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COMMENT

USP42 deubiquitinase in the arena of liquid–liquid phase separation and nuclear speckles

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First reported by Santiago Ramón y Cajal more than a century ago, nuclear speckles are mysterious interchromatin organelles that are known for their roles in regulating gene expression, mRNA splicing, and maturation, although the mechanisms that govern their function remained largely elusive. A very recent study by Liu et al. now shed light on the role of the deubiquitinase USP42 in nuclear speckle assembly and function through mechanisms of liquid–liquid phase separation (LLPS) [1].

Intracellular assemblies of proteins and other macromolecules forming foci, organelles, bodies, granules, or punctates have been known for decades and are associated with several cellular processes. In the last few years, these structures were revisited through investigation of the process termed LLPS, which emerged as a potential unifying regulatory mechanism of several cellular membrane-less organelles (hereafter, MLO) [2, 3]. LLPS corresponds to liquid phase demixing, i.e., liquid compartmentalization, that promotes the organization of molecules into droplets, condensates, or bodies in response to physicochemical and biochemical changes of the environment that surrounds these molecules [2, 3]. It has been recently evidenced that LLPS orchestrates a wide spectrum of cellular processes including membrane receptor-associated signaling, transcription, RNA maturation, and DNA repair [4–8]. Indeed, many proteins have been discovered as components of MLO, providing means for genetic and biochemical interventions to better understand the mechanisms involved in the formation and function of these organelles.

Multivalent bimolecular interactions involving folded protein domains (FPDs) and protein intrinsically disordered regions (IDRs), which are characterized by the absence of well-defined three-dimensional structures, have been recognized as key determinants of LLPS and MLO formation [3, 9]. Indeed, many examples of LLPS were shown to be governed by a wide range of weak intra- and inter-molecular interactions that involve IDRs [3, 7, 9]. However, interactions through FPDs or IDRs alone can hardly explain the highly dynamic nature of LLPS. Interestingly, bourgeoning evidence indicates that MLO might be dynamically regulated by posttranslational modifications (PTMs) including phosphorylation and ubiquitination [10–13]. Nonetheless, many unanswered questions remain regarding how signal transduction and PTMs might regulate MLO dynamics and what is the biological relevance of these signaling events. Remarkably, Liu et al. reported that the deubiquitinase (DUB) USP42 undergoes LLPS resulting in the formation of nuclear bodies that correspond to the previously established structures widely known as nuclear speckles [1]. Nuclear speckles are MLO that contain pre-mRNA splicing factors, transcription factors and RNAs, and are known to regulate mRNA splicing [14]. The authors provide evidence that USP42 regulates nuclear speckles morphology, mRNA splicing and cell growth and, finally, highlight a potential link between USP42, RNA splicing and cancer [1] (Fig. 1).

Ubiquitination is a highly dynamic PTM ensured by E3 ubiquitin ligases and removed by DUBs. It is involved in the initiation, execution, and termination of many cellular processes [15]. Therefore, this PTM intuitively offers a potential paradigm for defining how cell-signaling events might use LLPS mechanisms to govern biological processes. Indeed, recent findings established that ubiquitin signaling is associated with LLPS [11–13], raising the possibility that DUBs might be also involved in orchestrating MLO dynamics.

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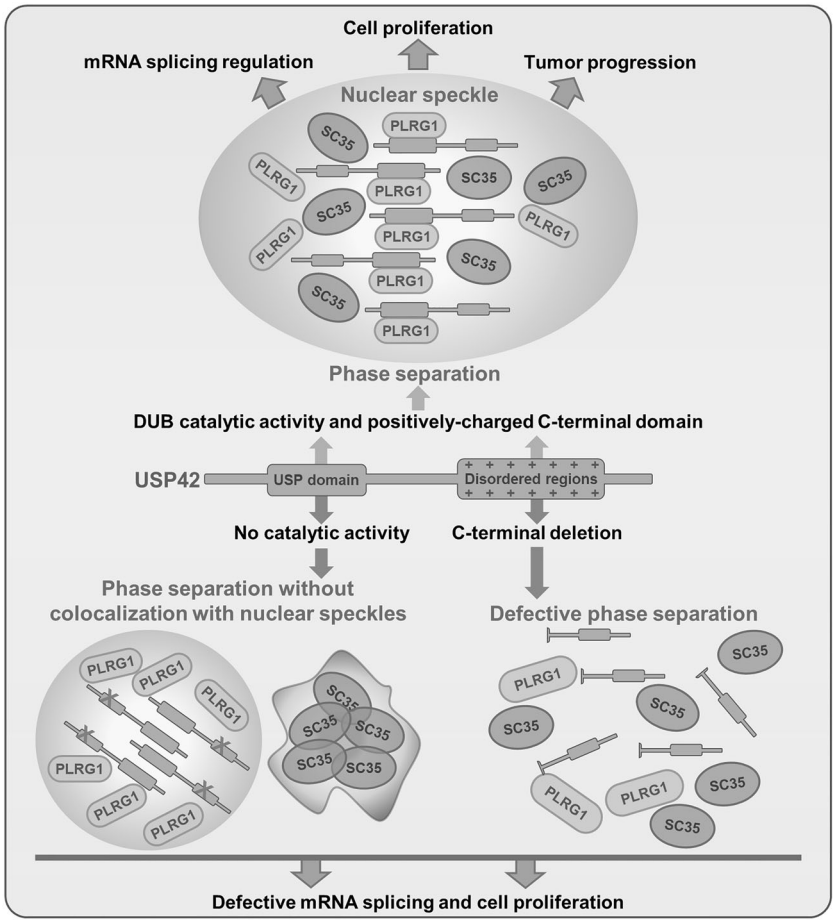

Fig. 1 Deubiquitinase USP42 undergoes liquid–liquid phase separation and controls nuclear speckles. USP42 drives nuclear liquid–liquid phase separation (LLPS) resulting in nuclear speckles containing the splicing protein PLRG1. The C terminus of USP42 is positively charged and is required for phase separation with PLRG1 and for efficient co-localization with the component of the nuclear speckles, SC35. USP42 catalytic domain is required for the proper co-localization of SC35 with PLRG1 and USP42. mRNA splicing regulation, cell proliferation and tumor progression are dependent on USP42 and its ability to undergo LLPS.

Liu et al., exploited this notion to determine whether certain DUBs could undergo LLPS, taking into account that a large family of these enzymes regulates diverse ubiquitin-signaling events. By conducting a DUB screening using the majority of mammalian DUBs, expressed as GFP-fusion proteins, they found that some DUBs constitutively form intracellular foci or condensates [1]. They subsequently used 1.6-hexanediol, a hydrophobic chemical generally used to inhibit LLPS, and found that USP42 is among a limited set of DUB candidates capable of undergoing LLPS.

The authors first confirmed that endogenous USP42 forms nuclear foci in different cell lines [1]. These structures are spherical, undergo fusion events, and FRAP experiments indicated their dynamic nature [1]. USP42 contains IDRs located in the C-terminal portion of the protein, outside the catalytic domain that are necessary and sufficient for foci formation. In further exploring the molecular determinants of the C-terminal region, several mutants targeting lysine- or arginine-rich motifs, were used and these indicated that positively charged clusters mediate USP42 foci assembly and LLPS in vitro [1]. Altogether, these result show that USP42 undergo LLPS under normal cell growth conditions.

In investigating the biological significance of USP42 LLPS, the authors serendipitously revealed that USP42 MLO coincide with nuclear speckles, as this DUB colocalizes with SC35 (also known as SRSF2), a known component of nuclear speckles involved in RNA splicing. Pre-treatment of cells with an excess of RNAse A did not affect USP42 MLO, arguing against a role of RNA as a driver of USP42 LLPS. Interestingly, USP42 catalytic dead mutant shows distinct DUB foci adjacent to, but not components of, SC35 foci, suggesting that catalytic activity is necessary for proper localization or maintenance of USP42 into SC35 nuclear speckles [1]. It was also found that the nuclear speckle protein PLRG1, which is another component of the spliceosome, interacts with the C-terminal part of USP42 and this interaction is responsible for LLPS of PLRG1 in the nucleus. Moreover, PLRG1 undergoes LLPS in vitro only in the presence of USP42 C-terminal domain, suggesting that USP42 might be a key factor for the recruitment of specific spliceosome factors and the proper assembly of nuclear speckles [1]. Inactivation of USP42 gene resulted in reduced numbers of nuclear speckles and altered morphology of the remaining speckles. Functionally, the absence of USP42 reduces the

cell colony-forming ability, as does the suppression of PLRG1, showing that these two proteins are important for cell growth and proliferation. At the molecular level, the authors found by RNA sequencing that the rate of splicing is altered in cells without USP42 and that USP42 and PLRG1 regulate mRNA splicing events [1].

USP42 MLO might also be associated with cancer pathogenesis. Consistent with protein interaction and localization studies, the authors show a correlation between USP42 and PLRG1 expression and cancer progression. Interestingly, USP42 appears to regulate the alternative splicing of cancer-associated genes. The authors present evidence that gene splicing is modified in tumors with high levels of USP42. In addition, overexpression of USP42 in lung tumors of patients is correlated with alterations in RNA splicing. Finally, high expression levels of USP42 and PLRG1 appear to be associated with a poor prognosis [1].

In summary, this interesting study underlines the role of LLPS in coordinating the function of the DUB USP42 in RNA maturation with implications for tumor progression, thus establishing a novel space for potential therapeutic treatments [1]. This study also raises many questions on how ubiquitin ligases and DUBs might regulate their substrates during LLPS. For instance, which substrates are potentially dynamically regulated by USP42 in nuclear speckles? Does USP42 regulate PLRG1 ubiquitination? How USP42 LLPS regulates splicing and cell proliferation? As mentioned above, recent studies also revealed important links between ubiquitin signaling and LLPS. For instance, ubiquitination can inhibit LLPS of stress granules in the cytoplasm [11], or promote LLPS of the proteasome in the nucleus [12]. Conversely, LLPS can in turn direct ubiquitin-signaling events, as shown for nucleosomal ubiquitination [13]. While it is still early to establish general principles, these studies, along with novel information provided by Liu et al. [1], strongly argue that ubiquitin signaling can play critical roles in a wide spectrum of cellular processes through LLPS-based mechanisms.

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Compliance with ethical standards

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References

1. Liu S, Wang T, Shi Y, Bai L, Wang S, Guo D, et al. USP42 drives nuclear speckle mRNA splicing via directing dynamic phase separation to promote tumorigenesis. Cell Death Differ. 2021.
2. Bergeron-Sandoval LP, Safaee N, Michnick SW. Mechanisms and consequences of macromolecular phase separation. Cell. 2016; 165:1067–79.
3. Alberti S, Gladfelter A, Mittag T. Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. Cell. 2019;176:419–34.
4. Hnisz D, Shrinivas K, Young RA, Chakraborty AK, Sharp PA. A phase separation model for transcriptional control. Cell. 2017;169: 13–23.
5. Emenecker RJ, Holehouse AS, Strader LC. Emerging roles for phase separation in plants. Dev Cell. 2020;55:69–83.
6. So C, Cheng S, Schuh M. Phase separation during germline development. Trends Cell Biol. 2021;31:254–68.
7. Tsang B, Pritisanac I, Scherer SW, Moses AM, Forman-Kay JD. Phase separation as a missing mechanism for interpretation of disease mutations. Cell. 2020;183:1742–56.
8. Ong JY, Torres JZ. Phase separation in cell division. Mol Cell. 2020;80:9–20.
9. Zhou HX, Nguemaha V, Mazarakos K, Qin S. Why do disordered and structured proteins behave differently in phase separation? Trends Biochem Sci. 2018;43:499–516.
10. Snead WT, Gladfelter AS. The control centers of biomolecular phase separation: how membrane surfaces, PTMs, and active processes regulate condensation. Mol Cell. 2019;76:295–305.
11. Dao TP, Kolaitis RM, Kim HJ, O'Donovan K, Martyniak B, Colicino E, et al. Ubiquitin modulates liquid-liquid phase separation of UBQLN2 via disruption of multivalent interactions. Mol Cell. 2018;69:965–78.e966.
12. Yasuda S, Tsuchiya H, Kaiho A, Guo Q, Ikeuchi K, Endo A, et al. Stress- and ubiquitylation-dependent phase separation of the proteasome. Nature. 2020;578:296–300.
13. Gallego LD, Schneider M, Mittal C, Romanauska A, Gudino Carrillo RM, Schubert T, et al. Phase separation directs ubiquitination of gene-body nucleosomes. Nature. 2020;579:592–7.
14. Galganski L, Urbanek MO, Krzyzosiak WJ. Nuclear speckles: molecular organization, biological function and role in disease. Nucleic Acids Res. 2017;45:10350–68.
15. Rape M. Ubiquitylation at the crossroads of development and disease. Nat Rev Mol Cell Biol. 2018;19:59–70.