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Getting in phase- DNA damage repair mechanisms and liquid-liquid phase separation of the plant-specific histone methyltransferase MtSUVR2

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Liquid-liquid phase separation (LLPS) has become a widely accepted mechanism for the dynamic compartmentalization of different cellular components into membraneless organelles or other cellular bodies. LLPS occurs when the concentration of a protein, nucleic acid or other molecule reaches a saturation concentration and its partition into high- and low-concentration phases is energetically favorable. This results in the demixing of the molecule and the formation of distinct droplets in the surrounding solution. The most well-studied membraneless compartments postulated to be formed via LLPS include P-bodies¹, the nucleolus², stress granules³ and photobodies⁴. These membraneless organelles exhibit the dynamic properties of liquids and concentrate specific macromolecules while excluding others. LLPS is thus an attractive mechanism proposed for many different foci or puncta observed within the cell, including different chromatin bodies such as the transient repair compartments that form on damaged chromatin. Recent work in yeast and mammalian cells has suggested that LLPS plays an important role during DNA damage response (DDR) by allowing the formation of distinct compartments that concentrate specific DNA-repair machinery.

In yeast, DNA repair enzymes such as RAD52 form liquid-like foci at double strand breaks (DSB), interact with DNA damage-inducible intranuclear microtubule filaments and transport DSB from hetero- to euchromatin, allowing access by repair enzymes and forming transient repair compartments.⁵ In humans, the protein 53BP1 is recruited to DSB and forms distinct chromatin domains via a putative phase separation mechanism. 53BP1 further acts as a scaffold for downstream repair factor assembly, shields the DNA from excessive nucleolytic cleavage.⁶ The formation of distinct foci with liquid-like characteristics critical for DNA repair in fungi and animal systems suggests that similar mechanisms exist in plants.

In plants, the maintenance of genome integrity is of particular importance in stem cells as these pluripotent cells are required for the growth and development of all subterranean and aerial organs. Regions of active gene transcription are located in loosely packed regions of euchromatin, whereas transposable elements and genes that are not under active transcription are located in regions of highly condensed heterochromatin. Structuring, packaging and dynamic reorganization mechanisms of euchromatin and heterochromatin are required to ensure the integrity of the genomic DNA as a function of changing physiological demands, environmental stresses, endogenous signals, and DNA damage events. Chromatin integrity involves the highly orchestrated activity of genetic and epigenetic factors remodeling the chromatin landscape, including chromatin interacting proteins, histone modifying enzymes and DNA repair enzymes.

In the article by Liu, *et al.*⁷, the authors propose an LLPS-driven chromatin remodeling and repair mechanism mediated by the plant-specific histone methyltransferase, MtSUVR2 from *Medicago truncatula*. MtSUVR2 deposits repressive di- and tri-methylation marks on histone 3 (H3K9me2/3), which is enriched in constitutive heterochromatin, acting to prevent transposon jumping and mediating gene silencing. Upon DNA-damage, MtSUVR2 colocalize with heterochromatin, acting to help compact DNA. MtSUVR2 also localizes to repair bodies at DSB upon DNA damage, forming foci at DSB sites and recruiting RAD51 to form DNA-repair bodies.

In many systems, intrinsically disordered regions (IDRs) have been shown to be drivers of LLPS via homotypic interactions that are satisfied intramolecularly in the dilute phase and intermolecularly in the protein dense liquid separated phase.^{8,9} MtSUVR2 possess two IDRs and the authors demonstrate the importance of these regions for nuclear localization, foci formation and RAD51 recruitment. IDR1, in conjunction with a low complexity domain (LCD), drives nuclear localization and foci formation. IDR2 is required for recruitment of RAD51. *In vitro* experiments demonstrate that MtSUVR2 is able to bind MtRAD51 in the dilute phase and recruit it to liquid droplets, however MtRAD51 is not able to enter MtSUVR2 droplets alone. This suggests an elegant mechanism to recruit specific factors via shuttling by MtSUVR2-bound cargoes into the droplet phase while excluding unbound proteins or proteins unable to interact with MtSUVR2.

This study furthers our understanding of DDR and suggests a novel mechanism for both the condensation of heterochromatin and efficient exposure of DSB for repair by a plant specific histone modifying enzyme of the SUVVR family. The work by Liu, *et al.* provides an attractive and exciting working model for the activity of MtSUVR2 via LLPS. This work opens important questions for future research. The formation of foci on both heterochromatin and euchromatin by MtSUVR2 and the different localization of RAD51 to only DSB-euchromatin foci is still to be addressed. Does MtSUVR2 determine the composition of the different foci in different regions of chromatin *in vivo*? Are other macromolecules required for foci formation *in vivo* and what role do they play? In addition, an important fundamental question that will require further study is whether LLPS is the driving force for *in vivo* DDR foci such as those observed for MtSUVR2. While LLPS has been demonstrated *in vitro* for many different macromolecules, *in vivo* LLPS is still highly controversial, with other mechanisms such as weak or transient protein-protein interactions, transient non-specific protein-DNA interactions and scaffolding by hub proteins or nucleic acids offering alternative explanations for the formation of *in vivo* foci.¹⁰ Answers to these questions will certainly come from this group and others, deepening our understanding of the complex DDR and chromatin landscape remodeling mechanisms that are essential for genome integrity.

Figure Legends

Figure 1. Schematic of a *Medicago truncatula* flower (yellow) and cell nucleus (dark blue sphere). MtSUVR2 activity in DNA damage repair and heterochromatin condensation is shown as blow-up images. MtSUVR2 localizes to heterochromatin and forms foci (red spheres) via liquid-liquid phase separation at double strand breaks, recruiting RAD51 to these foci (top blow-up). MtSUVR2 is shown as purple ovals, RAD51 as white spheres and DNA as green cartoons. MtSUV2 acts as a histone methyltransferase and plays a role in maintaining heterochromatin (bottom, blow-up). MtSUV2 deposits repressive marks on histone H3 (H3K9me2/3). Nucleosomes are shown as yellow cartoons with DNA in green and MtSUVR2 in purple.

References

1. Luo, Y., Na, Z. & Slavoff, S. A. P-Bodies: Composition, Properties, and Functions. *Biochemistry* **57**, 2424–2431 (2018).
2. Lafontaine, D. L. J., Riback, J. A., Bascetin, R. & Brangwynne, C. P. The nucleolus as a multiphase liquid condensate. *Nat Rev Mol Cell Biol* **22**, 165–182 (2021).
3. Kosmacz, M. *et al.* Protein and metabolite composition of Arabidopsis stress granules. *New Phytologist* **222**, 1420–1433 (2019).

4. Van Buskirk, E. K., Decker, P. V. & Chen, M. Photobodies in Light Signaling. *Plant Physiology* **158**, 52–60 (2012).
5. Oshidari, R. *et al.* DNA repair by Rad52 liquid droplets. *Nat Commun* **11**, 695 (2020).
6. Phase separation of 53BP1 determines liquid-like behavior of DNA repair compartments. *The EMBO Journal* **38**, e101379 (2019).
7. Liu, Q. *et al.* The histone methyltransferase SUVR2 promotes DSB repair via chromatin remodeling and liquid-liquid phase separation. *Mol Plant* **15**, 1157–1175 (2022).
8. Oldfield, C. J. & Dunker, A. K. Intrinsically Disordered Proteins and Intrinsically Disordered Protein Regions. *Annu. Rev. Biochem.* **83**, 553–584 (2014).
9. Borchers, W., Bremer, A., Borgia, M. B. & Mittag, T. How do intrinsically disordered protein regions encode a driving force for liquid–liquid phase separation? *Current Opinion in Structural Biology* **67**, 41–50 (2021).
10. McSwiggen, D. T., Mir, M., Darzacq, X. & Tjian, R. Evaluating phase separation in live cells: diagnosis, caveats, and functional consequences. *Genes Dev.* **33**, 1619–1634 (2019).

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Figure 1.

