/Cas9 system caused the fragmentation of the MATR3 meshwork in these cells.



【Fig. 5】 Artificial enhancement of the MATR3 meshwork leads to aberrant splicing associated with neurodegeneration

(A) Light-induced enhancement of the MATR3 meshwork. A white broken contour outlines the nucleus.
(B) A pie chart showing the numbers of affected splicing events by the enhancement of the MATR3 meshwork
(C) KEGG pathway analysis of the genes with the affected splicing events by MATR3-condensation.
The pathways associated with neurodegeneration are indicated in red. (*Mol Cell*, 2024, modified)

Research Summary and Future Perspective

We demonstrate that RBPs form meshworks that spread throughout the nucleus to drive RNA splicing. Core RBPs and accessory RBPs constitute two distinct meshworks that are adjacently but distinctly distributed, and mutually exclusive on pre-mRNA. The separation of the charged and uncharged IDRs in these RBPs achieves mutual exclusion. Our analysis revealed that the spatial organization of RBP networks is a functional regulator of RNA splicing.

We observed that the impaired formation of RBP meshworks causes aberrant splicing associated with neurodegeneration. Furthermore, we showed that the ALS causative mutation in the IDR affects the meshwork formation. We hope that our work will contribute to the understanding of the pathogenesis of neurodegenerative diseases.

Publication

Title: Blending and separating dynamics of RNA-binding proteins develop architectural splicing networks spreading throughout the nucleus

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