【Grant-in-Aid for Scientific Research (S)】

Broad Section H

Title of Project : Synthetic investigation of pathological phase separation of RNA-binding proteins

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Keyword : RNA binding protein, liquid-liquid phase separation, RNA processing

【Purpose and Background of the Research】

Liquid-liquid phase separation (LLPS) has been known for long time. However, recent discovery of phase separation of RNA binding proteins, and its effect on molecular complex structure underscored importance of LLPS in RNA biology. A hypothesis that membrane-less organelle plays role in functional regulation of cellular molecules attracted scientists, which further raised a question whether LLPS is also involved in pathology. In fact, recent findings suggest that LLPS is related to pathological conditions in such diseases as ALS and prion disease. In particular, LLPS will be important in cellular nuclear function, in which a condensed structure of nucleic acids, nucleoli, nuclear speckles, paraspeckles, Cajal body, PML body, and other membrane-less compartments co-exist. Evidences indicate that LLPS will be involved in genetic diseases, tumor metastasis, and viral infections, and our purpose is to investigate how those pathological conditions are understood from disordered LLPS.

【Research Methods】

(1) Understanding of RNA splicing regulation with LLPS of SRSF proteins.

Serine/arginine-rich splicing factor (SRSF) is a family of RNA binding proteins, mainly associated with alternative RNA splicing. SRSF family proteins consist of N-terminal RNA binding motif and C-terminal RS domain, in which serine and arginine residues are repeated. RS domain matches character of LLPS-related domain, which has low structural complexity. In fact, SRSF proteins form membrane-less organelle in cell nuclei, as nuclear speckles. In this section, we construct experimental tool for SRSF proteins function in LLPS, and investigate how phase separation of SRSF proteins influence cellular RNA splicing and gene expression.

(2) Regulation of transcriptional process by LLPS of RNAP complex

Transcription is mediated by an intricate regulation of RNA polymerase II (RNAPII) in complex with various proteins. C-terminal domain (CTD) of RNAPII is understood as a mediator of LLPS, but its functional importance, especially in complex with other proteins remains elusive. In this section, we investigate LLPS of RNAPII in detail toward understanding of physiological importance.

(3) LLPS in pathological gene expression conditions.

Although recent evidences suggest LLPS is associated with multiple diseases and viral infections, detailed insights of role of LLPS in those pathological conditions remain to be understood. In this section, we investigate how LLPS is connected to pathological gene expression, using disease models, such as triplet diseases, cancer, and viral infections. By these efforts, we would like to provide a concept of pathological LLPS

【Expected Research Achievements and Scientific Significance】

In this study, we investigate how LLPS is regulated in RNA processing, especially RNA splicing and transcription through RNA binding proteins. We expect to provide a novel concept toward understanding of diseases. Such new aspects yeaned through this study may also provide a clue for future drug development.

【Publications Relevant to the Project】

Therapeutic manipulation of IKBKAP mis-splicing with a small molecule to cure familial dysautonomia. Ajiro M, Awaya T, Kim YJ, Iida K, Denawa M, Tanaka N, Kurosawa R, Matsushima S, Shibata S, Sakamoto T, Studer R, Krainer AR, Hagiwara M. *Nat Commun*. **2021** Jul 23;12(1):4507.

Nuclear Import Receptor Inhibits Phase Separation of FUS through Binding to Multiple Sites. Yoshizawa T, Ali R, Jiou J, Fung HYJ, Burke KA, Kim SJ, Lin Y, Peeples WB, Saltzberg D, Soniat M, Baumhardt JM, Oldenbourg R, Sali A, Fawzi NL, Rosen MK, Chook YM. *Cell* **2018** Apr 19;173(3):693-705.e22.

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