Genes & Diseases (2023) 10, 1–3

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RESEARCH HIGHLIGHTS

Phase 'separating' 53BP1 from DSB repair

Youwei Zhang

Department of Pharmacology, Case Comprehensive Cancer Center, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA

Received 19 April 2022; accepted 25 April 2022
Available online 14 May 2022

Eukaryotic chromosomes occupy specific territories in the nucleus, which is key for genome stability and function. Based on the spatiotemporal organization and compaction pattern, eukaryotic chromatins can be recognized as transcriptionally active and loosely packed euchromatin in the nuclear interior and transcriptionally repressive and highly compacted heterochromatin at the nuclear periphery or around nucleoli (Fig. 1). Mounting evidence suggests that heterochromatin plays a critical role in maintaining genome stability and function by preserving the chromosome integrity and repressing or limiting transcription of repetitive DNA. Heterochromatin can be generally subcategorized into constitutive and facultative types. Constitutive heterochromatin is usually found at repetitive satellite DNA regions such as those at peri-centromeres and telomeres. On the other hand, facultative heterochromatin is rich in repetitive transposons and can lose its condensed structure and become transcriptionally active under specific developmental or environmental conditions. A hallmark of constitutive heterochromatin is tri-methylation at the 9th lysine residue of the histone protein H3 (commonly known as H3K9me3), which is carried out by the 'writer', suppressor of variegation 3–9 homolog 1/2 (SUV39H1/2), and will be recognized by the 'reader', heterochromatin protein 1 (HP1). Human HP1 has three paralogues, HP1α, β and γ, which are encoded by three different genes, chromobox homolog 5 (*Cbx5*), *Cbx1*, and *Cbx3*, respectively. It seems that HP1α is commonly associated with constitutive heterochromatin, whereas HP1β and HP1γ have both gene-silencing and gene-activating roles, probably due to the slight structural difference.

Liquid–liquid phase separation (LLPS) is a dynamic process by which proteins, often with nucleic acids (such as RNAs), form membraneless liquid droplets (also called condensates or inclusion bodies) in cells.[1] The concept of LLPS is particularly interesting in understanding nuclear events, as it has demonstrated great impact on the flow of the genetic information by, for instance, selectively enriching/precluding specific components and/or altering the local chemical/physical environment at euchromatin or heterochromatin. While HP1α facilitated the rapid compaction of DNA into LLPS droplets upon phosphorylation at the N-terminus or binding with DNA, HP1β or HP1γ did not, suggesting different properties of HP1 paralogs. Since HP1α requires phosphorylation or the addition of DNA for LLPS formation,[2] it is tempting to speculate that additional factors are likely required (or important) for this process. Consistent with this idea, other heterochromatin factors including the H3K9me2/3 'writer' SUV39H1 and a HP1α-interacting protein TRIM28/KAP1 also formed condensates.

53BP1 has been primarily known as a key mediator in determining the DNA double strand break (DSB) repair choice in the presence of DNA damage. However, we recently reported previously uncharacterized nuclear puncta of 53BP1 in the absence of DNA damage.[3] We confirmed that the 53BP1 puncta did not result from DNA damage foci, but instead localized at heterochromatin.[3] We showed that 53BP1 puncta rely on heterochromatin factor HP1α to form. However, depletion of 53BP1 reduced the

E-mail address: yxz169@case.edu.

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.04.024

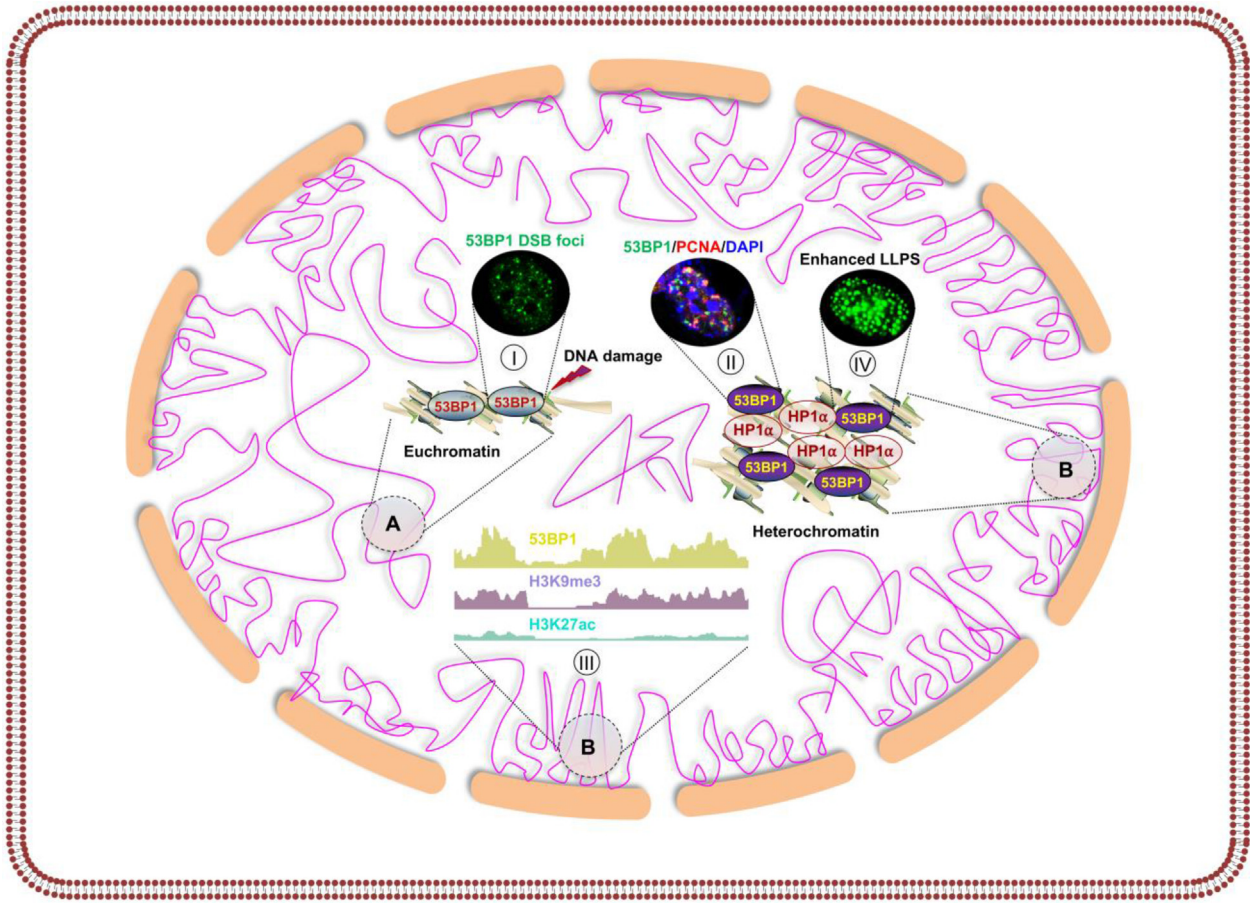

Figure 1 Model for the separate function of 53BP1 in DSB repair at euchromatin and LLPS at heterochromatin. For simplicity, the cytoplasmic content is omitted. Pink lines: chromatin/DNA in the nucleus, which can adopt into two compartmentalization patterns, A and B for euchromatin and heterochromatin, respectively. While type A is mainly in the interior of the nucleus, B type is largely localized at the nuclear periphery or adjacent to nucleoli. 53BP1 is distributed to both A and B types but demonstrates different functions. In type A, 53BP1 is involved in DSB repair choice by forming foci when the DNA is damaged (I), whereas in type B, it regulates the steady state function of heterochromatin under normal growth conditions through LLPS with HP1α and probably other heterochromatin factors (II, III). We identified a gain-of-function mutant of 53BP1, which, when expressed in cells, enhanced LLPS and promoted heterochromatin formation (IV), bearing the potential to be developed as a heterochromatin enhancer.

number of heterochromatin centers and resulted in the transcriptional de-repression of heterochromatic repetitive DNA, suggesting an important role of 53BP1 in maintaining both the compact structure and the transcriptional repression of heterochromatin (Fig. 1).

We then presented several lines of evidence to prove that the 53BP1 puncta are liquid droplets that are undergoing phase separation with HP1α.[1] The magnitude of the puncta formation generally depended on the protein level of 53BP1;[3] 53BP1 puncta temporarily resolve during mitosis but quickly re-form when cells enter G1 phase,[3] the same morphological change as HP1α nuclear bodies;[2] 53BP1 puncta are sensitive to temperature rise or chemical treatment that are known to disrupt the LLPS formation;[4] fluorescence recovery after photobleaching (FRAP), a commonly used method to assess LLPS in cells, shows that depletion of 53BP1 and HP1α reduced each other's mobility at heterochromatin, confirming their mutual dependence in LLPS;[5] purified 53BP1 and HP1α proteins facilitated each other's liquid droplet formation *in vitro*, a key readout for LLPS; 53BP1 and HP1α interacted with each other in cell cultures by co-immunoprecipitation and ChIP-seq. We further characterized separation-of-function mutants of 53BP1 that are differentially involved in LLPS and DSB repair. Importantly, 53BP1 mutant with LLPS function only also protected cells from stress-induced DNA damage, growth inhibition and senescence,[3] illustrating a protective function of 53BP1 LLPS on genome stability that is independent of its canonical role in DSB repair (Fig. 1).

Previously, the 53BP1 DSB foci were shown to also display LLPS features.[4,5] There is overlap between these two types of 53BP1 liquid droplets (i.e., at DSB site in the presence of DNA damage *vs* at heterochromatin in the absence of DNA damage), as both depended on the oligomerization domain (OD).[3,4] However, the differences are more significant. First, although both the OD and the following tandem Tudor domains are required for LLPS and DSB foci, their specific roles in these two functions are different. For LLPS at heterochromatin, the OD is absolutely essential, whereas the Tudor is playing a facilitating

role, but not essential. In contrast, both the OD and the Tudor domains are essential for foci formation at DSB sites. Second, even for the same OD, its requirement for these two types of LLPS is different. Four residues (H1237, T1248, Y1258, and V1269) in the OD that are conserved between human and yeast proteins are important for LLPS at heterochromatin, but not for foci formation at DSB site. Third, while the Asp1521 in the Tudor domain is absolutely essential for foci-type LLPS at DSB site through binding to methylated H4, it is dispensable for LLPS at heterochromatin. Fourth, H2A ubiquitination at K13/15 is not involved in LLPS at heterochromatin, but important for DSB foci formation of 53BP1. These findings collectively suggest that 53BP1 can form different types of liquid condensates in a context-depending fashion. An interesting question then is how the same protein knows when to form one type of LLPS but not the other. Further, whether there is a switch or competition of 53BP1 proteins between these two types of droplet formation remains to be determined. A possibility is that specific binding partners and/or posttranslational modifications of 53BP1 determine the LLPS outcome. These questions require further investigation, which will reveal new aspects of function of this important protein factor and its role in genome biology.

Author contributions

Y.Z. wrote the manuscript.

Conflict of interests

The author declares no conflict of interests.

Funding

Y. Z. is supported in part by US National Institutes of Health (NIH)/National Cancer Institute (NCI) (No. R01CA230453) and the Case Comprehensive Cancer Center grant (No. P30CA043703).

References

1. Boeynaems S, Alberti S, Fawzi NL, et al. Protein phase separation: a new phase in cell biology. *Trends Cell Biol*. 2018;28(6):420—435.
2. Larson AG, Elnatan D, Keenen MM, et al. Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin. *Nature*. 2017;547(7662):236—240.
3. Zhang L, Geng X, Wang F, et al. 53BP1 regulates heterochromatin through liquid phase separation. *Nat Commun*. 2022;13(1):360.
4. Kilic S, Lezaja A, Gatti M, et al. Phase separation of 53BP1 determines liquid-like behavior of DNA repair compartments. *EMBO J*. 2019;38(16):e101379.
5. Pessina F, Giavazzi F, Yin Y, et al. Functional transcription promoters at DNA double-strand breaks mediate RNA-driven phase separation of damage-response factors. *Nat Cell Biol*. 2019;21(10):1286—1299.